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Temperature-Sensitive Mutants of Chandipura Virus
II. Phenotypic Characteristics of the Six Complementation Groups

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Fifty temperature-sensitive (*ts*) mutants of the rhabdovirus Chandipura virus have been classified into six complementation groups designated ChI to ChVI. Group ChI contains 44 mutants, group ChII contains 2 mutants, and the remaining groups have 1 mutant each. Mutants in groups ChI, ChIII, ChIV, and ChVI had RNA-negative phenotypes in experiments measuring amplification of RNA synthesis at restrictive temperature. The two mutants in group ChII had RNA-positive phenotypes, and the virions were thermolabile. Mutant *ts* Ch851 of group ChV was also RNA positive, and the M polypeptide of this mutant appeared to be unstable in cells incubated at restrictive temperature. It is likely, therefore, that complementation groups ChII and ChV represent the genes coding for the two viral proteins of the virion envelope. No precise assignment can be made in the case of the four RNA-negative groups, since all the mutants examined showed some polymerase activity in vitro at restrictive temperature. An attempt to obtain polymerase mutants by screening for sensitivity to rifampin was not successful. Six temperature-dependent host range mutants (the *td*CE phenotype) of Chandipura virus failed to multiply in chicken embryo cells at restrictive temperature, but otherwise they differed in their host range properties from similar mutants of vesicular stomatitis virus.

Fifty temperature-sensitive mutants of Chandipura virus, a human rhabdovirus isolated in India, have been classified into six complementation groups. Group ChI contains 44 mutants, group ChII contains two mutants, and groups ChIII, ChIV, ChV, and ChVI have one mutant each. Weak complementation was observed between certain mutants in group ChI, and it was possible to distinguish two subgroups ChIA and ChIB by their interaction with mutant *ts* Ch1, an arbitrarily chosen prototype for group ChI (1).

We now describe characterization of the phenotype of the mutants of groups ChII, ChIII, ChIV, ChV, and ChVI and a representative sample of the mutants of subgroups ChIA and ChIB. The properties examined included RNA phenotype in vivo, RNA polymerase activity in vitro, thermal lability, and intracellular protein synthesis. Evidence is presented which associates complementation group ChV with the M protein transcriptional unit of the genome.

The isolation and host range of six temperature-dependent host range (*td*CE) mutants of Chandipura virus are also described. These mutants of Chandipura virus failed to multiply in chicken embryo (CE) cells at restrictive temperature, but otherwise were distinct in their host range properties from previously described *td*CE mutants of the Indiana and New Jersey serotypes of vesicular stomatitis virus (VSV) (10).

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MATERIALS AND METHODS

Cells. The BS-C-1 line of African green monkey kidney cells was propagated in roller bottle cultures in Eagle minimum essential medium (Glasgow modification) supplemented with 10% calf serum. BHK-21 clone 13 cells were supplied routinely by the Cytology Department of the Institute of Virology.

Bovine embryo kidney (BEK) cells and hamster embryo (HE) cells were obtained from Gibco Biocult, Glasgow. Feline embryo (FEA) cells were obtained from O. Jarrett, Department of Veterinary Pathology, University of Glasgow, and rat embryo (RE) cells were supplied by J. Macnab of the Institute of Virology. Mouse embryo (NIH/3T3) cells were obtained from S. A. Aaronson, National Institutes of Health, Bethesda, U.S.A.

Secondary CE cell cultures were prepared from eggs obtained from a leukosis-free flock maintained by the Poultry Research Institute, Edinburgh, Scotland.

Virus. Chandipura virus was obtained from F. Murphy, Center for Disease Control, Atlanta, Ga., and cloned by three sequential isolations from single plaques on BHK-21 cell monolayers. Isolation and classification of the *ts* mutants of Chandipura virus have been described in the preceding paper (1).

The rifampin-sensitive mutant (VSV-29) of VSV Indiana and its wild type (VSV-VJ) were kindly provided by Ch. Chany, INSERM U-43, Hôpital St. Vin-

cent de Paul, Paris, France. The Evans vaccine strain of vaccinia virus was obtained from H. Pennington, Institute of Virology.

Determination of RNA phenotype. BS-C-1 cell monolayers in 30-ml bottles were incubated at 37°C for 2 h in the presence of 10 μg of actinomycin D per ml (Cosmegen; Merck Sharp and Dohme, Rahway, N.J.). The cultures were then infected in duplicate at a multiplicity of 10 PFU/cell, and the virus was allowed to adsorb at +4°C for 30 min. Eagle medium containing 10 μg of actinomycin D per ml and 10 μCi of [^{3}H]uridine per ml (26 Ci/mmol; Radiochemical Centre, Amersham) was added to each culture bottle, and one set of cultures was immersed in a water bath at 39.5°C while the other set was incubated at 31°C. At 9 h after infection, 2 ml of a solution of 200-μg/ml proteinase K (Boehringen-Mannheim) in 0.01 M Tris-hydrochloride (pH 8.0) containing 0.01 M EDTA, 0.01 M NaCl, and 0.2% (wt/vol) sodium dodecyl sulfate was added to each monolayer, and the cells were digested at 20°C for 18 h. Trichloroacetic acid-precipitable radioactivity was measured for each sample in an Intertechnique SL30 liquid scintillation counter.

