JOURNAL OF VIROLOGY, Oct. 1973, p. 677-683

Vol. 12, No. 4
Printed in U.S.A.

Genetic and Physiological Properties of Temperature-Sensitive Mutants of Cocal Virus

C. R. PRINGLE AND W. H. WUNNER
MRC Virology Unit, Institute of Virology, Glasgow, G11 5JR, Scotland

Received for publication 14 May 1973

Temperature-sensitive (*ts*) mutants of Cocal virus (VSV Cocal) were isolated after treatment with the base analogue mutagen, 5-fluorouracil. These mutants could be classified into four mutually complementing groups. Weak complementation was detected between certain pairs of VSV Cocal *ts* mutants and *ts* mutants of vesicular stomatitis virus (VSV) Indiana, but no complementation was observed with *ts* mutants of VSV New Jersey. Two complementing *ts* mutants of Chandipura virus, an unrelated rhabdovirus, did not complement any VSV mutant. Thus, ability to complement in the VSV group appears to be correlated with serological relationships.

The RNA and protein-synthesizing capacities of these *ts* mutants have been determined, and it is possible to establish a correspondence between the VSV Cocal and the VSV Indiana complementation groups.

Cocal virus (VSV Cocal) is a rhabdovirus isolated originally from a pool of mites taken from rice rats in Trinidad and has been shown to be serologically related to vesicular stomatitis virus (VSV) (5). It is now considered to represent a subgroup of the Indiana serotype of VSV and to be unrelated to the other major serotype of the VSV group, VSV New Jersey (3). We have shown recently that VSV Cocal differs from VSV Indiana in the electrophoretic mobility of the G (glycoprotein) and M (matrix) proteins of the virion (17).

Temperature-sensitive (*ts*) mutants of the Indiana and New Jersey serotypes of VSV have been isolated and classified into five and six complementation groups, respectively (4, 9-11). Mutants from different wild-type strains of VSV Indiana complement in different host cells (D. V. Cormack, A. F. Holloway, and C. R. Pringle, J. Gen. Virol., in press; 4), but no complementation was observed between mutants from the different serotypes in any combination of complementation group (11). This specificity suggests that the *ts* mutational lesions affect synthetic and maturation functions rather than major structural components of the virion. The absence of interstrain complementation is in marked contrast to the frequently recorded phenotypic mixing between VSV and unrelated enveloped viruses (7, 18).

We now describe the isolation of *ts* mutants of VSV Cocal and their genetic and phenotypic characterization. These mutants complement the VSV Indiana mutants in certain combinations, and this information together with the physiological properties of the mutants enables us to establish the correspondence of the complementation groups of the two strains. No complementation was observed between the VSV Cocal mutants and the VSV New Jersey mutants, which further indicates that ability to complement within the VSV group follows serological relationships.

MATERIALS AND METHODS

Cells. All wild-type strains of VSV and *ts* mutants were propagated in BHK 21 clone 13 cells (6).

Virus. The derivation of the wild-type strains of VSV Indiana and VSV New Jersey and their respective *ts* mutants has been described previously (9-11).

The VSV Indiana mutants now carry the prefix G to distinguish them from *ts* mutants derived from different wild-type strains of the same serotype (D. V. Cormack et al., in press).

VSV Cocal was obtained from R. B. Somerville, Belvidere Hospital, Glasgow. This strain had been maintained in vitro by serial passage in Vero cells. For comparison, a strain of VSV Cocal, passaged through a susceptible bovine host, was obtained from R. B. Burrows, Animal Virus Research Institute, Pirbright, Surrey. The VSV Cocal *ts* mutants were isolated from the Vero-propagated strain after it had been cloned by three sequential isolations from monolayers of BHK 21 cells with single plaques to establish a genetically homogeneous wild-type stock.

