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FURTHER CHARACTERIZATION OF THE T-S MUTANT F207 OF BLUETONGUE VIRUS

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ABSTRACT

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Temperature-shift experiments verified that the t-s lesion of BTV mutant F207 is expressed late in the replication-cycle, that is, at a stage when all virus components have already been synthesized. All viral polypeptides were indeed found in the soluble but not in the particulate fraction of cytoplasmic extracts from infected cultures grown at the non-permissive temperature. This suggests that the t-s lesion could be a defect in one or both of the polypeptides P2 and P5, which are respectively reduced in amount and absent from the latter fraction. Alternatively, the lesion could be an inability of the core particle to bind these 2 outer capsid polypeptides.

Résumé

CARACTÉRISATION ULTÉRIEURE DU MUTANT T-S F207 DU VIRUS DE LA FIÈVRE CATARRHALE DU MOUTON

Des expériences basées sur des changements de température ont montré que la lésion t-s du mutant F207 du virus de la fièvre catarrhale du mouton (BTV) s'exprime à un stade tardif du cycle de réplication, c'est-à-dire à un stade où tous les composants du virus ont déjà été synthétisés. En effet tous les polypeptides viraux ont été trouvés dans la fraction soluble mais non dans la fraction en suspension d'extraits cytoplasmiques tirés de cultures infestées qu'on avait fait se développer à la température non-permissive. Ceci suggère que la lésion t-s pourrait être un défaut dans l'un ou dans les deux polypeptides P2 *et* P5, *qui sont respectivement réduit en quantité et absent de la dernière fraction. Une autre possibilité serait que la lésion consiste en une incapacité de la particule du noyau à lier ces deux polypeptides de la capside externe.*

INTRODUCTION

No detailed study of the replication pathway of bluetongue virus (BTV) in infected cell cultures has been made to date. It is uncertain therefore whether intermediate subviral structures, similar to those described for reovirus replication (Acs, Klett, Schonberg, Christman, Levin & Silverstein, 1971; Morgan & Zweerink, 1974; Morgan & Zweerink, 1975; Morgan & Zweerink, 1977; Sakuma & Watanabe, 1972), are synthesized during BTV morphogenesis. The isolation of intermediate subviral products is hampered by the very rapid turnover of viral products during normal replication. In reovirus this problem was solved by growing temperature-sensitive (t-s) mutants at the non-permissive temperature and studying the subviral particles which accumulate in mutant-infected cells (Fields, Raine & Baum, 1971; Matsuhisa & Joklik, 1974; Morgan & Zweerink, 1974).

Bluetongue virus t-s mutants have been isolated and genetically classified into 6 recombination classes (Shipham & De la Rey, 1976). One late mutant, (F207), belonging to group II, was found to express its t-s lesion very late in viral morphogenesis, and was shown to synthesize ssRNA at the non-permissive temperature (Shipham & De la Rey, 1979). Temperature-shift experiments were therefore carried out on F207 in order to determine more precisely the period during which the t-s lesion is expressed. Virus polypeptides in particulate and soluble fractions of F207-infected cells cultured at either permissive or non-permissive temperatures were also studied. The results reported here verify that the t-s lesion is, indeed, expressed late in the replication cycle. It is suggested that the t-s lesion could be a defect in one or both polypeptides P2 and P5. Alternatively, it could be an inability of the core particle to bind these 2 outer capsid proteins.

MATERIALS AND METHODS

Cells and media

L-strain mouse cells and BHK-21 cells, obtained from the American Type Culture Collection, were propagated as described by Verwoerd, Oellermann, Broekman & Weiss, 1967. Modified Eagle's medium (Macpherson & Stoker, 1962) was prepared as described by Verwoerd (1969).

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Virus

The isolation, culture and genetic classification of t-s mutants of BTV have been described elsewhere (Shipham & De la Rey, 1976).

Radioactive labelling of virus polypetides

BHK cell cultures were inoculated with virus at a multiplicity of infection (m.o.i.) of 20–30 plaque-forming units (PFU) per cell, and incubated at either 28 °C or 38 °C, as required. At 20 h post-infection (p.i.), the cultures were rinsed with amino-acid-free Eagle's medium and reincubated for 4 h at either 28 °C or 38 °C on a shaking platform with 10 mℓ of the same medium containing 0,5 μCi/mℓ ^{14}C protein hydrolysate(1). Cell cultures were subsequently rinsed and reincubated in Eagle's medium containing a twofold excess of unlabelled amino acids for a further 16 hours.

