Characterization of Temperature-Sensitive Mutants of Simian Rotavirus SA11: Protein Synthesis and Morphogenesis

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The synthesis of viral polypeptides, distribution of viral antigens, and morphogenesis of viral structures have been examined in cells infected with temperature-sensitive (*ts*) mutants of SA11 representing 10 recombination groups. At the permissive temperature (31°C) the synthesis of viral polypeptides and the distribution of viral antigens did not differ significantly from those of the wild type. At the nonpermissive temperature (39°C) some mutants (*ts*B, -C, -E, -F, and -G) synthesized significantly smaller amounts of viral polypeptides and had a very diffuse distribution of viral antigen. Several of the mutants synthesized one or more electrophoretically aberrant polypeptide species at both 31 and 39°C. All of the mutants, except *ts*F, assembled morphogenetic intermediates at 39°C. Aberrant intermediates were assembled in all mutants at 31 and 39°C. No specific morphogenic defect could be associated with any of the *ts* mutants.

Conditional-lethal temperature-sensitive (*ts*) mutants have been isolated from the rotavirus SA11 (16, 16a). The *ts* mutants were placed into 10 groups as defined by recombination. The segmented nature of the rotavirus genome, coupled with the observation that recombination occurred by the mechanism of reassortment (10, 13; Ramig, unpublished data), indicated that each of the mutant groups marked a different genome segment. Assignments have been made for the protein species encoded in 10 of the 11 genome segments of SA11 (2, 4, 12, 19). Although there are cases of disagreement between the coding assignments made in the different laboratories, they do agree that each segment encodes a single primary gene product. Thus, the mutants we have isolated from SA11 mark not only 10 of the 11 genome segments, but also 10 of the 11 primary gene products, with a *ts* lesion.

The SA11 *ts* mutants provide specific probes to examine the functions encoded in the genes they mark. Work in progress is aimed at assignment of each mutant group to a specific genome segment and, indirectly, to a specific gene product. These assignments, in conjunction with a biochemical characterization of the defects of each of the mutant groups, should lead to an understanding of the role of each viral gene and viral gene product in the virus replication cycle.

In this report we describe studies of the synthesis and function of the polypeptides synthesized by the SA11 *ts* mutants. Specifically, we examine the synthesis of viral polypeptides in cells infected with each of the mutants, the distribution of viral antigens within cells infected with each of the mutants, and the morphogenesis of the mutants.

MATERIALS AND METHODS

Media and buffers. Medium 199 with and without serum, trypsin, and Tris-buffered saline were prepared as previously described (1, 16).

Cells and virus. Propagation of MA104 cells and selection of SA11 wild-type and *ts* mutants have been described before (16, 16a). The following prototype *ts* mutants were used: group A, *ts*A(778); group B, *ts*B(339); group C,

*ts*C(606); group D, *ts*D(975); group E, *ts*E(1400); group F, *ts*F(2124); group G, *ts*G(2130); group H, *ts*H(2384); group I, *ts*I(2403) and group J, *ts*J(2131). All viruses were plaque purified from previous mutant stocks before use (total of three plaque purifications) and were used in these experiments at the second- or third-passage level. Virus was activated with trypsin (5 µg/ml, 30 min, 37°C) before all infections.