Two complementing mutants (*ts* Ch1 and *ts* Ch4) from a series of *ts* mutants isolated from the unrelated rhabdovirus, Chandipura virus (Pringle, unpublished data), were also used in one of the cross-complementation experiments. The wild-type stock was obtained from F. A. Murphy, Communicable Disease Center, Atlanta, Ga.

Mutagenesis. Monolayers of 3×10^6 BHK 21 cells in 30-ml screw-capped bottles were infected with wild-type VSV Cocal at low multiplicity (0.1 PFU/cell). The virus inoculum was allowed to adsorb at 4 C for 30 min. The infected monolayers were washed twice with 5 ml of cold Eagle medium containing 0.5% calf serum. Then 5 ml of the same medium containing concentrations of 5-fluorouracil (FU) (Koch-Light Ltd.) ranging from 5 to 500 μg/ml were added, and the infected monolayers were incubated until the cytopathic effect was maximal in control cells not exposed to the mutagen (~48 h). The entire cultures were frozen at −70 C, and the yield was analyzed subsequently for the presence of *ts* virus.

Complementation experiments. Complementation experiments were carried out by the procedure used with mutants of other serotypes of VSV (10). Briefly, monolayers of approximately 5×10^6 cells were infected at a multiplicity of approximately 10 PFU/cell for mixed inocula and 5 PFU/cell for self infections. Adsorption took place at +4 C for 20 min. The inoculum was then removed by two washes with cold incubation medium before the addition of 5 ml of the same medium. The infected monolayers were incubated totally immersed in a water bath at 39 C ± 0.05 C for 8 h. The samples were rapidly frozen and the yield of infectious virus was measured by plaque counting on BHK 21 cell monolayers at 31 C. Complementation indices were calculated according to the formula: yield $(m_1 + m_2)$/yield m_1 + yield m_2, where m_1 and m_2 are any two *ts* mutants. Values significantly greater than 1 indicate complementation. Each test was carried out in duplicate, and significance was tested by a procedure devised by R. A. Elton of the Institute of Virology, Glasgow, which tests the reliability of the complementation index estimate.

Test for significance of complementation. The test was carried out on the logarithms of the complementation indices, and it is assumed that these values (denoted by y) are normally distributed. If two replicates, y_1 and y_2, have been taken, the null hypothesis that their expected values are zero (no complementation) can be tested by a standard Student's t test with one degree of freedom, and the statistic can be expressed in the form: $t = y_1 + y_2/y_1 - y_2$. Critical values for this statistic are 6.31 (5% level) and 31.82 (1% level).

The test is apparently of low efficiency when y_1 and y_2 differ appreciably, and this is a consequence of the poor reliability of the estimate of variance of a sample of this size. Clearly, a more sensitive test can be obtained by using a larger sample size; the general formula for replicates $y_1, y_2 \ldots y_n$ would be: $t = \bar{y}\sqrt{s^2/n}$, with n-1 degrees of freedom, where $\bar{y} = (y_1 + y_2 + \ldots + y_n)/n$ and $s^2 = \sum (y_i - \bar{y})^2/(n-1)$.

The main assumption, that the y values are normally distributed, seems more plausible than assuming this distribution for the original indices themselves.

An alternative approach would be to use as the denominator a combined estimate of standard error from all the experiments, thereby providing a "cut-off" value for the complementation index in each cross. Although this would provide more degrees of freedom in the t test, it involves the additional assumption that the errors in different experiments are comparable.

Labeling virus-specified proteins in infected cells. BHK 21 cell monolayers were pretreated with actinomycin D (Cosmegen; Merck, Sharpe & Dohme) and infected as previously described (15). The infected cultures incubated at 31 or 39 C were labeled 4 h after virus adsorption with L-[^{3}H]leucine (6 μCi/ml) or L-[^{35}S]methionine (5 μCi/ml) (Radiochemical Centre, Amersham) by using the NaF procedure adopted in our previous investigations (15, 16) to obtain a differential inhibition of host protein synthesis. Protein was extracted with 2% sodium dodecyl sulfate, 0.2% dithiothreitol, and 0.5 M urea in 10 mM sodium phosphate buffer, pH 7.4, and analyzed by polyacrylamide gel electrophoresis as previously described (15).