Preparation of particulate fraction

Infected cell cultures labelled with ^{14}C amino acids were harvested by scraping and the cells pelleted at 1 200 × g for 15 minutes. The cell pellet was washed twice in PBS (isotonic salt solution in 0,05 M phosphate buffer) and resuspended in PBS containing 0,5% Nonidet P40 (Shell Chemicals). After 15 min. at 4 °C, the cells were disrupted in a Dounce homogenizer and the nuclei removed by centrifugation at 1 200 × g for 10 minutes. The cytoplasmic extract was centrifuged at 100 000 × g for 90 min and the pellet resuspended in 100 mM phosphate buffer containing 8 M urea, 4% 2-mercaptoethanol and 1,6% sodium dodecyl sulphate (electrophoresis buffer), prior to analysis by polyacrylamide gel electrophoresis.

Immune precipitation of virus polypetides in soluble fraction

The supernatant remaining after sedimentation of insoluble particles by centrifugation at 100 000 × g is referred to as the S100 fraction. Equal portions of

(1) Obtained from the Radiochemical Centre, Amersham, England

S100 fractions and BTV specific immune serum (kindly supplied by Dr H. Huismans, Veterinary Research Institute, Onderstepoort) were mixed and left overnight at 4 °C. The precipitate was collected by centrifugation at 10 000 rpm for 10 min in a SW 50 Beckman rotor, washed twice in PBS and resuspended in electrophoresis buffer prior to electrophoretic analysis.

Polyacrylamide gel electrophoresis

Electrophoresis [as described by Studier (1973)] was carried out in thin slab polyacrylamide gels with a final concentration of 7,5% acrylamide and 0,2% bisacrylamide in a 100 mM sodium phosphate buffer, pH 7,3. Acrylamide and bisacrylamide were recrystallized following the method of Loening (1967). Samples were suspended in electrophoresis buffer, heated at 70 °C for 1 min and electrophoresed at a potential of 3,5 volts/cm for 18 h at 20 °C. Gels were dried in a Hoefer gel slab dryer for 1 h and an autoradiogram prepared as described by Huismans (1979).

Temperature-shift experiments

Temperature-shift experiments were carried out in a series of Roux flasks containing BHK-cell monolayers infected with mutant F207. These cultures were incubated initially at either 28 °C or 38 °C as required. Thereafter, single successive infected cultures were shifted from 28 °C to 38 °C or vice versa at the end of every consecutive 4 h period. After a total incubation period of 48 h, the cultures were harvested by scraping off the cells and titrated for virus at 28 °C.

Control cultures of BHK-cell monolayers infected with mutant F207 were incubated throughout the infection cycle at 28 °C and 38 °C respectively, one culture incubated at each temperature being used every 4 h for titration at 28 °C.

Results

Temperature-shift experiments

It can be seen from Fig. 1 that a total incubation period of 28 h at 28 °C prior to a shift to 38 °C was required to ensure the production of fully infective virus particles of the t-s mutant F207. This period corresponds to the virus eclipse phase (control 28 °C), suggesting that the F207 t-s lesion is expressed very late in viral morphogenesis. In the case of shift-down experiments, a high concentration of infective viral progeny was still produced when cultures were incubated for 24 h at 38 °C prior to the shift to 28 °C. Incubation at 38 °C for periods exceeding 24 h results in a fall in progeny viral titres.

Virus polypetides in the particulate fraction of mutant-infected cells

The fact that the t-s lesion for mutant F207 is expressed at a late stage in the infection cycle (between 20 h and 28 h p.i.) (Fig. 1), raised the question as to whether, in mutant-infected cells, the capsid polypeptides required for production of full virions are available at a late stage (40 h p.i.), when no infective progeny can be demonstrated. BHK cell monolayers were therefore inoculated with mutant F207, incubated at either 28 °C or 38 °C and pulse-labelled for 4 h (20–24 h p.i.). After a total incubation period of 40 h the particulate fraction was isolated and fractioned on polyacrylamide gels. Cultures inoculated with wild-type BTV served as a control. The densitometer profiles prepared from the resulting autoradiograms are shown in Fig. 2.