Labeling of infected cells. Confluent monolayers of MA104 cells in 60-mm dishes (2.5×10^6 cells per dish) were prepared and incubated in medium without serum for 24 h before infection. The cells were infected with mutant or wild-type virus at a multiplicity of infection (MOI) of 25 PFU per cell. After adsorption for 1 h at 31°C, the inoculum was removed and the cells were fed with medium 199 containing 5 µg of actinomycin D per ml. Infected monolayers were incubated at 31 or 39°C. Thirty minutes before labeling, the cells were changed to minimum essential medium lacking amino acids and containing 5 µg of actinomycin D per ml. After amino acid depletion, the cells were labeled in medium lacking amino acids but containing 5 µg of actinomycin D and 25 µCi of L-³H-labeled amino acid mixture (ICN) per ml. Infected cells grown at 31°C were labeled from 10.5 to 13.5 h postinfection and, at 39°C, from 6.5 to 9.5 h postinfection. After labeling, the cell monolayers were washed with Tris-buffered saline, and the cells were lysed into 1.0 ml of RIPA buffer containing the protease inhibitors Trasylol (1%), phenylmethylsulfonylfluoride (2 mM), and leupeptin (100 µM). Samples of the infected cell lysates were either precipitated with 8 volumes of acetone or immunoprecipitated with antiserum, prepared in guinea pigs, to purified SA11. Acetone precipitates or *Staphylococcus aureus* immunocomplexes were collected by centrifugation, dissolved in sample buffer, and resolved by electrophoresis in 12% polyacrylamide gels containing 0.5 M urea (11). Gels were treated with Autofluor (National Diagnostics) and exposed to film at -70°C.

IF. Immunofluorescence (IF) tests and the preparation of guinea pig antiserum to SA11 have been described (7). MA104 cell monolayers on glass cover slips were infected (MOI = 5), and the infected cells were incubated at 31 or 39°C. Infected cells were prepared for IF at 20 h (31°C) or 14 h (39°C). Three representative fields from each cover slip

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TABLE 1. Summary of IF staining of SA11 *ts* mutants

Mutant and group	Temp (°C)	No. of cells examined	Antigen positive (%)	Viroplasms/cisternae (%) ^a	Diffuse stain (%)	Nuclear inclusion (%)	Cytoplasmic inclusion (%) ^b
<i>tsA</i> (778)	31	56	96	96	0	7	0
	39	90	62	14	48	12	1
<i>tsB</i> (339)	31	54	94	94	0	39	6
	39	62	39	3	35	6	3
<i>tsC</i> (606)	31	46	76	76	0	22	4
	39	88	60	24	36	2	1
<i>tsD</i> (975)	31	62	89	89	0	16	2
	39	75	84	31	53	7	3
<i>tsE</i> (1400)	31	46	87	87	0	20	0
	39	130	48	4	44	1	13
<i>tsF</i> (2124)	31	81	56	51	4	14	0
	39	73	53	4	53	1	1
<i>tsG</i> (2130)	31	36	86	81	5	28	0
	39	30	80	48	54	2	0
<i>tsH</i> (2384)	31	35	77	77	0	34	3
	39	75	80	72	8	4	8
<i>tsI</i> (2403)	31	48	92	92	0	17	2
	39	90	39	37	2	9	0
<i>tsJ</i> (2131)	31	30	60	60	0	3	0
	39	52	88	15	73	0	0
SA11-P	31	49	96	96	0	24	2
	39	51	94	94	0	18	0

^a Includes cells having an overall patchy fluorescence pattern and cells with a few bright, round cytoplasmic spots. In *tsG* and *tsF* some cells were scored as positive for both viroplasms/cisternae and diffuse stain.

^b Cells having the weakly fluorescent, cigar-shaped inclusions described in the text and illustrated in Fig. 2.

were photographed through a Zeiss fluorescent microscope equipped with epifluorescent optics. These photographs were used to obtain the data presented in Table 1.

EM. MA104 cells were infected with SA11 wild type or the various SA11 *ts* mutants (MOI = 10) and were incubated at 31 or 39°C. At various times postinfection, cells were prepared for electron microscopy (EM) and embedded in situ as previously described (1), except that the cells were stained en bloc with 2% aqueous uranyl acetate for 1 h at 60°C before dehydration and embedding. Thin sections were stained with lead citrate only. Four or five separate areas from each sample were mounted on plastic blocks, and the sections from each block were picked up on a single grid. These precautions were taken to ensure that each cell was counted only once in the microscopic examination. Representative micrographs were made of each sample.