Temperature shift experiments. The effect of temperature shift on the synthesis of RNA in *ts* mutant-infected cells was determined as follows. BS-C-1 cells in 30-ml bottles were infected at a multiplicity of 10 approximately, and the cultures were incubated at 31 or 39.5°C in 2 ml of medium containing 10 μg of actinomycin D per ml. After 3 h of incubation the culture medium was replaced with 2 ml of prewarmed (31 or 39.5°C) medium containing 10 μCi of [^{3}H]uridine per ml and 10 μg of actinomycin D per ml. Cultures previously incubated at 31°C were shifted to 39.5°C and incubated for 6 h at that temperature. Cultures previously incubated at 39.5°C were shifted to 31°C. Other cultures were maintained at 31 or 39.5°C throughout the experiment. All cultures were inoculated in duplicate and harvested at 9 h as described above.

In vitro polymerase assay. The polymerase activity of purified preparations of wild-type Chandipura virus and its *ts* mutants was assayed at 31 and 39°C under the conditions established for VSV Indiana (11). It was determined by experiment, however, that the optimum concentration of Triton N-101 in the reaction mixture was 0.08% for Chandipura virus. The reaction mixtures were warmed for 1 min at either 31 or 39°C prior to addition of 220 mM $MgCl_2$. Samples of 20 μl were withdrawn immediately before addition of $MgCl_2$ (the zero time sample) and after 30, 60, 90, and 120 min at 31 or 39°C, transferred to 2.5-cm^2 Whatman DE81 (ionic) paper disks, and air dried. The disks were then washed five times in 5% (wt/vol) sodium pyrophosphate at 20°C, followed by two washes in deionized water, ethanol, and diethyl ether. Residual ether was removed in a stream of air, and the disks were immersed overnight in toluene-based scintillant prior to counting (12).

Virus purification. Purified virions were obtained by inoculating BHK-21 clone 13 cells in rotating 2-liter bottles at a multiplicity of 0.1. The contents of 10 bottles were pooled and clarified by centrifugation at 1,500 rpm for 20 min at 4°C in a Mistral centrifuge (M.S.E. Ltd., London). Virus in the supernatant was sedimented through a 30% (vol/vol) glycerol layer using a rotor (10 by 100 ml) at 40,000 × *g* in a Superspeed 65 ultracentrifuge (M.S.E. Ltd., London) at 4°C. The resuspended pellet was layered on a 15 to 45% (wt/vol) linear sucrose gradient and centrifuged in a Beckman SW27 rotor at 50,000 × *g* for 60 min at 4°C. The opalescent band was collected by pipette and pelleted by centrifugation at 150,000 × *g* for 30 min at 4°C in a Beckman SW40 Ti rotor. The pellet was resuspended in a small volume of 20 mM Tris-hydrochloride (pH 8.0).

Determination of heat stability. Virus stocks were diluted 10-fold into phosphate-buffered saline and distributed into thin-walled glass vials. Heat exposure was carried out by totally immersing these vials in a water bath at 45 ± 0.1°C. Samples were transferred to an ice bath before heat exposure and at 45-min intervals after immersion at 45°C. Residual infectivity was assayed on BS-C-1 monolayers.

Analysis of intracellular proteins. BHK-21 cells in 30-mm petri dishes were infected at a multiplicity of 20 PFU/cell. The infected cells were labeled at 4.5 h after infection in Eagle medium containing one-tenth the normal concentration of methionine and 30 μCi of [^{35}S]methionine per ml (5 to 7 mCi/mmol; Radiochemical Centre, Amersham). The cells were either pulse-labeled for 15 min or continuously labeled for 3 h.

At 7.5 h after infection the medium from each culture plate was removed, 0.2 ml of a solubilization mixture (2% [wt/vol] sodium dodecyl sulfate and 5% [vol/vol] 2-mercaptoethanol in 0.14 M Tris-hydrochloride [pH 6.7] plus 10% [vol/vol] glycerol and bromophenol blue) was added directly to the monolayer, and the plates were left at 20°C for 15 min. Before electrophoresis, the samples were heated at 100°C for 5 min. Trichloroacetic acid-insoluble radioactivity was measured, and the concentration of the samples was adjusted so that each track in any single gel slab contained approximately equal counts.

Polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis was carried out in 5 to 12.5% gradient slab gels according to the method described by Marsden et al (5).

RESULTS

RNA phenotype. The RNA phenotype of the mutants was established as described in Materials and Methods. Eight mutants from subgroup ChIA and five mutants from subgroup ChIB were chosen at random to represent group ChI. Incorporation of [^{3}H]uridine was ≤1.3% of the wild-type value (Table 1). The group ChI mutants, therefore, were all considered to be RNA-negative mutants. The three mutants representing groups ChIII, ChIV, and ChVI also exhibited RNA-negative phenotypes, having levels of incorporation ≤0.15% of wild type. On the other hand, the two mutants belonging to group ChII showed good incorporation, 56 and 73% of wild type, and were designated RNA-positive mutants. Mutant *ts* Ch851 of group ChV

was also an RNA-positive mutant and appeared to incorporate more [^{3}H]uridine (329%) than wild type.

No significant incorporation of [^{3}H]uridine was observed when most of the RNA-negative *ts* mutant-infected cultures were shifted from permissive to restrictive temperature at 3 h after infection. These observations suggested that the input virion proteins, or those synthesized during the first 3 h at the permissive temperature, remained nonfunctional at the restrictive temperature. Cells infected with two RNA-negative mutants, *ts* Ch203 (ChIB) and *ts* Ch472 (ChIV), did show significant incorporation of [^{3}H]uridine after temperature up-shift (Table 2). This incorporation was not due to presence of wild-type virus in the inoculum, nor to reversion during multiplication, since the cultures maintained at restrictive temperature throughout the entire experiment showed no increase in [^{3}H]uridine incorporation between 3 and 9 h after infection. These results suggested that viral polypeptides synthesized during the first 3 h at the permissive temperature remained functional after the increase in incubation temperature.

TABLE 1. *RNA phenotype and heat stability of 19 ts mutants of Chandipura virus*

Complementation group	Mutant	[^{3}H]uridine incorporation (% of wild type)	RNA phenotype	Thermal inactivation at 45°C (V_t/V_o)[a]	Heat stability[b]
ChIA	*ts* Ch1	0.26	−	0.20	R
	ts Ch90	0.30	−	0.075	(R)
	ts Ch363	0.11	−	0.4	R
	ts Ch449	0.14	−	0.0005	L
	ts Ch482	0.9	−	0.29	R
	ts Ch484	0.03	−	0.17	R
	ts Ch540	0.11	−	0.01	(R)
	ts Ch867	0.16	−	0.20	R
ChIB	*ts* Ch128	0.3	−	0.14	R
	ts Ch188	0.02	−	0.03	(R)
	ts Ch203	0.35	−	0.4	R
	ts Ch315	1.30	−	0.01	(R)
	ts Ch808	0.25	−	0.0001	L
ChII	*ts* Ch4	73.2	+	0.0005	L
	ts Ch5	55.9	+	0.00001	L
ChIII	*ts* Ch465	0.09	−	0.4	R
ChIV	*ts* Ch472	0.14	−	0.5	R
ChV	*ts* Ch851	329.0	+	0.06	(R)
ChVI	*ts* Ch319	0.1	−	0.02	(R)
Wild type	*ts*$^+$	100	+	0.30	R

[a] V_o = the titer before and V_t = the titer after exposure at 45°C for 4.5 h.
[b] R, Resistant; (R), partially resistant; L, labile.

TABLE 2. *Effect of temperature shift on intracellular RNA synthesis*

Group	Mutant	[^{3}H]uridine incorporation (%)[a]		
		39.5°C	31°C → 39.5°C	39.5°C → 31°C
ChIA	*ts* Ch363	0.15	0.09	62.0
	ts Ch449	0.04	ND[b]	82.0
	ts Ch540	0.1	0.16	57.7
	ts Ch867	0.16	0.12	139.7
ChIB	*ts* Ch1	0.1	0.1	79.6
	ts Ch128	0.14	3.5	134.0
	ts Ch203	0.07	13.1	99.0
	ts Ch315	0.8	0.16	39.2
	ts Ch808	0.08	0.08	38.0
ChII	*ts* Ch4	35.4	32.1	221.9
	ts Ch5	10.5	39.4	96.0
ChIII	*ts* Ch465	0.1	0.1	59.5
ChIV	*ts* Ch472	0.15	17.7	83.8
ChV	*ts* Ch851	37.0	39.6	38.5
ChVI	*ts* Ch319	0.1	0.08	39.6
Wild type	*ts*$^+$	12.3	12.5	12.6

[a] [^{3}H]uridine incorporation between 3 and 9 h postinfection as percentage of the 31°C level.
[b] ND, No data.