RESULTS

Isolation of mutants. The first passage of VSV Cocal in BHK 21 cells either obtained from a susceptible bovine host or from routine culture in Vero monkey kidney cells in the laboratory had a very low efficiency of plating on BHK 21 cells at 39 C (Table 1). A strain of VSV Cocal with a high efficiency of plating at 39 C was obtained by isolating virus from the few plaques which appeared on plates incubated at 39 C. A wild-type stock designated *ts*$^+$39 was established from this stock by three repeated clonings at 39 C, and another wild-type stock designated *ts*$^+$37 was established from the original strain by cloning at 31 C.

Both wild-type viruses were mutagenized by growth in the presence of the base analogue FU. Induced mutants were isolated after screening for failure to replicate at the restrictive temperatures of 37 C for the *ts*$^+$37 stock and of 39 C for the *ts*$^+$39 stock. A marked difference was observed in the number of mutants induced when each wild-type stock was exposed to the same concentration of FU during incubation at the permissive temperature (31 C). Table 2 shows that FU had only a slight mutagenic effect on VSV Cocal *ts*$^+$37, whereas the effect on *ts*$^+$39 approached that observed previously with VSV Indiana (10). Graphical extrapolation of the values in Table 2 suggests that the spontaneous mutation frequency of *ts* mutants in both strains is not markedly different, and around 1%. Eight mutants, temperature sensitive at 37 C, were obtained from the *ts*$^+$37 virus, and 41

TABLE 1. *Plating efficiency (39 C/31 C) of cloned and uncloned VSV Cocal*[a]

Origin	History in BHK 21 cells	Titer at 31 C (PFU/ml)	Titer at 39 C (PFU/ml)	Efficiency of plating 39 C/31 C
First cattle passage 1 in bovine host	Passage 1 in BHK 21 at 31 C	2.5×10^9	5×10^2	2×10^{-6}
Passage 6 in Vero cells	Passage 1 in BHK 21 at 31 C	1.5×10^9	7.5×10^3	5×10^{-5}
	3X cloned at 31 C from passage 1	1.7×10^9	1.6×10^4	9×10^{-5}
	3X cloned at 39 C from 31 C cloned stock	1.0×10^9	4.5×10^8	0.45

[a] Monolayers of BHK 21 cells in 6-cm petri dishes were inoculated with 0.2-ml samples of each virus stock diluted in phosphate-buffered saline (pH 7.6) supplemented with 10% calf serum. The dishes were incubated at 31 C for 30 min for virus adsorption and then the monolayers were overlaid with 5 ml of Eagle medium containing 0.9% agar and 2% calf serum. The dishes were incubated in gassed incubators at 31 and 39 C for 48 h, and plaques were counted after staining with neutral red vital stain. The values in the table are the means of two separate experiments.

mutants, temperature sensitive at 39 C, were obtained from the *ts*$^+$39 virus.

Classification of VSV Cocal ts mutants into complementation groups. Complementation tests were carried out by the standard procedure described in Materials and Methods. None of the eight *ts* mutants from the *ts*$^+$37 wild-type stock complemented each other in any combination. It is possible, therefore, that all belong to the same complementation group.

The 41 *ts* mutants derived from the *ts*$^+$39 stock fell into four clearly demarcated complementation groups. Mutants of the same complementation group failed to complement one another in any combination, whereas they complemented mutants in all other groups. The pattern of complementation is similar to that observed with VSV Indiana (10) and VSV New Jersey (11). The four groups of VSV Cocal mutants have been designated α, β, γ, and δ to distinguish them from the complementation groups of VSV Indiana (groups I, II, III, IV, and V) and VSV New Jersey (groups A, B, C, D, E, and F). Individual mutants within a group are designated *ts* α1, α2, . . . αn, etc. Complementation indices obtained between representative mutants of the four complementation groups of VSV Cocal are shown in Table 3 (bottom panel).