RESULTS

Infected-cell polypeptide synthesis. Kinetic experiments showed that, in wild-type-infected cells (MOI = 25), viral polypeptide synthesis was maximal 10 to 14 h postinfection at the permissive temperature (31°C) and 6 to 10 h postinfection at the restrictive temperature (39°C; data not shown). Long-term labeling (6 to 30 h postinfection) of mutant polypeptides had shown that none of the *ts* mutants simply

had delayed kinetics of protein synthesis (data not shown). Therefore, cells were infected with wild type or each of the *ts* mutants, and polypeptides were labeled with ³H-amino acids at the time of maximal polypeptide synthesis at 31 and 39°C. Resolution of the polypeptide species on polyacrylamide gels revealed the profiles shown in Fig. 1. At 31°C, the wild type and each of the mutants synthesized the same species of viral polypeptides (125K, 94K, 88K, 53K, 41K, 38K or 36K, 35K, 34K, and 28K) previously reported for SA11-infected cells (5, 6). Each mutant, except *tsE*, synthesized the same polypeptide species at 39°C, although the synthesis of the high-molecular-weight polypeptides was reduced in all infections and in several cases the synthesis of all viral polypeptides was restricted to various degrees. Immunoprecipitation confirmed the synthesis of each of the structural polypeptide species at 31 and 39°C, even in cases where synthesis of viral polypeptides was poor (data not shown). *tsE* synthesized very little of the 35K nonstructural protein at 31°C and no detectable 35K at 39°C (Fig. 1A and B).

The most prominent feature of the polypeptide profiles of the mutants compared with the parental virus (SA11-P) was the synthesis of either the 38K or the 36K form of the structural glycoprotein, but not both. The 38K and 36K glycoproteins have been shown to be alternate forms of the same viral gene product and result from infection with a mixed virus population (6). Although the parental SA11

FIG. 1. Polypeptides of cells infected with SA11 *ts* mutants and controls at 31°C (A) and 39°C (B) analyzed by polyacrylamide gel electrophoresis. Infected cells were pulse-labeled with ^3H -amino acids from 10.5 to 13.5 h postinfection at 31°C and 6.5 to 9.5 h postinfection at 39°C. *ts* mutants A to J are indicated. UN, Uninfected cells; SA11-P, parental virus clone from which mutants were isolated; WT, a subclone of SA11-P synthesizing only the 38K glycoprotein. Estimated molecular weights ($\times 10^3$) of the SA11-specific polypeptides (5) are indicated on the left.

stock (SA11-P) from which the *ts* mutants were isolated had been subcloned by three plaque-to-plaque passages and was shown to have a homogeneous electrophoretic profile for its genomic RNA (16), it is clear that SA11-P was a mixture

synthesizing both 36K and 38K glycoproteins (Fig. 1, lanes SA11-P). Subsequent rounds of subcloning the SA11-P stock yielded predominantly clones that synthesized only the 38K or the 36K form of the structural glycoprotein, although a

few subclones synthesized both. Most subclones of the parental virus synthesized only 38K (Fig. 1, lanes WT). Thus, it appears that the alternate forms of the glycoprotein present in the various mutants result from further subcloning of a mixed parental virus population during the selection of the *ts* mutants. It is important to note that the wild type and each of the mutants were subcloned twice during mutant isolation (16, 16a) and were subcloned an additional time for these studies. Therefore, it is likely that the wild-type and *ts* mutant populations used here are homogeneous.

Several of the mutants (*tsF*, *tsG*, *tsH*, and *tsI*) either shut off the synthesis of cellular polypeptides much less efficiently than did wild-type virus or degraded some of the higher-molecular-weight polypeptides, since a significant background was seen at both temperatures with these mutants (Fig. 1A and B). The presence of these background bands in harvests performed in the presence of protease inhibitors and the inability to precipitate any of the background bands with anti-SA11 serum suggested that they represented cellular background. The failure to efficiently shut off host polypeptide synthesis was apparently a specific property of these mutants and not the result of low multiplicity, since titrations performed the day of infection showed that an MOI of 20 to 30 PFU per cell was obtained in every infection.

