Characterization of Two Recombination–Complementation Groups of Uukuniemi Virus Temperature-sensitive Mutants

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(Accepted 13 March 1984)

SUMMARY

With the aim of isolating temperature-sensitive (ts) mutants defective in virus maturation or glycoprotein transport, Uukuniemi virus, a bunyavirus, was mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine. Out of 13 initial clones unable to grow at 39 °C (non-permissive temperature), five mutants which grew to titres above 10^7 p.f.u./ml at 32 °C (permissive temperature) were selected for further studies. The mutants fell into two coinciding recombination–complementation groups. Three group I mutants (ts7, 8 and 12) and two group II mutants (ts6 and 11) synthesized all three RNA segments and were able to form the corresponding nucleoproteins at 39 °C. Thus, members of these two recombination groups had a RNA-positive phenotype. All five mutants showed immunofluorescence when cells were stained at 39 °C using a double-staining technique employing monoclonal antibodies against the glycoproteins G1 or G2, and polyclonal antibodies against the nucleoprotein, N. We have previously shown that in cells infected with wild-type virus both the G1/G2 and the N proteins accumulate in the Golgi complex, the site of virus maturation. In cells infected with ts12, accumulation of G1 and G2, but not N protein, was observed in the Golgi complex at 39 °C. The N protein was found evenly scattered in the cytoplasm, suggesting lack of interaction between the G1/G2 and N proteins. With ts6 and 11, G1 and G2 appeared to accumulate and aggregate in the endoplasmic reticulum (ER) at 39 °C. The location of the N protein coincided with that of the aggregated glycoproteins, suggesting that the N protein interacted with G1/G2 already in the ER. Thus, these mutants may prove valuable tools in studying the mechanism of Uukuniemi virus maturation.

INTRODUCTION

The Bunyaviridae family comprises more than 200 different arthropod-borne viruses grouped into the four genera Bunyavirus, Nairovirus, Phlebovirus and Uukuvirus. A number of possible bunyaviruses still remain unclassified (Bishop et al., 1980). All bunyaviruses so far studied have a common basic structure. They consist of a lipoprotein envelope containing two glycoproteins, G1 and G2, and a ribonucleoprotein core. The genetic information is divided among three single-stranded RNA segments of negative polarity, with which a nucleoprotein, N, and the L protein, the putative RNA polymerase, are associated (Bishop & Shope, 1979; Bishop et al., 1980).

Because of the segmentation of the genome, a high frequency of recombination due to reassortment of RNA segments has been observed (Bishop, 1979). Reassortment has been studied by dual infections of cells with temperature-sensitive (ts) mutants belonging to different recombination groups. Reassortment between RNA segments of serologically closely related viruses with the Bunyavirus genus has also been frequently found (Gentsch et al., 1977; Bishop, 1979; Iroegbu & Pringle, 1981; Pringle & Iroegbu, 1982). These analyses, in combination with translation in vitro of isolated mRNA species, have allowed the determination of the coding assignments for the three RNA segments (Gentsch & Bishop, 1978, 1979; Cash et al., 1979;
Ulmanen et al., 1981). Thus, the M RNA segment encodes G1 and G2 and, at least in the case of some members of the California encephalitis (CE) serogroup, also a non-structural (NSM) protein (Fuller & Bishop, 1982). The S RNA segment encodes the N protein and a non-structural (NSs) protein (Fuller & Bishop, 1982; Ulmanen et al., 1981). By deduction, and size considerations, the L RNA segment must encode the L protein.

As a model system, we have studied the structure and replication of one bunyavirus, namely Uukuniemi virus, the prototype of the Uukuvirus genus. This virus contains two envelope glycoproteins, G1 (Mr 70000) and G2 (Mr 65000) (Pettersson et al., 1971; von Bonsdorff & Pettersson, 1975), the glycan structures of which have recently been determined (Pesonen et al., 1982). The unglycosylated G1 and G2 are about the same size (Mr 54000) (E. Kuusmanen, unpublished results). They are synthesized as a 110000 mol. wt. (p110) precursor, which is cotranslationally cleaved in the middle (Ulmanen et al., 1981). The nucleocapsid consists of three circular single-stranded RNA species L (Mr 2.4 x 106), M (Mr 1.1 x 106) and S (Mr 0.5 x 106) (Hewlett et al., 1977; Pettersson et al., 1977), multiple copies of the N protein (Mr 25000) (Pettersson et al., 1971) and a few copies of the L protein (Mr about 200000) (R. F. Pettersson, unpublished results).