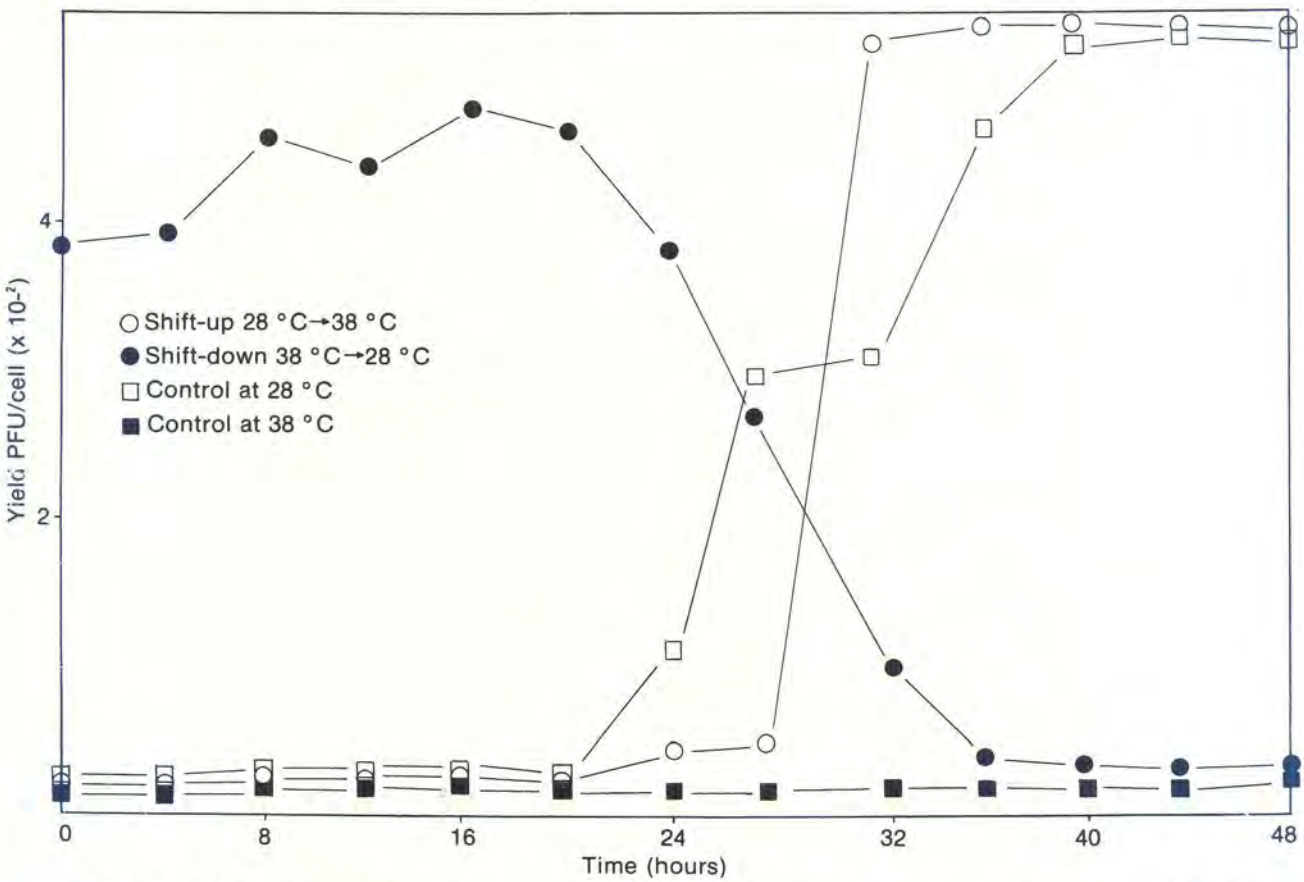

FIG. 1 The effect of temperature-shift on the production of infective mutant F207 virus. The controls are growth curves at 28 °C and 38 °C.