In the reciprocal experiment, cultures infected with any of the RNA-negative *ts* mutants showed significant [^{3}H]uridine incorporation when incubated for 3 h at restrictive temperature and then labeled after transfer to permissive temperature for 6 h (Table 2). These results suggested that the mutant genomes remained functional during the 3-h sequestration at restrictive temperature.

As expected, the RNA-positive *ts* mutants induced significant incorporation of [^{3}H]uridine in both the up-shift and down-shift experiments (Table 2).

Heat stability. Preliminary experiments showed that the mutants could be differentiated best by exposure at 45°C for 4.5 h. Table 1 lists the reduction in infectivity for each mutant and wild type. Both mutants belonging to group ChII and mutants *ts* Ch449 (ChIA) and *ts* Ch808 (ChIB) appeared to be thermolabile (>2 $\log_{10}$ units inactivation). The remaining mutants were fully or partially heat resistant.

In vitro polymerase activity. The in vitro polymerase activity of purified preparations of Chandipura virus and its *ts* mutants was determined as described in Materials and Methods. Wild-type virus and RNA-positive mutants *ts* Ch4 (group ChII), *ts* Ch5 (group ChII), and *ts* Ch851 (group ChV) showed considerably more

in vitro polymerase activity than the remaining mutants at both 31 and 39°C; nevertheless, an unequivocally RNA-negative phenotype was not detected among the remaining mutants (data not shown).

Since none of the *ts* mutants showed any clear defect in polymerase activity in vitro, an attempt was made to obtain polymerase mutants by screening clones of mutagenized Chandipura virus for sensitivity to rifampin. Previously, Moreau (6) had reported the isolation of a rifampin-sensitive mutant of VSV Indiana which displayed atypical polymerase activity in vitro (7). Rifampin was obtained from both Sigma Chemicals (London) and, as aged and fresh preparations, from G. Tocchini-Valentini, Lancini-Lepetit, Milan, Italy. The antibiotic was dissolved by adding 200 mg to 2 ml of dimethyl sulfoxide and diluting to 10 ml with sterile distilled water. This stock solution was diluted 1:100 in solid or liquid incubation medium to give a final concentration of 200 μg/ml. A total of 593 clones of Chandipura virus grown in the presence of 100-μg/ml 5-fluorouracil were tested for ability to multiply in the presence of 200 μg of rifampin per ml, but none was sensitive to the drug. All the preparations of rifampin reduced vaccinia virus yields by more than 4 $\log_{10}$ units at this concentration. VSV was slightly more rifampin sensitive than Chandipura virus, but it was not possible to clearly differentiate the rifampin-sensitive mutant (VSV-29) isolated by Moreau from its wild type (VSV-VJ) in BS-C-I cells under these conditions, and the attempt to obtain polymerase mutants by this procedure was abandoned.

Intracellular protein synthesis. BHK-21 cell monolayers were used for radiolabeling of intracellular polypeptides in preference to BS-C-1 cells, because there was less loss of cells by detachment during the labeling period. Figure 1 shows the [^{35}S]methionine-labeled polypeptides

