Proteins and Glycoproteins Specified by Bunyamwera Virus and by Belmont Virus, a Possible Bunyavirus, in Mammalian Cells

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SUMMARY

Purified preparations of Belmont virus were shown to be very similar morphologically and biochemically to those of Bunyamwera virus. Comparisons of the synthesis of virus-specified proteins in Vero and BHK-21 cells confirm the close taxonomic relationship. Total protein synthesis was inhibited 95% by 23 h post-infection with Belmont virus; a similar reduction occurred earlier in Bunyamwera virus-infected cells. This inhibition was multiplicity dependent, and synthesis of the host protein component was inhibited more severely. When cells were labelled late during infection at an m.o.i. of 1, the four structural proteins were readily resolved by gel electrophoresis. A small possibly non-structural protein (p14 for Belmont, p13 for Bunyamwera) was also identified late in infection in both hosts. As in the virion, the small envelope protein (P28) of Belmont virus was not glycosylated, whereas the large envelope protein G107, and the corresponding G1 and G2 of Bunyamwera virus, were labelled intracellularly in mannose, galactose and glucosamine. The kinetics of synthesis of the proteins for both viruses were similar, the events occurring earlier in Bunyamwera virus-infected cells. The nucleoprotein N was the most prominent at 3 to 5 h post-infection and remained so; G1 or the large envelope protein was also prominent early, but later more label was apparently incorporated into G2 or the small envelope protein. Pulse–chase experiments provided no evidence of precursor proteins. The relationship of the four or five identified virus-specified translation products to the three bunyavirus messenger RNAs remains obscure.

INTRODUCTION

Belmont virus was originally isolated from the mosquito Culex annulirostris in Queensland in 1968 (Doherty et al., 1972) and more recently from Culex species collected in the Northern Territory in 1978 (H. A. Standfast, personal communication). The virus is serologically unrelated to all known arboviruses and the preferred vertebrate hosts are marsupials unique to Australia (Doherty et al., 1972). Results presented in the accompanying paper (McPhee & Westaway, 1981) show that the size of the virion proteins and RNA species of Belmont virus are very similar to those of Bunyamwera virus, the type species of the bunyavirus genus within the Bunyaviridae family. However, Belmont virus particles are larger than those of Bunyamwera and the small envelope protein of Belmont virus is

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apparently not glycosylated. Although Belmont virus appears to be a *Bunyaviridae* species, its generic status needs further consideration (McPhee & Westaway, 1981).

The virions of Belmont and Bunyamwera each comprise four proteins (total mol. wt. about $300 \times 10^3$) but only three species of RNA (total mol. wt. $5.9 \times 10^6$ and $5.0 \times 10^6$ respectively). Three species of messenger RNA (mRNA) complementary to the L, M and S species of the virion RNA have been identified in cells infected with Lumbo and snowshoe hare bunyaviruses (Bouloy & Hannoun, 1976; Cash *et al.*, 1979). The S mRNA species identified for snowshoe hare virus was $3.8 \times 10^5$ mol. wt. compared to $4.5 \times 10^5$ for the complementary virion RNA species and no evidence was obtained for more than one species of M mRNA (Cash *et al.*, 1979). Gentsch & Bishop (1978, 1979) showed by genetic reassortment analyses of the bunyaviruses that the S and M genomic RNA species code for the nucleoprotein and the two envelope proteins respectively; from these results it seems likely that the L RNA species codes for the L protein. *In vitro* translation studies with the S mRNA species have confirmed its coding function for the nucleoprotein (Cash *et al.*, 1979). The strategy required to produce the two envelope proteins coded for by the one piece of M RNA is unknown; there is obviously an excess of coding information in the M and the L RNA species. To further characterize Belmont virus as a possible bunyavirus, this paper presents evidence of similar translational control of the proteins and glycoproteins specified by Belmont virus and by Bunyamwera virus in Vero cells and BHK-21 cells.

**METHODS**

*Virus and cell culture.* The sources of Belmont virus (R 8659) and of Bunyamwera virus, and of the Vero and BHK-21 cells, are described in the accompanying paper (McPhee & Westaway, 1981).