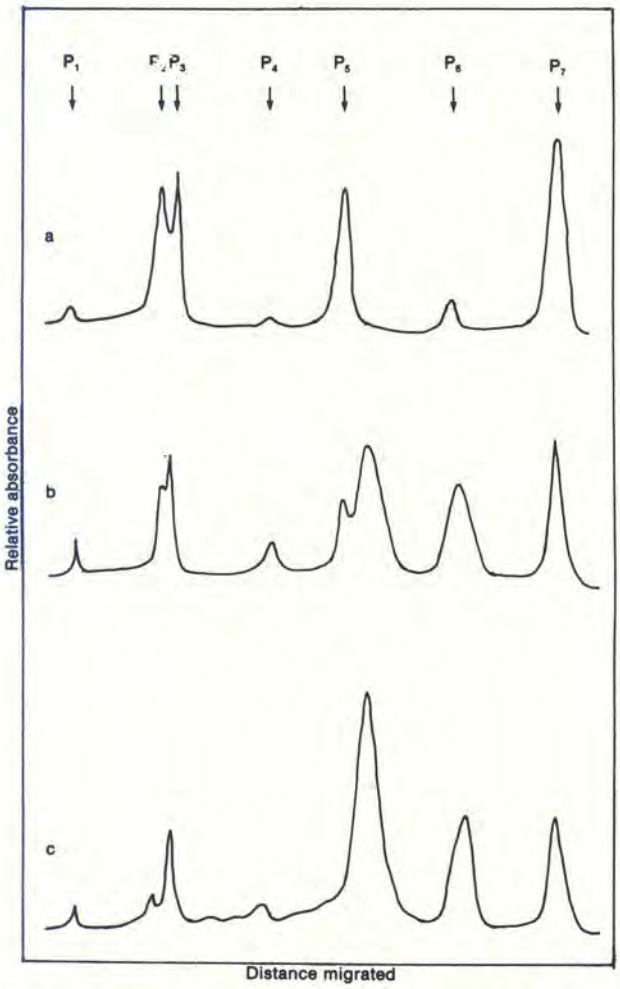

FIG. 2 Densitometer profiles of polyacrylamide gel electrophoretic fractionation patterns of polypeptides present in the particulate fraction isolated from F207 infected cultures. Migration is from left to right. (a) Control (b) 28 °C (c) 38 °C.

The profile of the BTV wild-type control (Fig. 2-a) illustrates the electrophoretic migration pattern of the 7 capsid polypeptides, numbered P1–P7 corresponding to the increase in mobility. The presence of all 7 capsid polypeptides was demonstrated in the particulate fraction isolated from F207-infected cultures incubated throughout at the permissive temperature (28 °C) (Fig. 2-b). Also present in this fraction was a non-capsid polypeptide designated P5A migrating slightly faster than P5. Capsid polypeptide P5 could not be demonstrated in the particulate fraction from mutant F207-infected cultures incubated for 40 h at the non-permissive temperature (38 °C) (Fig. 2-c).

In order to compare the relative amounts of the polypeptides P1 through P7, the integration indices for the respective fractions were calculated and recorded in Table 1.

It can be seen that, in addition to the absence of P5, the relative amount of P2 (20) is also considerably reduced in infected cells incubated throughout at the non-permissive temperature (38 °C) as compared to that (60) isolated from infected cells incubated at the permissive temperature 28 °C. P5A on the other hand is present in relatively higher concentrations in extracts from cultures incubated at 38 °C compared with those of cultures incubated at 28 °C.

Polypeptides in the soluble fraction of mutant infected cells

The polypeptides in the soluble fraction of the infected cells were investigated next. The densitometer profiles prepared from the autoradiograms are given in Fig. 3. For reference the migration pattern of the 7 polypeptides of the BTV wild-type control is shown in Fig 3-a. In contrast to the particulate fraction, polypeptide 5 was present in the soluble fraction of mutant infected cells incubated at both 28 °C (3-b) and 38 °C (3-c).

The integration indices of the densitometer profiles (Table 2) show that very similar amounts of the different capsid polypeptides were present in the soluble fraction from mutant-infected cells incubated at either the permissive or the non-permissive temperature. At both temperatures P4, a minor polypeptide, appeared to be absent. The presence of P5 and P2 in the soluble fraction of the mutant-infected cells, incubated at 38 °C, clearly shows that the absence of P5 and the reduced amounts of P2 in the particulate fraction of these cells was not because these polypeptides had not been synthesized. The polypeptides that had been synthesized could have been defective, however.