The 41 mutants are distributed unequally between the groups; groups α, β, γ, and δ contained 29, 6, 3, and 2 mutants, respectively. One mutant remains to be classified. No complementation was observed in mixed infections of representative mutants of these four groups and a mutant of the *ts*$^+$37 wild-type stock.

TABLE 2. *Mutagenic action of FU on two wild-type strains of VSV Cocal*[a]

FU concn (μg/ml)	Frequency of *ts* clones (%)	
	VSV Cocal *ts*$^+$ 37	VSV Cocal *ts*$^+$ 39
500	4.0	ND[b]
300	7.0	ND
200	2.6	28.1
150	ND	19.0
100	3.1	15.0
50	0.5	ND
25	ND	ND
0	1.2[c]	0.8[c]

[a] The *ts*$^+$ 37 stock was isolated from a Vero cell-passaged strain of VSV Cocal by cloning at 31 C. Clones were isolated at 31 C from mutagenized stock and scored for ability to multiply at 37 C. The *ts*$^+$ 39 stock was isolated from the *ts*$^+$ 37 stock by three repeat clonings at 39 C. Clones were isolated from mutagenized stock of *ts*$^+$ 39 incubated at 31 C and scored for ability to multiply at 39 C. Each frequency estimate is based on screening of at least 100 clones.

[b] ND, Not done.

[c] Estimated by graphical extrapolation.

Relationship of complementation groups of VSV. Complementation tests were arranged between mutants representing the three serotypes of VSV in all combinations to determine the relationship of the VSV Cocal mutants to those of VSV Indiana and VSV New Jersey. The

TABLE 3. *Complementation indices obtained in inter- and intrastrain mixed infection of VSV ts mutants*[a]

Strains	Complementation group	Mutant	VSV Cocal-ts⁺ 39; mutant			
			ts α1	*ts* β1	*ts* γ1	*ts* δ1
VSV Indiana	I	*ts* G11	0.37	1.06	0.62	0.78
	II	*ts* G22	0.90	0.65	0.80	0.60
	III	*ts* G31	*12.66*	*5.50*	1.19	*12.10*
	IV	*ts* G41	0.62	0.46	0.76	1.03
	V	*ts* 045	*12.50*	*9.29*	1.40	1.24
VSV New Jersey	A	*ts* A1	0.96	0.60	0.79	1.16
	B	*ts* B1	0.10	0.16	0.14	0.15
	C	*ts* C1	0.33	0.26	0.29	0.22
	D	*ts* D1	0.42	0.59	0.36	1.05
	E	*ts* E1	0.99	1.12	1.20	0.73
	F	*ts* F1	0.97	0.77	1.06	1.20
VSV Cocal-*ts*⁺39	α	*ts* α1		*195*	*29*	*116*
	β	*ts* β1			*28*	*24*
	γ	*ts* γ1				*6*
	δ	*ts* δ1				

[a] The complementation indices are the means of duplicate determinations obtained in a single experiment. The significant values are italicized.

result of an individual experiment in which the five groups of VSV Indiana and the six groups of VSV New Jersey are compared with the VSV Cocal mutants is shown in Table 3. No significant complementation was obtained with any combination of mutants of VSV Cocal and VSV New Jersey. Certain combinations of VSV Cocal and VSV Indiana mutants, however, gave significant complementation indices. These all involve *ts* G31 (VSV Indiana group III) and *ts* 045 (VSV Indiana group V). The results suggest that group III may be homologous with group γ. The complementation pattern, however, is not symmetrical (e.g., *ts* γ1 failed to complement any VSV Indiana mutant); consequently, the precise homology of any of the groups cannot be established by a genetic criterion alone. Therefore, some physiological properties of the mutants have been examined to allow further comparison of the groups.