*Labelling of infected cells.* The techniques for labelling infected cell monolayers with amino acid (methionine) or carbohydrate labels have essentially been described previously (Westaway, 1973, 1975; Boulton & Westaway, 1976). Confluent cell monolayers in 60 mm plastic Petri dishes were inoculated with approx. 1 p.f.u./cell of cloned virus stocks. After adsorption for 1 h at 37 °C, the inoculum was removed and replaced with Eagle's minimal essential medium (MEM) supplemented with 0.2 % bovine serum albumin (BSA). Two to 4 h before starting labelling, the medium was changed to a labelling medium which was equivalent to maintenance medium containing 3 μg/ml actinomycin D (Act. D) but which was deficient in methionine (1 % of the normal concentration) when using $^{35}$S-methionine label, and totally deficient in glucose when using $^3$H-mannose or $^3$H-glucosamine label. The Act. D was a gift from Merck, Sharp and Dohme (Sydney, Australia). Following treatment of the cells with Act. D, the appropriate label was added in the same medium for a 2 to 4 h period; the radiochemicals (The Radiochemical Centre, Amersham) were $^3$H-mannose-2-H3 (2 Ci/mmol), $^3$H-glucosamine (13.4 to 19 Ci/mmol) and L-$^{35}$S-methionine (225 to 790 Ci/mmol). The cells were harvested in 0.3 to 0.5 ml 2 % SDS and then stored at −20 °C. Pulse–chase experiments were performed in a room maintained at 37 °C; cells were pretreated as above with methionine-deficient medium, and $^{35}$S-methionine (20 μCi/ml) was then added in the same medium for 5 min. The label was then removed, the cells washed immediately and chased for specific periods with normal maintenance medium containing a 400-fold excess of unlabelled methionine.

*Polyacrylamide gel electrophoresis.* Labelled cytoplasmic proteins were separated by electrophoresis in polyacrylamide slab gels, using 8 % gels with a SDS–phosphate buffer system (Westaway & Shew, 1977) or 10 % gels with a discontinuous buffer system (Laemmli, 1970) as described in the accompanying paper (McPhee & Westaway, 1981).
RESULTS

Virus-specified proteins in infected cells

Cells were labelled during successive periods post-infection to further compare Belmont with Bunyamwera virus, and also to search for non-structural proteins which might account for the apparent excess of protein-coding information (see Introduction). Belmont virus grows readily in infected Vero cells treated with Act. D at early times post-infection (3 μg/ml, 2 to 6 h post-infection). To follow the kinetics of total protein synthesis, confluent Vero cell monolayers were infected with Belmont virus at m.o.i.s of 0-1, 1 and 10. Cells were pretreated with Act. D and labelled for specific periods with 35S-methionine; the monolayers were then processed for precipitation, with 10% cold trichloroacetic acid (TCA), in situ of labelled proteins. The results show a variable decrease in the total protein synthesis with different m.o.i. (Fig. 1 a). At an m.o.i. of 10 the decrease in total protein synthesis with increasing time was not as great as at m.o.i.s of 0-1 and 1.

It was of interest to investigate whether these variations with m.o.i. were due to changes in the proportion of virus protein synthesis relative to host cell protein synthesis. As host cell protein synthesis is switched off most effectively late during infection (see later) this time period was investigated at the different m.o.i. The results indicate (Fig. 1 b) that switch-off of
Fig. 2. Kinetics of protein synthesis in Belmont and Bunyamwera virus-infected Vero cells. Infected and mock-infected (U) cells were labelled with $^{35}$S-methionine (5 $\mu$Ci/ml) at 2 to 4 (lane F), 4 to 6 (lanes A, B and G), 10 to 12 (lanes C and H), 16 to 18 (lanes D and J) and 22 to 24 (lanes E and J) h post-infection; the hour shown over each lane is the midpoint of the labelling period. In this and all subsequent figures, cells were infected with cloned virus stock at an m.o.i. of 1. After labelling, the cells were disrupted in 2% SDS and the treated samples were electrophoresed in an 8% SDS–phosphate slab gel. Sample volumes were adjusted to yield equivalent counts in each well (A to E sample sizes are 0.5, 0.6, 1.3, 6 and 14 $\mu$l; F to I are 0.7, 0.7, 3 and 8 $\mu$l; and J is 0.9 $\mu$l). In this and subsequent figures Belmont (•) and Bunyamwera (<) virus-specified proteins are designated by their mol. wt. $\times 10^{-3}$ using the prefix P, G or p for non-glycosylated proteins, glycosylated proteins and non-structural proteins respectively. The labelled bands of p13 and p14 are diffuse and are seen more clearly in Fig. 3.

host protein synthesis relative to virus-specified protein synthesis is most effective at an m.o.i. of 1. Identification of virus-specified proteins is explained later. With an m.o.i. of 0–1 one might expect a relatively higher background of host protein synthesis as not all cells would be infected during the first cycle, but with an m.o.i. of 10 such a result is not expected. The high host cell background at an m.o.i. of 10 could be explained by autointerference; this has been found to occur for several members of the Bunyaviridae (David-West & Porterfield, 1974; Porterfield et al., 1975/6).