Mutants *tsB*, *tsC*, *tsE*, *tsF*, and *tsG* synthesized lesser amounts of the viral polypeptides at 39°C (Fig. 1B) than at 31°C (Fig. 1A). In most cases all polypeptide species synthesized at 31°C were also observed at 39°C. In *tsA* and *tsB* the synthesis of 35K was greatly reduced, and in *tsE* no detectable 35K was synthesized at 39°C (Fig. 1B). Synthesis of the high-molecular-weight polypeptides (125K, 94K, and 88K) was also reduced at 39°C in all viruses examined except *tsD*, although faint bands were detected (Fig. 1B). Synthesis of the high-molecular-weight polypeptide species was confirmed by immunoprecipitation (data not shown). The low amounts of the high-molecular-weight polypeptides seen in samples processed in the presence of protease inhibitors suggested that degradation did not account for the poor synthesis of these polypeptides at 39°C. Small amounts of high-molecular-weight polypeptides, especially 125K, were also observed at 31°C with some of the viruses. Synthesis of high-molecular-weight species at 31°C was confirmed by immunoprecipitation (data not shown).

With three exceptions, the mutants synthesized polypeptides that were electrophoretically indistinguishable from those of the wild type. *tsJ* synthesized a 41K polypeptide that had an electrophoretic mobility slightly greater than that of the wild type. *tsC* and *tsH* synthesized 125K and 34K polypeptides, respectively, that migrated more slowly than the corresponding polypeptides of the wild type (Fig. 1A and B). In all three cases the aberrant migration was observed at both 31 and 39°C.

Several polypeptide species smaller than 28K have been observed in short pulse-labels or in cells treated with tunicamycin and have been shown to be precursors to higher-molecular-weight viral polypeptides (5). The failure to observe these polypeptide species in the longer-term label of the mutants suggests that none of the mutants have defects in the processing of these precursors.

Distribution of viral antigens. SA11 polypeptides were localized by IF within cells infected with each of the mutants at 31 and 39°C (Table 1; Fig. 2). The patchy cytoplasmic fluorescence seen with the parental virus at either temperature and with all of the mutants at 31°C probably represents both viroplasmic inclusion bodies and virus-filled cisternae

of the endoplasmic reticulum (see reference 1; Fig. 3). This pattern is typical for rotavirus-infected cells. In addition, weakly fluorescent, "cigar-shaped" inclusion bodies were observed in some of the infected cell nuclei and occasionally in the cytoplasm (arrows, Fig. 2). These probably correspond to the tubular or ribbon-like inclusions described below. It is unlikely that these are related to any *ts* lesions, since they occur most often with the wild-type virus and with the mutants at 31°C.

When grown at 39°C, many of the mutant-infected cells displayed a diffuse cytoplasmic fluorescence rather than the patchy pattern described above for 31°C. This shift in the distribution of viral antigens at 39°C was greatest with *tsB*, *tsE*, and *tsF* mutants and was undetectable with *tsH* and *tsI*. Some cells infected with *tsG* appeared to have an intermediate type of antigen distribution characterized by small bright fluorescent spots on a diffusely stained background (Fig. 2). None of the cells infected with SA11-P showed the diffuse staining pattern at 31 or 39°C. For three of the mutants, *tsB*, *tsE*, and *tsI*, significantly fewer cells contained detectable amounts of viral antigens when grown at 39°C.