More recently, we have studied the mechanism of virus maturation and intracellular glycoprotein transport. A unique feature of the bunyaviruses, including Uukuniemi virus, is that the virus particles mature intracellularly by a budding process at the smooth-surfaced vesicles in the Golgi region (Murphy et al., 1973; Bishop & Shope, 1979; Kuusmanen et al., 1982). Using immunofluorescence and immunoelectron microscopy, the glycoproteins and the nucleoprotein were found to accumulate in the Golgi complex (Kuusmanen et al., 1982). The nucleoprotein was closely associated with the cytoplasmic face of the smooth-surfaced vesicles, suggesting binding of the nucleocapsid to a cytoplasmic extension of one or both of the glycoproteins. We have interpreted these results to mean that the accumulation of the glycoproteins in, and the progressive vacuolization of, the Golgi complex determine the site of bunyavirus maturation (Kuusmanen et al., 1982, 1984). What causes the glycoproteins to accumulate in the Golgi apparatus is not known at present.

As one approach to the study of the maturation process and glycoprotein biosynthesis, I have isolated ts mutants of Uukuniemi virus that are defective at the non-permissive temperature in either glycoprotein transport or nucleoprotein–glycoprotein interaction. In this report, I describe the isolation and preliminary characterization of five such ts mutants. These mutants have RNA-positive phenotypes and fall into two recombination–complementation groups.

METHODS

Cells and virus. All experiments were carried out in secondary chicken embryo fibroblasts (CEF) grown as monolayers in plastic dishes or on coverslips at 37 °C or, in the ts mutant work, at 32 °C or 39 °C. The origin and cultivation of the cells and the preparation of stock virus from the prototype strain S23 of Uukuniemi virus after several successive plaque-purification cycles have been described previously by Pettersson & Kääriäinen (1973). The titre of the original stock virus was 2 x 108 p.f.u./ml.

Infectivity assay, plaque purification and preparation of wild-type stock virus. Infectivity assays of virus preparations diluted in tenfold series were carried out in CEF under an agar overlay as described previously (Pettersson & Kääriäinen, 1973). The dishes were incubated at 32 °C, 37 °C or 39 °C and plaques counted without staining after 9 to 10, 5 to 6 and 4 to 5 days, respectively.

For cloning of the virus, individual well-separated plaques were picked from endpoint dilutions through the agar overlay using a Pasteur pipette. Each plaque was resuspended in 1 ml of Dulbecco's phosphate-buffered salt solution containing 0.2 % bovine serum albumin (BSA) (Armour Pharmaceutical Co.). The virus was eluted from the agar plug after one cycle of freezing and thawing.

Before the isolation of ts mutants, the original stock virus was adapted to growth at the permissive (32 °C) and non-permissive (39 °C) temperatures by three consecutive plaque purifications (twice at 39 °C and once at 32 °C). At each cloning cycle a plaque yielded 104 to 105 p.f.u./ml. From the final plaque a primary wild-type (wt) stock virus was prepared using a multiplicity of about 0.01 p.f.u./cell. The primary stock was stored at −70 °C in 0.5 ml aliquots. A working stock virus used to isolate the ts mutants was prepared from the primary stock virus using a multiplicity of about 0.05 p.f.u./cell. The working stock had an infectivity titre of 2 x 108 p.f.u./ml.