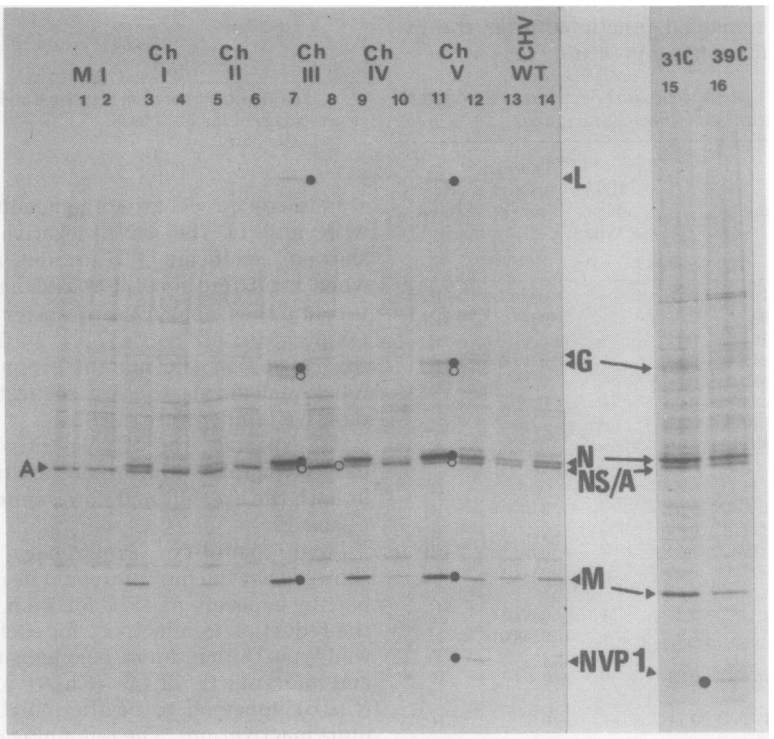

FIG. 1. *Polypeptide synthesis in BHK-21 cells infected with ts mutants ts Ch1, ts Ch4, ts Ch465, ts Ch472, and ts Ch851, from complementation groups ChI, ChII, ChIII, ChIV, and ChV, respectively. The odd-numbered tracks are the polypeptide profiles of samples from cultures labeled at 31°C, and the even-numbered tracks are samples from cultures labeled at 39°C. Monolayers of BHK-21 cells (or BS-C-1 cells for tracks 15 and 16) were infected at 20 PFU/cell and labeled between 4 and 7 h with 30 μCi of [^{35}S]methionine per ml. The cultures were harvested and proteins were extracted as described in the text. Samples containing equal amounts of radioactivity were electrophoresed on a 5 to 12.5% polyacrylamide gel for 3 to 4 h at 40 mA. The viral polypeptides are labeled L, G, N, NS, and M; A is actin, NVP1 is nonviral polypeptide 1, and MI is a mock infection. Tracks 15 and 16 were taken from another gel to show that NVP1 was also resolved in BS-C-1 cells infected with ts Ch851 (group ChV) at 39°C.*

synthesized in cells infected with wild-type virus and mutants *ts* Ch1, *ts* Ch4, *ts* Ch465, *ts* Ch472, and *ts* Ch851, representing complementation groups ChI, ChII, ChIII, ChIV, and ChV, respectively. All the *ts* mutants, including *ts* Ch319 of group ChVI (not shown), synthesized less viral protein at 39°C (even-numbered tracks). The L polypeptide was not resolved in the cultures incubated at 39°C, except in *ts* Ch851 (group ChV)-infected cells. The G protein was resolved into two bands in most of the mutant-infected cells at 31°C. These two bands probably correspond to the G1 and G2 polypeptides of VSV and represent two stages in glycosylation (2; R. M. L. Buller, Ph.D. thesis, University of Glasgow, Glasgow, Scotland, 1975). The pulse-chase experiments illustrated in Fig. 2, 3, and 4 confirmed that a shift in electrophoretic mobility of the G protein occurred during incubation, as with VSV Indiana (2). The N polypeptide of Chandipura virus migrated just above the cellular actin band and was clearly resolved in all the mutant-infected cells. The NS polypeptide was not resolved, since it migrated in the region of cellular actin. The M polypeptide was present in all the virus-infected cell extracts.

A [^{35}S]methionine-labeled polypeptide designated NVP1 (nonviral polypeptide 1) was observed in both BHK-21 (track 12) and BS-C-1 (track 16) cells infected with mutant *ts* Ch851 and incubated at 39°C. NVP1 was not observed in *ts* Ch851-infected cells at 31°C (tracks 11 and 13), or in cells infected with wild-type virus and other *ts* mutants at either 31 or 39°C. NVP1 was also not resolved in cells infected with either of two spontaneous revertants of *ts* Ch851 (Fig. 4).