TABLE 1 The integration indices of the densitometer profiles shown in Fig. 2

Polypeptide	P1	P2	P3	P4	P5	P5A	P6	P7
BTV (Wild-type)	8	130	125	7	134	0	16	222
F207 at 28 °C	7	60	77	13	100	210	158	168
F207 at 38 °C	6	20	100	10	0	600	230	177

TABLE 2 Integration indices of the densitometer profiles shown in Fig. 3

Polypeptide	P1	P2	P3	P4	P5	P5A	P6	P7
BTV (Wild-type)	8	110	116	4	102	0	11	220
F207 at 28 °C	0	445	445	0	79	30	116	780
F207 at 38 °C	0	320	280	0	40	28	142	526

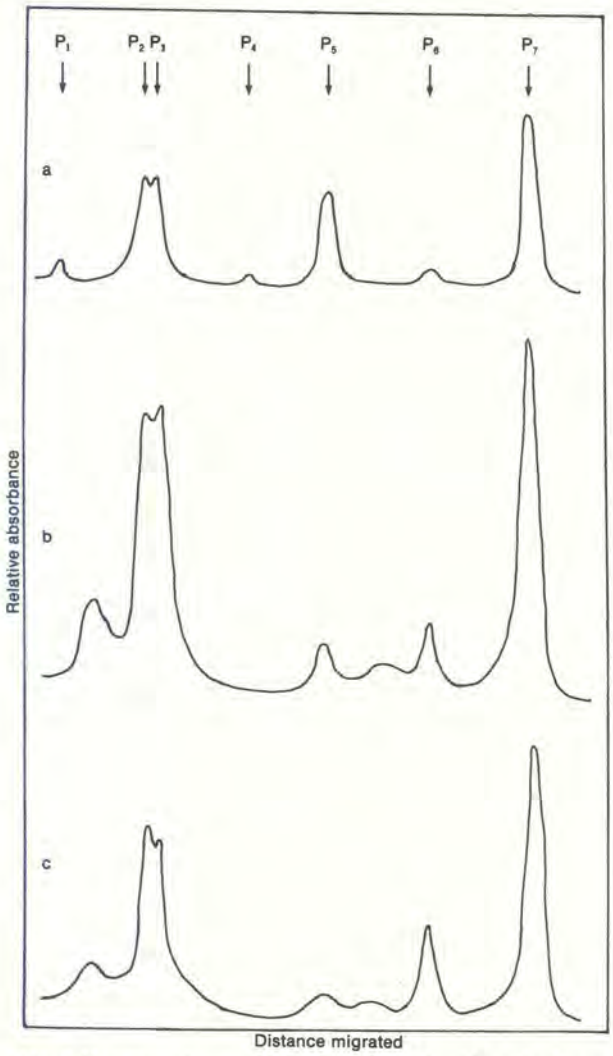

FIG. 3 Densitometer profiles of polyacrylamide gel electrophoretic fractionation patterns of polypeptides present in the soluble fraction isolated from F207 infected cultures. Migration is from left to right. (a) Control (b) 28 °C (c) 38 °C.

Discussion

The mutant F207 expresses its t-s lesion only late in the infection cycle and the viral function inhibited at the non-permissive temperature is therefore one which occurs at the end or near the end of viral morphogenesis. After 20 h at 28 °C (permissive temperature) all viral products, and even subviral products, should already have been produced. A shift from the permissive to the non-permissive temperature at this stage should therefore result in the production of fully infective viral progeny unless the final stages of virus encapsidation cannot occur at 38 °C (non-permissive temperature). The temperature-shift experiments therefore suggest that the encapsidation step of F207 morphogenesis is temperature-sensitive.

This suggestion is supported by the finding that all the capsid polypeptides are present in infected cells incubated for 40 h at 38 °C. The relative amounts of the various polypeptides do differ, however, in the supernatant and the particulate fractions. The particulate fraction contains a mixture of full virions, subviral particles and other virus and cell-associated complexes. At the permissive temperature all 7 virus capsid polypeptides can be demonstrated, most likely as a result of the presence of full virions in the particulate fraction. At the non-permissive temperature, however, no P5 and very few P2 polypeptides are found in a similar fraction, suggesting that these 2 polypeptides are not associated with the viral or subviral forms present. Polypeptides P2 and P5 are outer capsid proteins (Verwoerd, Els, De Villiers & Huismans, 1972) and it is therefore possible that the t-s defect in F207 replication may be due to a defect in either or both of these 2 proteins. A second possibility is that the core-particle which is produced in mutant-infected cells incubated at 38 °C (Shipham, 1977) is defective and unable to bind the outer capsid proteins available.

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