Mutagen treatment and isolation of ts mutants. N-Methyl-N'-nitro-N-nitrosoguanidine (NTG) treatment (100 or
200 µg/ml in TN buffer (0.1 M-NaCl, 0.05 M-Tris–HCl pH 7.4) of the wt virus was performed as described by Burge & Pfefferkorn (1966). The mutagen treatment was done at 20 °C for 30 min. Immediately after the incubation, a sample was diluted 1:1000 and an infectivity assay carried out at 32 °C. The infectivities recovered after NTG treatment were 8% (100 µg/ml) and 2% (200 µg/ml) compared to untreated wt controls. The diluted mutagenized virus was stored at −70 °C for subsequent use. The ts mutants were isolated from two separate mutagenizations using a dilution giving about five plaques per 20 cm² dish. The dishes were incubated at 32 °C and plaques well-separated from each other were collected into 1 ml of Dulbecco’s salt solution containing 0-2% BSA. The clones were screened for their ability to form plaques at 39 °C. Those clones which produced few or no plaques at 39 °C were re-titrated at 32 °C and 39 °C. Clones which showed an efficiency of plating (e.o.p.; ratio of plaques at 39 °C/plaques at 32 °C) of 0.01 or less were selected for further analysis. To obtain a primary stock, virus from one plaque was grown for 48 h at 32 °C at a multiplicity of about 0.01 p.f.u./cell. From each primary stock a working stock was grown for 72 h for only one further passage using an m.o.i. of 0.05. The ts mutants were numbered serially.

The leak yield was determined for each mutant by growing the virus at 39 °C and 32 °C using an infectivity of 5 p.f.u./cell. The medium was collected after 24 h (39 °C) or 48 h (32 °C) and the infectivity titres of both harvests were determined by assaying at 32 °C.

**Recombination (reassortment) assay.** Monolayers of CEF in duplicate 20 cm² dishes were infected with a ts mutant at a multiplicity of 5 p.f.u./cell or with pairs of ts mutants at a combined multiplicity of 10 p.f.u./cell. After adsorption for 1 h at 32 °C the unadsorbed virus was aspirated and the cells were washed three times with prewarmed growth medium (M199 supplemented with 5% newborn calf serum and 10% tryptose phosphate broth). Four h after infection the medium was removed, the cells were washed once, fresh medium was added and incubation continued at 32 °C. Samples for infectivity assays were taken 48 h after infection and the recombination (reassortment) frequency (RF) was calculated as follows: \( RF(\%) = \frac{((AB)^{39 \text{ °C}} - (A + B)^{39 \text{ °C}}) \times 100}{(AB)^{32 \text{ °C}}}, \) where A and B are any pair of ts mutants and the superscript is the temperature of assay.

**Complementation assay.** Duplicate 20 cm² dishes were infected singly or with pairs of ts mutants at 39 °C as described above. Incubation was continued at 39 °C for 24 h. Samples from duplicate dishes were pooled and assayed for infectivity at 32 °C and 39 °C. Complementation indices were measured as follows: CI = \( \frac{(AB)^{32 \text{ °C}} - (AB)^{39 \text{ °C}}}{(A + B)^{32 \text{ °C}}}, \) where A and B are any pair of ts mutants and the superscript is the temperature of assay. A CI value greater than 2 was considered an indication of complementation.

**Isolation of RNA and ribonucleoprotein.** Secondary CEF on 75 cm² dishes were infected with ts mutants or wt virus at a multiplicity of 5 p.f.u./cell at 39 °C. Mock-infected cells were used as controls. Actinomycin D (2 µg/ml) was added to the cultures at 11 h (wt) or 13 h (ts mutants) and the virus-specific RNA was labelled between 12 and 16 h (wt) or 14 and 19 h (ts mutants) with [3H]uridine (Amersham; 50 µCi/ml, sp. act. 25 to 30 Ci/mmol).

To prepare cytoplasmic extracts, the culture medium was removed and the cells were washed once with ice-cold phosphate-buffered saline (PBS). The cells were scraped off the dishes into 1 ml PBS, collected by centrifugation, and washed once with an isotonic buffer (Iso-B: 0.15 M-NaCl, 10 mM-Tris–HCl pH 7.8, 1.5 mM-MgCl₂). The cells were disrupted by incubation at 0 °C for 10 min in Iso-B containing 1% Triton X-100, and 40 µg/ml polyvinyl sulphate (PVS). The nuclei were removed by centrifugation at 1500 r.p.m, for 10 min at 0 °C. One-half of the supernatant was treated with SDS at a final concentration of 2%. The RNA was fractionated on a 15 to 30% (w/w) sucrose gradient made in TNE buffer (140 mM-NaCl, 10 mM-Tris–HCl pH 7.4, 1 mM-EDTA) containing 0.1% SDS. Centrifugation was for 17 h at 25000 r.p.m. and 23 °C using a SW28 rotor.