Morphogenesis. Electron micrographs of cells infected with parental virus at 31 and 39°C and cells infected with each of the *ts* mutants at 31°C revealed the unusual features of rotavirus morphogenesis (Fig. 3a). Large, granular viroplasms formed in the cytoplasm and newly assembled virus particles passed from these into the lumen of the endoplasmic reticulum via a budding process. The membrane envelope thus acquired by the virus particles was subsequently lost in the endoplasmic reticulum cisternae. Several previously described aberrant structures (1) were also found in both the parental SA11- and *ts* mutant-infected cells, including nuclear and cytoplasmic inclusions of small tubules or ribbon-like structures surrounded by electron-lucent space (Fig. 3e), large tubules resembling elongated virus particles (Fig. 3c), and elaborations of smooth membrane-like material within the endoplasmic reticulum (Fig. 3f). In addition, accumulations of granular material resembling viroplasms, but smaller and more electron dense, were often present in areas of viral replication (Fig. 3a, c, and d). The frequency of these structures and other parameters of viral replication are presented in Table 2.

At 39°C only three of the mutants, *tsA*, *tsD*, and *tsI*, produced near-normal quantities of virus particles. Most of the mutant-infected cells contained greatly reduced numbers of virus particles within the endoplasmic reticulum and some cisternae were filled with amorphous material (Fig. 3d). In general, viroplasmic inclusions were smaller in cells grown at 39°C, especially in those infected with *tsH*, *tsI*, and *tsJ* (Fig. 3b and f). There were fewer viroplasms per infected cell (very few in the case of *tsF*) and those often appeared to be more irregular in shape and less well organized than those in cells infected with the wild type. At 39°C cisternae containing a few virus particles were observed in areas void of viroplasmic inclusions (Fig. 3e), most frequently in cells infected with *tsB* and *tsE*. Conversely, in cells infected with *tsC*, *tsG*, *tsH*, and *tsJ*, viroplasms sometimes occurred in the near absence of particle formation (Fig. 3b). Three of the mutants, *tsC*, *tsE*, and *tsH* (Fig. 3g), showed a large fraction of "empty" particles which lack the electron-dense nucleoid when grown at 39°C. In addition, *tsH*-infected cells often had a large proportion of enveloped particles. Although *tsF*-infected cells were virus negative at 39°C, they often produced large tubules resembling aberrant virus at 31°C (Fig. 3c), suggesting that viral replication was disturbed even at the permissive temperature.

FIG. 2. Distribution of viral proteins seen by IF within cells infected by SA11-P and the *ts*C mutant at 31°C and all *ts* mutants at 39°C. Arrows indicate nuclear and cytoplasmic inclusions.

DISCUSSION

This examination of the synthesis of polypeptides, distribution of antigens, and morphogenesis of particles in *ts* mutant-infected cells defines several of the characteristics of the SA11 prototype *ts* mutants. In general, there was good agreement between the results obtained for a mutant by the different methods. Mutants that synthesized viral polypeptides poorly at 39°C correspondingly produced less antigen

and assembled fewer viral structures at 39°C. Conversely, mutants that synthesized polypeptides normally at 39°C also produced near-normal amounts of antigen and viral structures at 39°C.

The mutants examined in these experiments were the prototypes of the 10 mutant groups currently identified in SA11. In groups for which more than one *ts* mutant has been isolated (groups A to E) (16, 16a), only the mutant that has been used as the group prototype in other experiments was

FIG. 3

selected for analysis. Since these mutants were isolated after mutagenesis (16, 16a), it is likely that each mutant contains, in addition to the characterized *ts* mutation, mutations for which no phenotype has yet been identified. Thus, some of the properties reported here for the prototypes may be mutant specific and not related to the *ts* lesion upon which grouping was based. The aberrant migration rates noted for some polypeptide species in some mutants may represent the expression of the non-*ts* mutations, especially if the aberrant migration was not expressed in a temperature-

sensitive manner. The aberrantly migrating protein species could result from a number of mutational effects, including premature termination of translation, altered protein secondary structure, and altered post-translational modification.