The latent period of Belmont virus in Vero cells is about 10 h, about 4 h longer than that of Bunyamwera virus (McPhee & Westaway, 1981). For comparative purposes, proteins in Belmont and Bunyamwera virus-infected Vero cells were labelled with $^{35}$S-methionine during the latent period and at later periods (until extensive c.p.e. was observed) using an m.o.i. of 1 (Fig. 2). At least four virus-specified proteins were observed for each virus. The mol. wt. for the Belmont virus proteins were $147 \times 10^{3}$ (P147), $107 \times 10^{3}$ (G107), $28 \times 10^{3}$ (P28) and $25 \times 10^{3}$ (P25); for Bunyamwera virus the values were $145 \times 10^{3}$ (P145), $104 \times 10^{3}$ (G104), $32 \times 10^{3}$ (G32), and $22 \times 10^{3}$ (P22). The mol. wt. of the presumptive structural proteins in the
cytoplasm were identical with those of the corresponding four virion proteins for each virus determined under the same conditions (i.e. electrophoresis of virus-specified proteins with seven standard proteins in the same slab gel) as in the accompanying paper (McPhee & Westaway, 1981). We also showed previously that for Bunyamwera virus, P145 represents the L protein, G104 the large envelope protein G1, G32 the small envelope protein G2 and that P22 represents the nucleoprotein N in the current nomenclature for bunyavirus proteins (Obijeski & Murphy, 1977). The Belmont virus proteins corresponding to L, G1, G2 and N are P147, G107, P28 and P25 respectively (McPhee & Westaway, 1981).

By 17 h post-infection in Vero cells infected with Bunyamwera virus, host protein synthesis was almost completely switched off (total protein synthesis was reduced to 10%) and similarly by 23 h in Belmont virus-infected Vero cells (total protein synthesis was reduced to 4%) (Fig. 2). The four virion proteins were thus easily identified and in addition there was a small amount of a low mol. wt. possibly virus-specified protein (p14 for Belmont and p13 for Bunyamwera; see also Fig. 3 and 4) not detected in purified virus preparations (McPhee &
The kinetics of protein synthesis are similar for both viruses, the events occurring earlier in Bunyamwera virus-infected cells. The nucleoprotein P22 and the large envelope protein G104 of Bunyamwera virus were prominent early in infection (3 h post-infection), whereas the corresponding Belmont proteins P28 and G107 were not prominent until several hours later. The envelope proteins for both viruses (G107 and P28 for Belmont, and G104 and G32 for Bunyamwera) were detected in increasing amounts with time. Synthesis of the very large minor protein (P147 for Belmont, and P145 for Bunyamwera) was detected with difficulty (see also Fig. 5), possibly because its presence was masked early in infection by a high background of host cell protein in this region; its synthesis finally decreased relative to other virus-specified proteins very late during infection (23 h and 17 h for Belmont and Bunyamwera respectively). Late in infection, a small amount of a protein of mol. wt. about 8000 was also detected in Bunyamwera virus-infected Vero cells, after long exposures of autoradiograms (not shown).

Like Belmont virus, Bunyamwera virus grows to relatively high titres in BHK-21/13 cells; the latent periods are similar, 6 to 7 h for both viruses. It was of interest to see if all the virus-specified proteins found in Vero cells were synthesized in BHK-21/13 cells. Accordingly, the proteins labelled late during infection in both cell lines were compared by electrophoresis (Fig. 3). Clearly, switch-off of host cell protein synthesis also occurred late during infection in Belmont and Bunyamwera virus-infected BHK-21/13 cells, although for Belmont virus it was not as great as that observed in Vero cells. The profiles show that the virus-specified proteins common to both sets of infected cells are the four virion proteins and the small possibly non-structural protein (i.e. P147, G107, P28, P25 and p14 for Belmont, and P145, G104, G32, P22 and p13 for Bunyamwera). The protein of mol. wt. 8000 observed in Bunyamwera virus-infected Vero cells was not found in infected BHK-21/13 cells, and hence it is probably not virus specified.