The other half of the cytoplasmic extract was layered directly on a 15 to 40% sucrose gradient made in TNE buffer. A 3 ml cushion of 60% sucrose was added at the bottom of the gradient. Centrifugation was for 16 h at 21000 r.p.m. at 4 °C. Fractions of 1 ml were collected from the bottom, and the TCA-precipitable radioactivity was determined by samples of each sucrose gradient fraction by counting in a toluene-based scintillation fluid using an LKB-Wallac 1211 Rackbeta scintillation counter.

**Immunofluorescence.** Non-confluent monolayers of cells grown on coverslips were infected with Uukuniemi virus wt or ts mutants at 32 °C or 39 °C. Mock-infected cells were used as controls. At 16 h after infection, cells were washed once with prewarmed PBS (32 °C or 39 °C) and then fixed with 3% paraformaldehyde in 0.1 M-PBS (pH 7.2) at room temperature for 15 min. For intracellular staining, fixed cells were permeabilized with 0.05% Triton X-100 in PBS at room temperature for 30 min.

Cells were double-stained using an indirect immunofluorescence method as described previously by Kuismannen et al. (1982). A polyclonal antisera directed against the nucleocapsid protein (N) prepared in rabbit (Kuismannen et al., 1982) and monoclonal antibodies directed against the virus glycoproteins G1 and G2 prepared in mice (Kuismannen et al., 1984) were used. For the staining, swine anti-rabbit IgG conjugated to fluorescein isothiocyanate (FITC) (Dako, Copenhagen, Denmark) and rabbit anti-mouse IgG conjugated to rhodamine isothiocyanate (TRITC) (Cappel Laboratories, Cochranville, Pa., U.S.A.) were used. The fluorescence was examined with a Leitz Dialux 20 microscope fitted with a 100 x or 63 x oil immersion objective and filters for FITC and TRITC fluorescence.
RESULTS

Isolation and selection of ts mutants

The wt Uukuniemi virus was plaque-cloned three times at 32 °C and 39 °C before mutagenization to obtain a genetically homogeneous virus able to grow and plate efficiently at both temperatures. The growth curves of the wt virus at 32 °C and 39 °C are shown in Fig. 1. At 32 °C the virus grew somewhat more slowly and to a slightly lower titre than at 39 °C. The wt virus was mutagen-treated with 100 or 200 μg/ml NTG for 30 min. To analyse the inactivation of the virus, samples were taken at different time points and the infectivity titres determined. Untreated virus served as a control. As shown in Fig. 2, 92% and 98%, respectively, of the virus was inactivated at the two concentrations of NTG after 30 min. Initially, two ts mutants (0.5%) were isolated from 401 clones analysed after treatment with 100 μg/ml NTG for 30 min. In a separate experiment 11 ts mutants (1.0%) were obtained out of 1049 clones analysed after treatment with 200 μg/ml NTG for 30 min. The frequency of spontaneously arising ts mutants was not determined.

The efficiency of plating and leak yield were determined for the mutants. Out of the 13 initial ts mutants, only five grew to a sufficiently high titre (> 10⁷ p.f.u./ml) and had acceptable values for leak yield (< 10⁻³) and reversion frequency (< 10⁻²) (Table 1). Eight mutants had either too high a reversion frequency, leakiness, and/or grew too poorly and were therefore not further analysed.

Recombination (reassortment)

When ts mutants were grown in pairs, a high frequency of recombination was observed and
The mutants fell into two recombination groups (Table 2). Group I comprised ts mutants 7, 8 and 12, and group II ts6 and ts11. No recombination was observed between ts mutants within either group.

Analysis of recombinant progeny clones isolated at 39 °C after dual infection with ts11 and ts12 (22 clones analysed) and ts8 and ts11 (22 clones analysed) showed wt e.o.p. values (i.e. close to 1) for all clones (data not shown). This indicated that true reassortment of RNA segments had occurred, resulting in genetically stable progeny viruses with wt phenotypes. The growth curves of one ts mutant of recombination group I (ts12) and one of group II (ts11) were determined at 32 °C (Fig. 1). The ts12 mutant grew to the same titre and with about the same kinetics as the wt virus, whereas ts11 grew to a somewhat lower titre and with a slower kinetics.