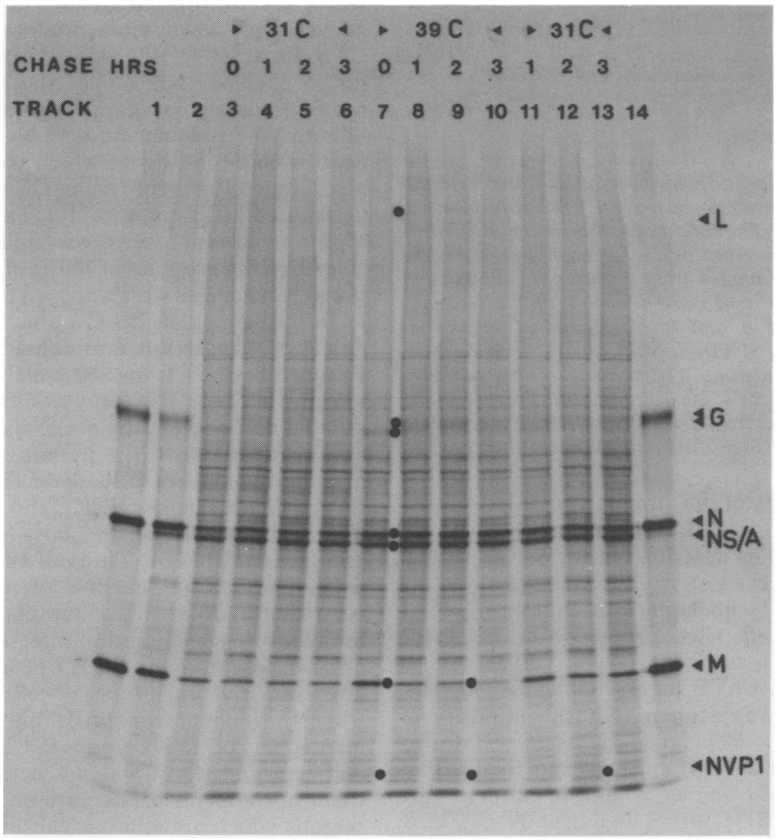

FIG. 2. *The polypeptides of ts Ch851-infected cells in a pulse-chase experiment. BHK-21 cell cultures were infected with mutant ts Ch851 at 20 PFU/cell and incubated for 4 h at 31 or 39°C. The cultures were labeled for 15 min with 30 μCi of [^{35}S]methionine per ml and then returned to ordinary medium. Cultures were harvested at 0, 1, 2, and 3 h after labeling as described in the text. Samples containing equal counts were electrophoresed on a 5 to 12.5% polyacrylamide gel. Tracks 3, 4, 5, and 6, Cultures incubated at 31°C throughout and harvested at 0, 1, 2, and 3 h, respectively, after labeling; tracks 7, 8, 9, and 10, cultures maintained at 39°C throughout; tracks 11, 12, and 13, cultures labeled at 39°C and subsequently incubated at 31°C for 1, 2, and 3 h, respectively. Tracks 1 and 14 show marker polypeptides from partially purified ts Ch851, and track 2 shows partially purified wild-type virus. The letters are identified in the legend to Fig. 1.*

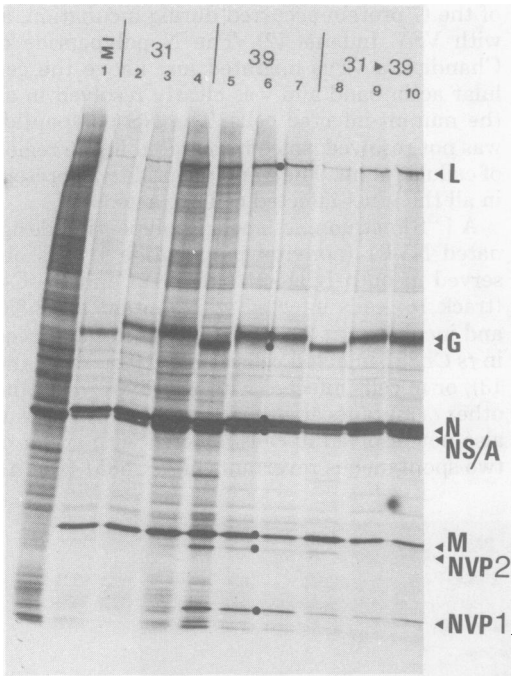

FIG. 3. *Polypeptide synthesis in ts Ch851-infected cells after transfer to restrictive temperature at 4 h after infection. The conditions of infection and labeling were as described in the legend to Fig. 2. Tracks 2, 3, and 4, Samples from cultures incubated and labeled at 31°C and chased for 0, 1, and 2 h, respectively; tracks 5, 6, and 7, corresponding cultures incubated at 39°C throughout; tracks 8, 9, and 10, cultures incubated at 31°C and subsequently labeled and chased at 39°C for 0, 1, and 2 h. Track 1 shows the polypeptides from a mock-infected culture at 31°C. The letters are identified in the caption to Fig. 1.*

The stability of the viral polypeptides synthesized at 39°C was examined in pulse-chase experiments (3) as described in the legend to Fig. 2. No degradation of viral polypeptides was detected in cells infected with mutants *ts* Ch1 (group ChI), *ts* Ch4 (group ChII), *ts* Ch465 (group ChIII), *ts* Ch472 (group ChIV), and *ts* Ch319 (group ChVI). In *ts* Ch851-infected cells, however, it was evident that the intensity of labeling of the M polypeptide decreased during incubation at 39°C (Fig. 2, tracks 7 to 10). This effect was not observed when the cultures were incubated at 31°C after labeling at 39°C (Fig. 2, tracks 11 to 13). However, if the cells were incubated at 31°C and then labeled and chased at 39°C, degradation of M was observed (Fig. 3, tracks 8 to 10). NVP1 was apparent in *ts* Ch851-infected cells, provided the cultures were incubated at 39°C during or after pulse-labeling (Fig. 2, tracks 7 to 13, and Fig. 3, tracks 5 to 10). An additional polypeptide (NVP2) has been resolved in Fig. 3, but the nature of this polypeptide or the band between N and M in track 5 is unknown. Neither was observed consistently in other gels.