Viral RNA and protein synthesis at 31 and 39 C. Previously it was found that VSV protein synthesis was correlated directly with RNA phenotype (15, 16). Mutants unable to synthesize viral RNA at 39 C also failed to synthesize viral structural proteins, and mutants able to synthesize RNA at 39 C invariably synthesized all viral proteins. Preliminary experiments (I. B. Duncan, unpublished data) involving two mutants from each group indicated that the mutants belonging to groups α and β failed to synthesize viral RNA at 39 C (<7.0% and <5.2%, respectively, of the amount of actinomycin-resistant RNA synthesis induced by wild-type virus under identical conditions). The two mutants of group γ had RNA-positive phenotypes (301 and 357% of wild-type synthesis). One mutant of group δ was RNA positive (66% of wild-type synthesis), and the other was RNA negative (<2.6% of wild-type synthesis).

The ability of these mutants to induce viral protein synthesis at 39 C was then examined. Polyacrylamide gel profiles of protein synthesized at 31 and 39 C in cells infected with the RNA-negative phenotype mutants of groups α (*ts*α1), β (*ts*β1), and δ (*ts*δ1) are shown in Fig. 1 along with profiles from mock-infected BHK 21 cells. Mutants *ts*α1 and *ts*δ1 failed to induce viral proteins in appreciable amounts at 39 C. Mutant *ts*β1, on the other hand, induced a limited amount of viral-specified protein synthesis at 39 C.

A methionine-rich protein, probably specified by the host cell genome, migrates slightly behind the nonstructural (NS) proteins and is located between fractions 24 and 28 of all four profiles. Other experiments using reversed label showed that this methionine-rich protein was synthesized equally well at 31 and 39 C.

The similarity in profiles between *ts*δ1 and uninfected cells at 39 C suggested that little change had occurred in host protein synthesis of cells infected with this mutant at the restrictive temperature. Increasing the multiplicities of infection to 50 PFU/cell did not produce any further depression of host protein synthesis, and mutant *ts*δ1 appeared to be less effective in depressing host protein synthesis than the other mutants and wild type.