**Complementation**

Pairs of mutants were also tested for complementation. As shown in Table 3, the CI values varied between 15-8 and 146. The two complementation groups observed coincided with the recombination groups I and II (Table 2). No complementation between ts mutants belonging to the same recombination group was observed.
Fig. 3. Double immunofluorescence staining of ts11-infected cells using G2-specific monoclonal antibodies and polyclonal anti-nucleoprotein antiserum. (a) Cells grown at 32 °C and stained with monoclonal anti-G2 antibodies. (b) Same cells as in (a) grown at 32 °C and stained with anti-nucleoprotein antiserum. (c) Cells grown at 39 °C and stained with anti-G2 antibodies. (d) Same cells as in (c) grown at 39 °C and stained with anti-nucleoprotein antiserum. In (a) and (c), TRITC-conjugated anti-mouse IgG was used, and in (b) and (d), FITC-conjugated anti-rabbit IgG was used.
The primary purpose of this study was to isolate \textit{ts} mutants that would be able to synthesize large amounts of viral structural proteins at the non-permissive temperature. Such mutants should be useful in studying virus maturation and glycoprotein transport. Preliminary immunofluorescence studies using antibodies directed against the structural proteins indicated that all five mutants readily synthesized the G1/G2 and N proteins. To be able to localize G1 G2 and the N protein in the same cell, an indirect double-staining technique was used. In this method, \textit{ts} mutant-infected cells were indirectly stained using G1- or G2-specific monoclonal antibodies and TRITC-conjugated rabbit anti-mouse IgG. The same cells were then stained with rabbit polyclonal anti-N antiserum and FITC-conjugated swine anti-rabbit IgG.

\textit{Immunofluorescence of cells infected with \textit{ts} mutants}

Fig. 4. Double immunofluorescence staining of \textit{ts}12-infected cells using G2-specific monoclonal antibodies and polyclonal anti-nucleoprotein antiserum. (a), (b), (c) and (d): Conditions as in Fig. 3.
As shown in Fig. 3(a, b) and Fig. 4(a, b) for cells infected at 32 °C with ts11 and ts12, both the glycoproteins and the N protein were localized throughout the cytoplasm and were heavily concentrated in the Golgi region. These staining patterns were indistinguishable from those obtained for the wt virus (Kuismanen et al., 1982, 1984). Accumulation of the viral structural proteins in the Golgi complex is typical of Uukuniemi virus-infected cells and is an indication of a close association of the nucleocapsid with the glycoproteins at the site of virus maturation (Kuismanen et al., 1982). At 39 °C the distribution of the glycoproteins of ts11 was different from that observed at 32 °C or of the wt virus-infected cells (Fig. 3c). Most of the glycoproteins were localized outside the juxtanuclear region as large heavily stained spots, possibly large aggregates. The nuclear membrane was also fluorescent. This staining pattern suggests that the glycoproteins remain in the endoplasmic reticulum (ER) at 39 °C. The staining of the N protein coincided with that of the glycoproteins (Fig. 3d), suggesting that the nucleocapsid may associate with the glycoproteins in the ER. The staining pattern of G1 or G2 and the N protein of cells infected with ts6 belonging to the same recombination group as ts11 was similar (data not shown).

At 39 °C, the distribution of the glycoproteins in tsl2-infected cells was similar to the pattern observed at 32 °C or in cells infected with wt virus (Fig. 4c). The N protein, however, showed no concentration in the Golgi region, but was distributed throughout the cytoplasm, indicating lack of interaction with the glycoproteins in the Golgi complex (Fig. 4d). In cells infected at 39 °C with ts7 (recombination group I) the staining patterns were different from those observed with ts6, ts11 and ts12 in that the glycoproteins were concentrated in the Golgi region but the N protein appeared to be scattered throughout the cytoplasm and accumulated in patches outside the Golgi region (Fig. 5a, b). Because cells infected with ts8 were weakly fluorescence-positive at 39 °C, the staining properties of this mutant were not further characterized.