Analyses of densitometer traces of the gel profiles of methionine-labelled Vero cells infected with Bunyamwera virus showed that the ratio of the areas under the peaks G1 : G2 decreased progressively as follows: 1.5 at 4 to 6 h, 1.2 at 10 to 12 h, 0.8 at 16 to 18 h (data from Fig. 2), and subsequently was stable at 0.4 to 0.5 during the periods 20 to 24 h (from Fig. 3, and two unpublished experiments) and 23 to 27 h (from Fig. 4). Similar comparisons were not possible for Belmont virus infections because of the poor resolution of P28 from P25. In contrast, the ratio of methionine incorporation for G1 : G2 of virions harvested later in infection was about 1 (mean of the values 0.9, 1.0, 1.0 and 1.2 in unpublished experiments).

Comparison of virus-specified glycoproteins in infected cells

A unique property of Belmont virus, when compared to Bunyamwera virus, is that the small envelope protein (P28) is not glycosylated in virions grown in the presence of labelled glucosamine (McPhee & Westaway, 1981). Infected cells were therefore labelled with radioactive carbohydrates to compare intracellular glycosylation of the envelope proteins of both viruses.

Belmont and Bunyamwera virus-infected Vero cells were labelled late in infection with $^3$H-2-mannose or $^3$H-6-glucosamine and the products electrophoresed in parallel with samples of $^3$S-methionine-labelled infected cells (Fig. 4). Analysis in an 8 % SDS–phosphate slab gel revealed that the Bunyamwera envelope glycoproteins G104 and G32 incorporated labelled mannose and glucosamine in infected cells, but relatively less glucosamine was incorporated into G32 (lane E, Fig. 4). In contrast, only the large envelope protein of Belmont virus (G107) incorporated both glucosamine and mannose in infected cells (lanes C and I respectively, Fig. 4); as in the virion, carbohydrate label is not incorporated into P28, the small envelope protein. The same results were obtained in infected cells labelled with $^3$H-galactose (results not shown). The large glycoproteins (G107 for Belmont and G104 for Bunyamwera), when
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Fig. 4. Comparison by electrophoresis of proteins labelled with $^{35}$S-methionine (met) (2 $\mu$Ci/ml), $^3$H-mannose (man) (10 $\mu$Ci/ml) or $^3$H-glucosamine (glu) (10 $\mu$Ci/ml) in Belmont (BEL) and Bunyamwera (BUN) virus-infected Vero cells, and of similarly labelled proteins of mock-infected Vero cells (U). Belmont virus-infected cells and mock-infected cells were labelled 32 to 36 h post-infection and Bunyamwera virus-infected cells were labelled 23 to 27 h post-infection. Samples were electrophoresed in an 8% SDS-phosphate slab gel. The arrows indicate additional carbohydrate-labelled products which may be virus induced. $\blacktriangleright$, Belmont virus-specified labelled products; $\triangleleft$, Bunyamwera virus-specified labelled products.

labelled with either of the carbohydrates, appeared to migrate slightly but perceptibly faster than the amino acid-labelled counterparts. This is apparently due to the relatively larger amounts of protein being loaded on to the gel (approx. 100 $\mu$l of cell extract for $^3$H-mannose- and $^3$H-glucosamine-labelled cells compared with 5 to 10 $\mu$l for $^{35}$S-methionine-labelled cells).

There were several additional products which incorporated labelled glucosamine or mannose in Vero cells infected with both viruses (indicated by arrows in Fig. 4). These bands do not apparently correspond, by electrophoretic migration, to any carbohydrate-labelled products in mock-infected cells, nor to any in amino acid-labelled infected cells. The possibility that they are virus specified cannot be excluded in the absence of data from immunoprecipitation experiments or from glycopeptide maps obtained after limited proteolytic digestion; if they are, for example, glycosylated precursors of envelope protein(s), they must be remarkably stable during the long labelling period of 4 h, yet are not detectable by methionine label. Alternatively they may be host cell glycoproteins whose synthesis is induced in infected cells. Furthermore, there is a considerable amount of small mol. wt. material labelled in carbohydrate and migrating near the dye front, more so than in mock-infected cells (Fig. 4). Most of this material disappeared on dialysis of the sample
before electrophoresis (not shown), suggesting that the material comprised small glycopeptides or glycolipid associated with increased membrane synthesis in infected cells.