The corresponding profiles obtained at 31 and

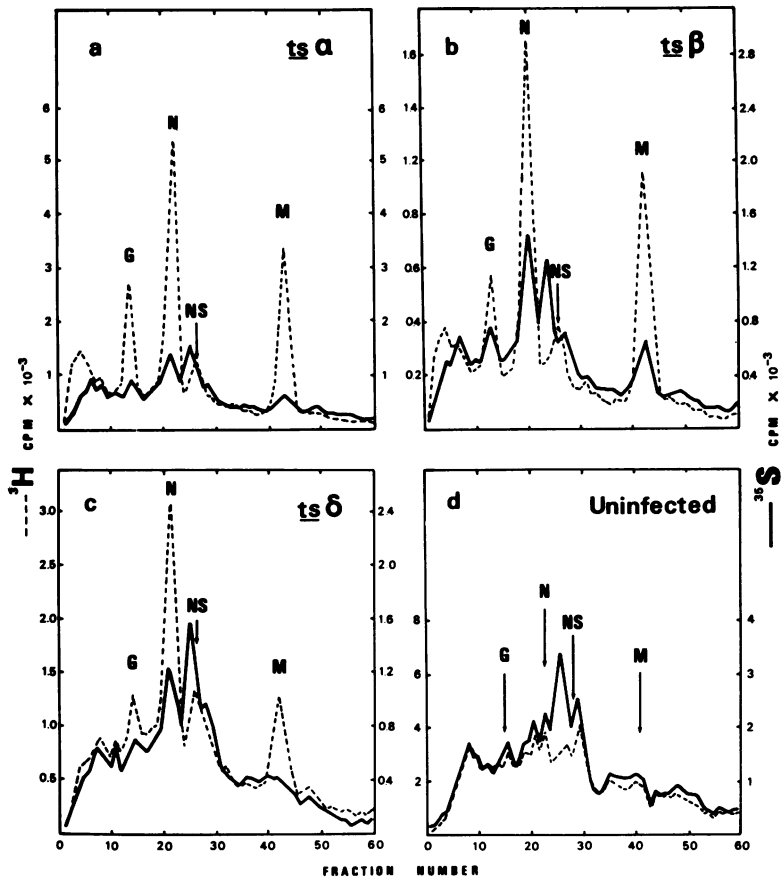

FIG. 1. *Synthesis of virus-specified proteins in BHK 21 cells infected with ts mutants of complementation groups α, β, and δ. Monolayers infected with mutants tsα1, tsβ1, and tsδ1 were labeled with L-[^{3}H]leucine (31 C cultures, - - - - -) or L-[^{35}S]methionine (39 C cultures, ——) in a medium without calf serum and added amino acids during a 5-min pulse, 20 min after NaF treatment (15). Uninfected BHK 21 cells were labeled similarly. Proteins extracted from the 31 and 39 C incubated cells were compared by co-electrophoresis in 10% polyacrylamide gels. Virion structural proteins corresponding to the intracellular virus-specified proteins are marked by G for glycoprotein, N for nucleoprotein, M for matrix protein, and NS for an unidentified nonstructural protein (14).*

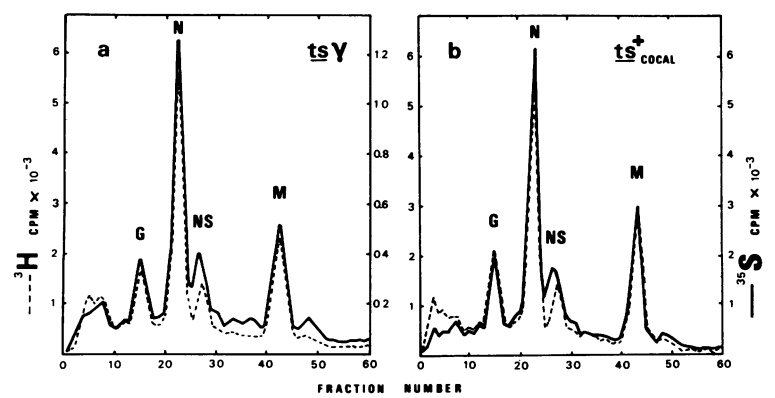

FIG. 2. *Synthesis of virus-specific proteins in BHK 21 cells infected with a ts mutant of complementation group γ (a) and the ts$^+$39 wild type (b). Cells were infected and labeled, and protein was compared as described in legend to Fig. 1. Mutant ts γ1 represented group γ.*

39 C with the RNA-positive phenotype *ts* mutant of group γ (*ts*γ1) are shown in Fig. 2 along with wild type (*ts*$^+$39 virus). Virus-specified proteins were present at both 31 and 39 C in near-equivalent amounts in the two types of infection.

Similar results confirming these observations were obtained with one other mutant from each of groups α, β, and γ. Mutant *ts*δ2, the RNA-positive mutant of group δ, induced viral-specific protein synthesis at 39 C, although in lesser amount than obtained with mutants *ts*γ1 and *ts*γ2.

DISCUSSION

The four complementation groups of VSV Cocal described here can be equated provisionally with four of the five established complementation groups of VSV Indiana, despite the absence of interstrain complementation. One complementation group predominates numerically in each serotype: group α in VSV Cocal and group I in VSV Indiana. It is reasonable to equate these two groups since the phenotype of the mutants in both groups is similar; little or no viral RNA or protein is synthesized at 39 C. Groups δ (VSV Cocal) resembles group II (VSV Indiana), because these are the only two groups which contain mutants of grossly different phenotypes. Groups γ (VSV Cocal) and group III (VSV Indiana) both induced synthesis of viral RNA and protein at 39 C; furthermore, mutant *ts* G31 (VSV Indiana, group III) complements VSV Cocal mutants belonging to groups α, β, and δ, but not group γ. Group β (VSV Cocal) and group IV (VSV Indiana) rank second in terms of numbers of mutants, and in each group the mutants are defective for viral RNA synthesis at 39 C.