Several *ts* mutant group-specific features were noted in the examination of polypeptide synthesis at 31 and 39°C. (i) At 31°C the mutants synthesized the major polypeptide species reported for wild-type-infected cells (5). At 39°C several of the mutants synthesized significantly lesser amounts of all viral polypeptides. These results correspond

FIG. 3. Thin sections of cells infected with several *ts* mutants at 31 or 39°C, as indicated. Representative examples of various structures are labeled as follows: large asterisks, viroplasms; small asterisks, dense viroplasm-like material; small arrows, virus particles within the endoplasmic reticulum; arrowhead, ribbon-like cytoplasmic inclusion; large arrow, smooth membrane proliferation; double arrows, large tubules. Bars, 0.5 μ m.

to a double-stranded RNA-negative phenotype for those mutants (*ts*B, -C, -E, -F, and -G; J. Gombold and R. Ramig, unpublished data) and are similar to results reported for *ts* mutants of the bovine rotavirus UK (8). In addition, one mutant (*ts*E) synthesized little 35K nonstructural polypeptide at 31°C and none of this species at 39°C. The basis of this specific restriction in synthesis was not understood. (ii)

Several examples of mutant-specific aberrant migration of polypeptide species were noted among the mutants. The 38K/36K glycoprotein heterogeneity described in Results appeared to be the result of isolation of the *ts* mutants from a mixed parental virus population. It was surprising that the parental virus clone (SA11-P) was heterogeneous since it had been cloned by three plaque-to-plaque passages and had a

TABLE 2. Summary of EM examination of SA11 *ts* mutants

Mutant and group	Temp (°C)	No. of cells examined	No. of cells infected	Virus production ^a	No. of cells examined with the following features:						
					Viroplasm	>30% empty ^b	>30% enveloped ^b	Large tubules ^c	Nuclear inclusions ^d	Cytoplasmic inclusions ^d	Membrane proliferation ^e
<i>tsA</i> (778)	31	21	21	+++	19	0	0	0	0	0	0
	39	20	17	++	13	0	2	0	6	3	3
<i>tsB</i> (339)	31	20	20	+++	17	4	0	1	2	1	0
	39	28	20	+	9	3	2	1	5	3	0
<i>tsC</i> (606)	31	20	19	+++	19	3	3	2	1	0	0
	39	27	15	+/-	10	6	0	0	2	4	6
<i>tsD</i> (975)	31	10	10	+++	10	0	3	3	0	0	3
	39	25	16	++	10	0	1	3	1	5	5
<i>tsE</i> (1400)	31	8	8	+++	5	0	0	0	0	0	2
	39	22	9	+/-	2	8	5	1	1	1	1
<i>tsF</i> (2124)	31	15	13	+++	12	0	7	9	3	2	0
	39	16	8	-	5	0	0	0	0	0	0
<i>tsG</i> (2130)	31	19	18	++	16	0	7	0	5	1	0
	39	10	6	+/-	6	1	2	0	0	0	0
<i>tsH</i> (2384)	31	19	18	+++	19	0	9	6	1	1	1
	39	20	19	+	14	11	15	4	1	2	4
<i>tsI</i> (2403)	31	23	21	+++	21	0	11	5	7	0	2
	39	22	17	++	17	0	3	0	3	0	4
<i>tsJ</i> (2131)	31	13	11	++	11	0	1	0	2	0	0
	39	15	14	+/-	12	0	2	0	0	0	8
SA11-P	31	19	19	+++	15	0	0	1	2	0	0
	39	20	17	+++	16	0	0	0	9	1	6

^a Indicates overall level of virus production. Considerable variation between cells was observed.

^b More than 30% of the virus particles within the endoplasmic reticulum in a given cell either were lacking the dense central nucleoid or were enveloped as indicated.

^c Resemble elongated virus particles as in Fig. 3c.

^d The type of inclusion illustrated in Fig. 3e.