**Pulse labelling of virus-infected cells**

The results above show that Belmont virus-specified protein synthesis is similar to that of Bunyamwera virus, with four, possibly five, virus-specified proteins being recognized for both viruses in infected Vero or BHK-21/13 cells, well within the coding capacity of each virus. Pulse–chase experiments were performed in infected Vero cells to search for evidence of post-translational cleavage and precursor–product relationships. The times post-infection chosen for Bunyamwera and Belmont viruses were soon after the latent periods (8 and 12 h respectively) and also 8 h later, before development of extensive c.p.e. (16 and 20 h respectively). The cells were pulse labelled for 5 min and then chased for periods of 1 to 60 min. After treatment the samples were electrophoresed in 10% SDS discontinuous Laemmli slab gels (Fig. 5). In this gel system the Belmont virus proteins P28 and P25 were more clearly resolved than in 8% SDS–phosphate gels, but the possibly non-structural proteins p14 (Belmont) and p13 (Bunyamwera), were not resolved at the dye front.

In Belmont virus-infected cells (Fig. 5a), the nucleoprotein P25 and the larger envelope protein G107 were prominently labelled after only a 1 min chase. P147 was barely detectable in the 12 h samples only. Densitometer traces showed that both envelope proteins incorporated additional label relative to P25 when the chases were extended beyond 1 min, the greater increase being in G107. Presumably these increases occurred because of slower rates of elongation and/or completion of any post-translational processes, relative to synthesis of P25. No putative precursors nor post-translational cleavages were detected. Analyses of these samples in an 8% SDS discontinuous slab gel yielded the same results (not shown). Samples were also electrophoresed in 8% SDS–phosphate slab gels to detect the possible non-structural protein p14; this protein was prominent after a 1 min chase and did not appear to increase or decrease with increasing chase periods.

In Bunyamwera virus-infected cells, the results (Fig. 5b) were similar to those obtained for Belmont virus. G104 and G32 were labelled after only a 1 min chase. As with Belmont virus, densitometer traces showed that additional label was incorporated in the envelope proteins (mainly into G104) when the chase period was extended. P22 was labelled rapidly without any apparent delay, and P145 was prominent only at 8 h post-infection. Because of the decrease noted earlier in the ratio of Bunyamwera G1:G2 in intracellular pools late in infection, similar comparisons were made after extended chase periods. Thus, in cells labelled with $^{35}$S-methionine for 5 min at 18 h post-infection, the G1:G2 ratio decreased from 1.3 for a 1 h chase to 0.9 for a 4 h chase, but remained constant at 1.7 after corresponding pulse and chase periods from 9 h post-infection (results not shown).

**DISCUSSION**

These results remove any possible doubt regarding the classification of Belmont virus as a member of the *Bunyaviridae* (McPhee & Westaway, 1981). Apart from the slower sequence, the kinetics of synthesis of Belmont virus-specified proteins are completely in accord with those of Bunyamwera. In a similar study of Bunyamwera virus-infected BSC-1 cells using an m.o.i. of 40, Pennington *et al.* (1977) detected synthesis of the large (L) protein and the nucleoprotein N at 2 to 3 h post-infection and of the envelope proteins G1 and G2 at 4 to 5 h. L and G2 were not always detected nor resolved from host proteins in gel electrophorograms and were never prominent; synthesis of all proteins declined markedly after 8 h. Similar kinetics of synthesis of Bunyamwera virus proteins were observed in our experiments, except that G1 was detected earlier and G2 was easily identified (Fig. 2). The advantages of resolution of proteins in slab gels is clear from comparisons with recent results of Lazdins &
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Fig. 5. Electrophoresis of proteins pulse labelled with $^{35}$S-methionine (20 μCi/ml) in (a) Belmont and (b) Bunyamwera virus-infected Vero cells. Virus-infected cells were pulsed for 5 min at 12 and 20 h post-infection for Belmont and at 8 and 16 h post-infection for Bunyamwera, then chased for 1, 5, 30 and 60 min (shown above each lane) in medium containing an excess of unlabelled methionine. All sample sizes loaded on to the 10% SDS discontinuous Laemmli gel were identical (20 μL). Mock-infected samples (U) and samples of Belmont (BEL) and Bunyamwera (BUN) virus-infected Vero cells labelled from 24 to 28, and from 16 to 20 h post-infection respectively were included for comparisons.