According to this assignment, therefore, group V (VSV Indiana) does not correspond to any of the VSV Cocal groups. This may reflect some mutagen specificity, since all the VSV Indiana group V mutants are of spontaneous origin and none have been isolated from wild-type strains treated with mutagens, mainly 5-fluorouracil (D. V. Cormack et al., in press; 4). The homologies of the complementation groups of the two serotypes are tentative and are intended merely to provide a basis for further experimentation. Ability to cross-complement within the VSV group appears to be correlated with serological relationships. Significant complementation was observed only in mixed infections of strains of related serotypes (VSV Cocal and VSV Indiana). This finding is reinforced by other experiments in which two complementing *ts* mutants of Chandipura virus failed to complement mutants of any complementation group of all three VSV serotypes (Table 4). Chandipura virus is a rhabdovirus isolated from man in India (1) and was initially thought to be serologically related to VSV (8). Recent work has cast doubt on this serological relationship (2), and our experiments show no evidence of a close genetic relationship.

Mutants of group α, β, γ, and δ failed to complement the noncomplementing mutants isolated from the *ts*$^+$37 strain or even to rescue the wild type itself in mixed infection. This suggests that the difference between the *ts*$^+$37 and *ts*$^+$39 strains involves more than one genome function.

The experiments described in this paper do not directly provide information about the gene functions affected by the various mutations. The indirect evidence suggests that the mutants belonging to group α are all virion transcriptase mutants, if it is accepted that group α corresponds to group I of VSV Indiana. Szilágyi and Pringle (13) concluded from in vitro experiments that the site of the group I mutational lesions was in the structural gene for the virion transcriptase. By analogy with VSV Indiana, it can be presumed that VSV Cocal mutants belonging to the other three groups are likely to have more or less normal transcriptase activities. It is interesting that mutant *ts*δ1, which

TABLE 4. *Complementation indices obtained in inter- and intrastrain mixed infection of VSV ts mutants and mutants ts Ch1 and ts Ch4 of Chandipura virus*[a]

Strain	Complementation group	Mutant	Chandipura *ts* mutant	
			ts Ch1	*ts* Ch4
VSV Indiana	I	*ts* G11	1.03	0.73
	II	*ts* G22	0.83	0.46
	III	*ts* G31	0.83	1.05
	IV	*ts* G41	0.57	0.93
	V	*ts* 045	1.06	0.86
VSV Cocal-*ts*$^+$39	α	*ts* α1	0.96	1.09
	β	*ts* β1	0.55	1.50
	γ	*ts* γ1	0.55	0.74
	δ	*ts* δ1	0.91	1.00
VSV New Jersey	A	*ts* A1	0.85	1.63
	B	*ts* B1	1.30	0.70
	C	*ts* C1	0.56	0.23
	D	*ts* D1	0.64	0.61
	E	*ts* E1	0.76	0.58
	F	*ts* F1	0.76	0.58
Chandipura	I	*ts* Ch1		*240*
	II	*ts* Ch4		

[a] The only significant complementation index is italicized. The complementation indices are the means of duplicate determinations obtained in a single experiment.

failed to inhibit host protein synthesis effectively, falls into this category and might be presumed to possess a normal RNA transcriptase. This may indicate that primary transcription alone is not sufficient for the suppression of host protein synthesis which follows VSV infection of cells.