^e Proliferation of smooth membrane-like material within the endoplasmic reticulum as in Fig. 3f.

homogeneous electrophoretic profile for its genome RNA (16). This result, along with the results of others (6, 17), indicates that multiple cycles of plaque purification are not always adequate to resolve mixtures of rotaviruses. Mutants *tsC*, *-H*, and *-J* had aberrantly migrating species, 125K, 34K, and 41K, respectively. Since none of the aberrant polypeptide migrations was affected by temperature, they may not result directly from the *ts* lesion. However, these aberrant migrations may provide useful markers that could be exploited in a manner analogous to those in reovirus (3). (iii) The failure to note significant amounts of any of the SA11 glycoprotein precursors (5) at either 31 or 39°C indicated that none of the mutants had specific defects in the processing of the viral glycoproteins. (iv) The failure of some mutants to efficiently shut off host protein synthesis at either 31 or 39°C suggested that these mutants had a non-*ts* defect in host cell shutoff and further suggested that a specific viral gene(s) may be associated with this phenotype as was found for the related reovirus (18).

As noted above, there was general agreement among the protein synthesis, IF, and EM data. The proportion of cells infected was higher in the EM experiments than in the IF experiments. This probably resulted from a higher MOI in

the EM experiments and from greater sensitivity of EM, where a cell was considered infected if any virus was present in the endoplasmic reticulum, if any viroplasm was seen, or if any nuclear or cytoplasmic inclusions or large tubules were present. Also, the proportion of cells with viroplasms was apparently higher in EM experiments, possibly because the diffuse fluorescence in many *ts*-infected cells obscured the smaller viroplasms.

The data in Table 2 do not define specific morphogenic blocks for any of the mutant groups since no accumulation of any specific type of morphogenetic intermediate was noted. This result was in contrast to the accumulation of empty inner or outer capsids seen with certain *ts* mutants of reovirus (9). However, the high percentage of *tsE*- and *tsH*-infected cells with >30% empty particles at 39°C may result from a block in the production of RNA in these mutants. In addition, many *tsH*-infected cells contained >30% enveloped particles (compared to 10% enveloped in wild-type-infected cells; 14). These are believed to represent an immature stage of rotavirus morphogenesis and have been associated with a block in glycosylation of the 28K nonstructural glycoprotein (14). However, the increased proportion of enveloped particles in *tsH*-infected cells does not appar-

ently result from a glycosylation defect since no accumulation of nonglycosylated 28K precursors was noted.

Although aberrant morphogenetic intermediates were more common than previously reported for SA11 (1) in the *ts* mutant-infected cells, they occurred at both 31 and 39°C and in the cells infected with SA11-P. It is therefore unlikely that they are the direct result of any of the *ts* lesions. It is more likely that the parental clone of SA11 (SA11-P) produces more of the various tubules and inclusions than other SA11 clones or that these structures result from growth of the infected cells at either suboptimal (31°C) or supraoptimal (39°C) temperature. Similar arguments apply to the appearance of the denser viroplasm-like inclusions which have not been previously reported.

At the present time, little is known about morphogenetic events that take place within the viroplasms. Indeed, the composition of the viroplasms is not completely known. The 94K and 41K structural proteins have been localized to these structures and the structural 38K glycoprotein has been shown to be absent (15). Additional light microscopic and EM immunocytochemical analysis of the *ts* mutant-infected cells should aid in elucidating the early stages of rotavirus assembly and in defining the morphogenetic functions altered in each of the mutant groups.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants CA09197 from the National Cancer Institute and AI16687 from the National Institute of Allergy and Infectious Diseases.

We thank Judith Carinhas for excellent technical assistance, Edward Calomeni for thin sectioning and EM, and Mary Estes for anti-SA11 serum.