**Synthesis of viral RNA and RNP in cells infected with ts mutants**

The ability of the five ts mutants described above to synthesize structural proteins in quantities sufficient for visualization by immunofluorescence suggested that they were capable of secondary transcription at the non-permissive temperature. To confirm this, [3H]uridine-
Fig. 6. Sucrose gradient analysis of Uukuniemi virus-specific RNA made in wt- and ts mutant-infected cells. The wt virus or ts mutants were labelled with [3H]uridine in the presence of actinomycin D and cytoplasmic extracts were prepared. The RNAs were fractionated on 15 to 30% (w/w) sucrose gradients. One ml fractions were collected from the bottom and assayed for acid-precipitable radioactivity. (a) wt, (b) ts6, (c) ts11, (d) ts12. Arrows indicate the positions of the L, M, S1 and S2 RNA species.

labelled virus-specific RNA synthesized in infected cells at 39 °C in the presence of actinomycin D was analysed by sucrose gradient centrifugation. As shown in Fig. 6 for ts6 (panel b), ts11 (panel c) and ts12 (panel d), the ts mutants were able to synthesize the three RNA species L, M and S co-sedimenting with the wt virus RNA species (Fig. 6a). The ts7 and ts8 mutants also synthesized all RNA species (data not shown). Mutants ts12 (Fig. 6d), ts7 and ts8 (data not shown) synthesized more L and less M RNA compared to ts6, ts11 and wt virus. In addition to the L, M and S RNA species, a small amount of the S2 mRNAs (Ulmanen et al., 1981) transcribed from the S segment coding for the N and NS proteins was observed in all cases.

Cells infected with ts6 (Fig. 7b), ts7 (data not shown), ts11 (Fig. 7c) and ts12 (Fig. 7d) were all able to form the three ribonucleoproteins (RNPs) found in cells infected with wt virus (Fig. 7a). The ratio of the RNPs in the different ts mutant-infected cells roughly corresponded to the ratio of the RNA species (Fig. 6).
Fig. 7. Sucrose gradient analysis of Uukuniemi virus RNPs made in wt- and ts mutant-infected cells. The RNPs, labelled with [3H]uridine in the presence of actinomycin D, were isolated from a cytoplasmic extract and fractionated on a 15 to 30% (w/w) gradient (see Methods). One ml fractions were collected from the bottom and assayed for acid-precipitable radioactivity. (a) wt, (b) ts6, (c) ts11, (d) ts12. Arrows indicate the positions of the L, M and S RNPs.

DISCUSSION

Here, I have reported the isolation and preliminary characterization of conditional lethal ts mutants of Uukuniemi virus, the prototype of the genus Uukuvirus within the Bunyaviridae family (Bishop et al., 1980). To date, nearly 200 ts mutants representing the Bunyamwera and the California encephalitis (CE) serogroups within the Bunyavirus genus have been isolated (Bishop, 1979; Bishop & Shope, 1979; Iroegbu & Pringle, 1981; Ozden & Hannoun, 1978, 1980; Pringle & Iroegbu, 1982).

Consistent with the presence of a tripartite segmented genome, high frequencies of 'homologous' recombination between ts mutants of the same virus and 'heterologous' recombination between ts mutants belonging to the same serogroup have been observed (Gentsch & Bishop, 1976; Gentsch et al., 1977; Bishop, 1979; Iroegbu & Pringle, 1981; Pringle & Iroegbu, 1982). In agreement with these results, we also observed a high frequency of 'homologous' recombination between Uukuniemi virus ts mutants.

Although the mutagen, nitrosoguanidine, was shown to inactivate the Uukuniemi wt virus by 92% and 98% under the conditions used (Fig. 2) a surprisingly low frequency (0.5 to 1%) of ts mutants was obtained. To avoid the generation of a high proportion of double mutants we did not want to use a harsher treatment. Bishop & Shope (1979) and Iroegbu & Pringle (1981) observed a frequency of spontaneous ts mutants of 2.3% (snowshoe hare virus) and 2.7%
Uukuniemi virus ts mutants

(Maguari virus), respectively. Thus, the low frequency of ts mutants obtained for Uukuniemi virus could indicate that most of the mutants may have been spontaneous ts mutants. On the other hand, the three consecutive purification steps at 39 °C, 32 °C and 39 °C should have efficiently eliminated the background of spontaneously arising ts mutants, which were possibly present in the original plaque-purified wt stock virus. It is unlikely that our procedure, which was based on plaque formation at 39 °C and 32 °C and not on cytopathic effect, would have failed to detect some fraction of the ts mutants.