Many investigators, including ourselves, have looked for precursor-product relationships in VSV protein synthesis. No evidence of accumulation of precursor or post-translational cleavage has been found in *ts* mutant-infected cells in pulse/chase experiments with or without inhibitors of protein synthesis. A ^{35}S-methionine-rich protein is synthesized at 39 C in cells infected with the RNA-negative *ts* mutants. This protein probably corresponds to a methionine-rich protein of uninfected cells, but is particularly obvious when NS is not synthesized at 39 C. If the incubation temperature is shifted from 39 to 31 C, synthesis of the methionine-rich protein appears to decrease, and protein corresponding to NS is synthesized (unpublished data). Further studies are in progress to determine whether there is any relationship between these two proteins.

ACKNOWLEDGMENTS

We thank Robert Gibson and Carole Main for excellent technical assistance and Ian Duncan for permission to include his data on mutant RNA synthesis. We thank J. H. Subak-Sharpe for advise and discussion.

LITERATURE CITED

1. Bhatt, P. N., and F. M. Rodriques. 1967. Chandipura: a new arbovirus isolated in India from patients with febrile illness. Indian J. Med. Res. **55**:1295-1305.
2. Cartwright, B., and F. Brown. 1972. Serological relationships between different strains of vesicular stomatitis virus. J. Gen. Virol. **16**:391-398.
3. Federer, K. E., R. Burrows, and J. B. Brooksby. 1967. Vesicular stomatitis virus—the relationship between some strains of the Indiana serotype. Res. Vet. Sci. **8**:103-117.
4. Flamand, 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.
5. Jonkers, A. H., R. E. Shope, T. H. G. Aitken, and L. Spence. 1964. Cocal virus, a new agent in Trinidad related to vesicular stomatitis virus, type Indiana. Amer. J. Vet. Res. **25**:236-242.
6. Macpherson, I. A., and M. G. P. Stoker. 1962. Polyoma transformation of hamster cell clones—an investigation of genetic factors affecting cell competence. Virology **16**:147-151.
7. McSharry, J. J., R. W. Compans, and P. W. Choppin. 1971. Proteins of vesicular stomatitis virus and of phenotypically mixed vesicular stomatitis-simian virus 5 virions. J. Virol. **7**:722-729.
8. Murphy, F. A., and R. E. Shope. 1971. Workshop on Bridging groups of viruses, p. 261. Proc. Second Int. Congr. Virol. Budapest.
9. Pringle, C. R. 1970. The induction and genetic characterisation of conditional lethal mutants of vesicular stomatitis virus, p. 567-582. *In* R. D. Barry and B. W. J. Mahy (ed.), The biology of large RNA viruses. Academic Press Inc., New York.
10. 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.
11. Pringle, C. R., I. B. Duncan, and M. Stevenson. 1971. Isolation and characterization of temperature-sensitive mutants of vesicular stomatitis virus, New Jersey serotype. J. Virol. **8**:836-841.
12. Stampfer, M., D. Baltimore, and A. S. Huang. 1969. Ribonucleic acid synthesis of vesicular stomatitis virus. I. Species of ribonucleic acid found in Chinese hamster ovary cells infected with plaque-forming and defective particles. J. Virol. **4**:154-161.
13. 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.
14. 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.
15. Wunner, W. H., and C. R. Pringle. 1972. Protein synthesis in BHK 21 cells infected with vesicular stomatitis virus. I. *ts* mutants of the Indiana serotype. Virology **48**:104-111.
16. Wunner, W. H., and C. R. Pringle. 1972. Protein synthesis in BHK 21 cells infected with vesicular stomatitis virus. II. *ts* mutants of the New Jersey serotype. Virology **50**:250-253.
17. Wunner, W. H., and C. R. Pringle. 1972. Comparison of structural polypeptides from vesicular stomatitis virus (Indiana and New Jersey serotypes) and Cocal virus. J. Gen. Virol. **16**:1-10.
18. Zavada, J. 1972. Pseudotypes of vesicular stomatitis virus and the coat of murine leukemia and of avian myeloblastosis viruses. J. Gen. Virol. **15**:183-191.