A spontaneous revertant (Ch851/R2) of mutant *ts* Ch851 was isolated and compared with the parental mutant in the same experiment (Fig. 4). The instability of the M polypeptide in *ts* Ch851-infected cells at 39°C was again apparent (tracks 5 to 7), but in cells infected with the revertant there was less degradation of M (tracks 11 to 13). Densitometer tracings of the tracks in Fig. 4 showed that during the chase period radioactivity in the M peak declined to 60% of the initial level for revertant Ch851/R2 and 33% for mutant *ts* Ch851, standardized in each case to the N peak. Similar results were obtained with another revertant clone of *ts* Ch851.

Host range properties of *ts* and *td*CE mutants of Chandipura virus. The wild-type and conventional *ts* mutants produced plaques on CE cells at 31°C, and only the wild type produced plaques at 39°C, i.e., the *ts* mutants retained their phenotype in avian cells. Mutants of both VSV Indiana and VSV New Jersey have been isolated, however, which were conditionally temperature sensitive in CE cells and were designated *td*CE mutants (10). Therefore Chandipura wild-type virus was mutagenized by growth in the presence of 100 μg of 5-fluorouracil per ml, and clones were screened for their ability to form plaques on BS-C-1 and CE cells at 31 and 39°C. The six *td*CE mutants listed in Table 3 were obtained from 509 isolates, i.e., a frequency of 1.2%. No mutants completely restricted on CE cells (the *hr*CE phenotype) were obtained. The six *td*CE mutants were titrated also on monolayers of hamster (HE), rat (RE), feline (FEA), bovine (BEK), and mouse (NIH/3T3) embryo cells and on mouse L929 cells at 31 and 39°C (Table 3). Three of the six mutants were temperature sensitive on CE cells only, whereas the other three mutants showed some temperature sensitivity on mammalian embryo cells. Indeed, mutant *td*CE Ch530 was temperature sensitive in all types of cells except BS-C-1 cells. None of the *td*CE mutants complemented any of the six groups in CE cells at 39°C, and consequently it was not possible to assign them to complementation groups. However, for reasons which are still unclear, little significant complementation was observed between the conventional *ts* mutants of Chandipura mutants in CE cells (data not shown).

DISCUSSION

The phenotypic properties of the *ts* mutants of Chandipura virus suggest that complementa-

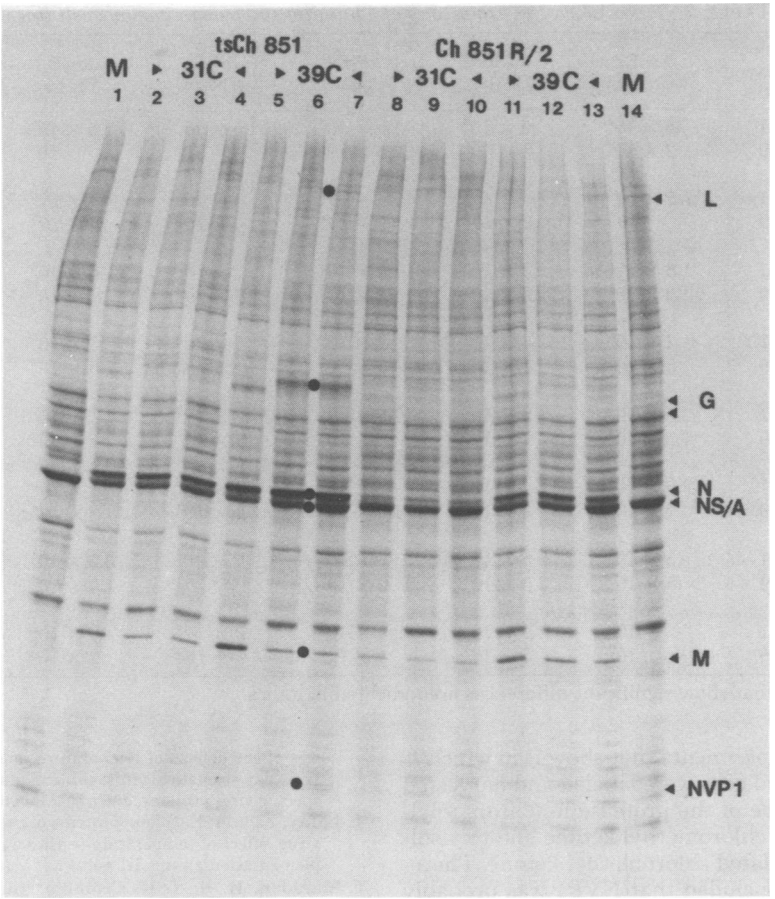

FIG. 4. *Polypeptide synthesis in cells infected with clone Ch851/R2, a non-temperature-sensitive revertant of ts Ch851. The conditions of infection and labeling were as described in the legend to Fig. 2, except that the cultures were harvested at 0, 1.5, and 3 h after labeling. Tracks 2, 3, and 4, Samples from cultures infected with ts Ch851 and incubated throughout at 31°C; tracks 5, 6, and 7, corresponding cultures incubated at 39°C; tracks 8, 9, and 10, cultures infected with the revertant clone Ch851/R2 and incubated throughout at 31°C; tracks 11, 12, and 13, corresponding cultures incubated at 39°C. Tracks 1 and 14 show polypeptides in mock-infected cells at 31°C. The letters are identified in the caption to Fig. 1.*