The genome of bunyaviruses consists of three unique RNA segments coding for four structural proteins and one or two non-structural proteins. Accordingly, three recombination (reassortment) and five or six complementation groups should exist. Despite the fact that more than 100 ts mutants within the CE serogroup have been isolated, only two recombination–complementation groups, representing mutations in the M (group I) and the L (group II) segments, have been identified. The third recombination group has remained elusive. A third recombination group representing a mutation in the S segment has not been identified (Bishop, 1979). This may be due to the fact that this RNA segment apparently contains two overlapping genes translated in two different reading frames (Fuller et al., 1983). Iroegbu & Pringle (1981) have also identified two main recombination groups within Bunyamwera serogroup viruses. However, they tentatively assigned the mutations to the S (group I) and the M (group II) RNA segments. The discrepancy between the Bunyamwera and CE serogroup assignments has remained unsolved. More recently, Pringle & Iroegbu (1982) have been able to isolate a ts mutant of Maguari virus, MAGts23, representing the third expected recombination group. This ts mutant with a host-restricted phenotype is the only example of a third recombination group reported so far.

The group I and group II ts mutants of Uukuniemi virus reported here were able to synthesize all RNA species and structural proteins as well as to form the three RNPs at the non-permissive temperature. This suggests that they could carry out primary and secondary transcription as well as genome replication.

At present it is not possible with certainty to assign the two recombination groups to any one of the L, M or S RNA segments. The present results suggest that the virus glycoproteins in ts6- and ts11-infected cells are arrested in the ER, suggesting a defect in their transport from the ER to the Golgi complex. This could mean that the ts defect might reside in either of the two glycoproteins. The co-staining of the capsid protein and the glycoproteins in the ER in ts6- and ts11-infected cells suggests that processing of the primary N-linked glycans and terminal glycosylation may not be necessary for the glycoprotein–nucleocapsid interaction. The unglycosylated glycoproteins synthesized in tunicamycin-treated cells infected with Uukuniemi wt virus also accumulate in the ER (Kuismanen et al., 1984). Under these conditions, the nucleoproteins likewise associate with the glycoproteins in the ER (Kuismanen et al., 1984).

The preliminary results presented here suggest that the glycoproteins in cells infected with ts7 and ts12 (group I) are transported apparently normally from the ER to the Golgi complex and also to some extent to the plasma membrane (N. Gahnberg, unpublished results), whereas the nucleocapsids failed to associate with the glycoproteins in the Golgi apparatus, an event that normally occurs in wt virus-infected cells. These results indicate that the interaction between the nucleocapsids and the glycoproteins is defective. The reason for the difference in the distribution of N protein in ts7-infected (aggregates) and ts12-infected cells (even distribution) is not known at present.

A typical feature of the bunyaviruses is that they mature in the Golgi complex (Murphy et al., 1973; Bishop & Shope, 1979). We have recently shown that the Uukuniemi virus glycoproteins G1 and G2 as well as the nucleocapsid, accumulate in the Golgi region (Kuismanen et al., 1982). A concentration of the glycoproteins in, and a progressive vacuolization of, the Golgi complex appears to be a prerequisite for the association of the nucleoproteins with the Golgi complex (Kuismanen et al., 1984). The reasons for the morphological changes of the Golgi complex and the accumulation of the glycoproteins in this area are so far unknown. It is possible that these events are due to an inefficient transport of the glycoproteins from the complex to the plasma membrane. To confirm this hypothesis it would be important to study the kinetics of transport of the glycoproteins from their site of synthesis in the ER to the plasma membrane in the absence
of virus maturation. In this respect ts12 appears to be a promising tool. Detailed biochemical and immuno-electron microscopic analyses are, however, necessary before the usefulness of this and other similar ts mutants can be evaluated for studies on the various aspects of virus maturation.

I wish to thank Tuula Rusi and Annikki Kallio for their skilled technical assistance, Kirsti Tuominen for secretarial help, and Drs Ralf Pettersson, Sirkka Keränen, Leevi Kääriäinen and Esa Kuusmanen for valuable advice. This study was supported by the Sigrid Juselius Foundation.

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(Received 24 January 1984)