LITERATURE CITED

1. Altenburg, B. C., D. Y. Graham, and M. K. Estes. 1980. Ultrastructural study of rotavirus replication in cultured cells. *J. Gen. Virol.* **46**:75-85.
2. Arias, C. F., S. Lopez, and R. T. Espejo. 1982. Gene products of SA11 simian rotavirus genome. *J. Virol.* **41**:42-50.
3. Cross, R. K., and B. N. Fields. 1976. Use of an aberrant polypeptide as a marker in three-factor crosses: further evidence for independent reassortment as the mechanism of recombination between temperature-sensitive mutants of reovirus type 3. *Virology* **74**:345-362.
4. Dyll-Smith, M. L., and I. H. Holmes. 1981. Gene-coding assignments of rotavirus double-stranded RNA segments 10 and 11. *J. Virol.* **38**:1099-1103.
5. Ericson, B. L., D. Y. Graham, B. B. Mason, and M. K. Estes. 1982. Identification, synthesis, and modifications of simian rotavirus SA11 polypeptides in infected cells. *J. Virol.* **42**:825-839.
6. Estes, M. K., D. Y. Graham, R. F. Ramig, and B. L. Ericson. 1982. Heterogeneity in the structural glycoprotein (VP7) of simian rotavirus SA11. *Virology* **122**:8-14.
7. Estes, M. K., D. Y. Graham, E. M. Smith, and C. P. Gerba. 1979. Rotavirus stability and inactivation. *J. Gen. Virol.* **43**:4:3-409.
8. Faulkner-Valle, G. P., A. V. Clayton, and M. A. McCrae. 1982. Molecular biology of rotaviruses. III. Isolation and characterization of temperature-sensitive mutants of bovine rotavirus. *J. Virol.* **42**:669-677.
9. Fields, B. N., C. S. Raine, and S. G. Baum. 1971. Temperature-sensitive mutants of reovirus type 3: defects in viral maturation as studied by immunofluorescence and electron microscopy. *Virology* **43**:569-578.
10. Greenberg, H. B., A. R. Kalica, R. G. Wyatt, R. W. Jones, A. Z. Kapikian, and R. M. Chanock. 1981. Rescue of noncultivable human rotavirus by gene reassortment during mixed infection with *ts* mutants of a cultivatable bovine rotavirus. *Proc. Natl. Acad. Sci. U.S.A.* **78**:420-424.
11. Mason, B. B., D. Y. Graham, and M. K. Estes. 1980. In vitro transcription and translation of simian rotavirus SA11 gene products. *J. Virol.* **33**:1111-1121.
12. Mason, B. B., D. Y. Graham, and M. K. Estes. 1983. Biochemical mapping of the simian rotavirus SA11 genome. *J. Virol.* **46**:413-423.
13. Matsuno, S., A. Hasegawa, A. R. Kalica, and R. Kono. 1980. Isolation of a recombinant between simian and bovine rotaviruses. *J. Gen. Virol.* **48**:253-256.
14. Petrie, B. L., M. K. Estes, and D. Y. Graham. 1983. Effects of tunicamycin on rotavirus morphogenesis and infectivity. *J. Virol.* **46**:270-274.
15. Petrie, B. L., D. Y. Graham, and M. K. Estes. 1982. Localization of rotavirus antigens in infected cells by ultrastructural immunocytochemistry. *J. Gen. Virol.* **63**:457-468.
16. Ramig, R. F. 1982. Isolation and genetic characterization of temperature sensitive mutants of simian rotavirus SA11. *Virology* **120**:93-105.
- 16a. Ramig, R. F. 1983. Isolation and genetic characterization of temperature-sensitive mutants that define five additional recombination groups in simian rotavirus SA11. *Virology* **130**:464-473.
17. Sabara, M., D. Deregt, L. A. Babiuk, and V. Misra. 1982. Genetic heterogeneity within individual bovine rotavirus isolates. *J. Virol.* **44**:813-822.
18. Sharpe, A. H., and B. N. Fields. 1982. Reovirus inhibition of cellular RNA and protein synthesis: role of the S4 gene. *Virology* **122**:381-391.
19. Smith, M. L., I. Lazdins, and I. H. Holmes. 1980. Coding assignments of double stranded RNA segments of SA11 rotavirus established by in vitro translation. *J. Virol.* **33**:976-982.