tion groups ChII and ChV correspond to the genes coding for the two proteins of the viral envelope. The virions of both mutants in group ChII were thermolabile (Table 1), and the intracellular M polypeptide of *ts* Ch851 (group ChV) was unstable at restrictive temperature (Fig. 2, 3, and 4). Reversion of the temperature sensitivity of *ts* Ch851 was accompanied by an increase in stability of the intracellular M polypeptide (Fig. 4). Mutant *ts* Ch851 was also an overproducer of RNA at restrictive temperature, like the group III mutants of VSV Indiana which are also considered to be M protein mutants (3, 4, 9). It is not possible to suggest any gene assignment in the case of the four RNA-negative groups. Group ChI, which contains 88% of the *ts* mutants of Chandipura virus, may correspond to group I of VSV Indiana (containing 77% of mutants), which is considered to represent the gene coding for the L protein (9). However, none of the *ts* mutants of Chandipura virus, including the thermolabile *ts* Ch449 of group ChIA and *ts* Ch808 of group ChIB, had defective RNA polymerase activity in vitro at the restrictive temperature.

The apparent mutant specificity of NVP1 suggested that it might be a minor virus-coded polypeptide which accumulated in conditions where maturation was blocked but transcription continued unrestricted. However, NVP1 could not be chased into virions by labeling at 39°C and incubation at 31°C (data not shown). In addition, NVP1 did not appear to be derived by proteolytic cleavage from any of the five viral

TABLE 3. *Titers of six tdCE mutants of Chandipura virus in various cell lines*[a]

Cells	Temp of incubation (°C)	*td*CE Ch514	*td*CE Ch516	*td*CE Ch525	*td*CE Ch530	*td*CE Ch565	*td*CE Ch859
BS-C-1	31	5×10^8	5×10^8	1.5×10^8	1.5×10^8	1.5×10^8	2×10^8
	39	2×10^8	10^8	2.5×10^7	10^7	10^7	5×10^7
CE	31	*1.5×10^7*	*10^7*	*10^7*	*10^7*	*5×10^6*	*3×10^7*
	39	*10^4*	*2×10^3*	*10^2*	*10^2*	*5×10^3*	*2×10^4*
HE	31	1.5×10^7	3×10^6	1.5×10^7	*10^7*	5×10^6	1.5×10^7
	39	3×10^6	2.5×10^6	3×10^6	*4×10^4*	10^6	10^6
RE	31	2×10^6	3×10^6	2×10^6	*5×10^5*	3×10^5	*5×10^6*
	39	5×10^5	4×10^5	5×10^5	*10^3*	1.5×10^4	*5×10^4*
FEA	31	2.5×10^7	2×10^7	2.5×10^7	*2×10^7*	10^7	3×10^7
	39	7.5×10^6	10^7	10^6	*5×10^3*	10^6	10^6
BEK	31	2.5×10^6	10^6	2×10^6	*2×10^6*	*1.5×10^6*	*2.5×10^6*
	39	7×10^5	5×10^5	1.5×10^5	*2×10^3*	*3.5×10^4*	*5×10^4*
L929	31	5×10^6	5×10^6	5×10^6	2×10^6	3.5×10^6	*3×10^6*
	39	5×10^5	3.5×10^5	2×10^5	*10^2*	1.5×10^5	*5×10^2*
NIH/3T3	31	10^6	2×10^6	2.5×10^6	*2×10^6*	5×10^5	*5×10^6*
	39	10^6	5×10^5	5×10^5	*10^2*	2×10^5	*1.5×10^4*

[a] The titers that show significant differences are indicated in italics.

proteins in experiments (not shown) in which *ts* Ch851-infected cells were labeled and harvested in the presence of the protease inhibitors tolylsulfonyl lysyl chloromethyl ketone and tolylsulfonyl phenylalanyl chloromethyl ketone. Therefore it was concluded that NVP1 was probably a host protein which accumulated at 39°C as a consequence of the defective maturation of mutant *ts* Ch851. Occasionally a faint band was observed in the NVP1 position in mock-infected cells (e.g., Fig. 3, tracks 1 to 4). Stimulation of a specific host protein by a rhabdovirus has not been reported previously, but enhanced synthesis of two host proteins has been observed in simian virus 40-infected CV1 cells (8).

Finally, the isolation of *td*CE mutants of Chandipura virus suggests that host factors are involved in the replication of all rhabdoviruses, not only VSV (10).

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