Holmes (1979) who detected the same sequence of synthesis of Bunyamwera virus proteins in Vero cells but at much later periods (N and G1 at 10 to 12 h, followed by G2 at 16 to 18 h) using sliced cylindrical gels.

In Vero cells infected at an m.o.i. of 1 with either Belmont or Bunyamwera virus, there was a marked reduction in host cell protein synthesis after 11 h post-infection (Fig. 2); this decline was relatively greater than that of virus-specified protein synthesis which also decreased late
in infection (compare the sample sizes in Fig. 2). Lazdins & Holmes (1979) also noted that total protein synthesis was dramatically reduced early post-infection by Bunyamwera virus at an m.o.i. of 1, but in contrast to our results with Belmont virus (Fig. 1), an even greater reduction at a higher multiplicity. Surprisingly, no switch-off of total protein synthesis relative to the control occurred in cells infected with Uukuniemi virus, another *Bunyaviridae* member, at a very high m.o.i. of 30 to 200 (Pettersson, 1974).

Electrophoresis in two gel systems of material from two host cells did not reveal any Belmont or Bunyamwera virus-specified proteins additional to the four structural proteins, except for the small possibly non-structural protein, p14 and p13 respectively. The latter is the first possible non-structural protein reported for the bunyaviruses apart from one of similar mol. wt. noted in a recent abstract on Bunyamwera virus-specified proteins (Pickup *et al.*, 1978). It was present in two host cell species with both viruses late in infection when host cell protein synthesis was switched off. The relationship of the four or five protein products to the three presumptive messenger RNAs (Bouloy & Hannoun, 1976) complementary to the three pieces of genomic RNA is intriguing. Obijeski & Murphy (1977) reported preliminary pulse–chase experiments which indicate that G1 and G2 of La Crosse virus may be formed by post-translational cleavage but these results await confirmation. Pennington *et al.* (1977) found no evidence of high mol. wt. protein precursors in Bunyamwera virus-infected cells in labelling experiments using amino acid analogues, pulses as short as 4 min, or a 1 h pulse followed by a 3 h chase. While no precursor proteins were detected in our experiments a delay appeared to occur in the accumulation of label for both envelope proteins following a 5 min pulse (Fig. 5). The simplest interpretation is that nascent polypeptides were being completed during the early chase periods. Relatively more of the intracellular pool of G1 may be incorporated into virions (as the yield increases) than the pool of G2, indicated by the greater G1:G2 ratio of methionine label in virions (about 1) compared to the ratio in cells late in infection (0.4 to 0.5). The low G1:G2 ratio in cells late in infection may be characteristic of the Bunyavirus genus, because we found that in infections with Thimiri virus (a member of the Simbu group), which achieves switch-off of host protein synthesis, the G1:G2 ratio of methionine incorporation is 1.2 at 22 to 26 h and 0.42 at 46 to 50 h (D. A. McPhee & E. G. Westaway, unpublished results).

In summary, the syntheses of Belmont virus-specified proteins are remarkably similar to those of Bunyamwera virus, confirming our proposal that Belmont virus is a new member of the *Bunyaviridae* (McPhee & Westaway, 1981). However, the small envelope protein P28 retains its unique character in that it could not be labelled in glucosamine or in mannose, under conditions in which G2 of Bunyamwera virus was clearly labelled. A possible non-structural protein, in addition to the four structural proteins, was identified in cells infected with either virus. No evidence of precursor protein(s) was obtained. The S species of RNA (mol. wt. 3 × 10^3; McPhee & Westaway, 1981) codes appropriately on a size basis for the N protein of mol. wt. 22 × 10^3 to 25 × 10^3, as determined by genetic reassortment experiments (Gentsch & Bishop, 1978); similarly, the M species of RNA (mol. wt. 2 × 10^6; McPhee & Westaway, 1981) codes for the two envelope proteins (total mol. wt. about 150 × 10^3) (Gentsch & Bishop, 1979); the L species of RNA (mol. wt. approx. 3 × 10^6; McPhee & Westaway, 1981) must therefore code for the remaining (largest) structural protein (mol. wt. approx. 150 × 10^3). If p13 or p14 is virus specified, adequate coding information is still available in the L or M RNA. Synthesis of the L and N proteins appears to occur independently of others. The two envelope proteins could arise from rapid post-translational cleavage of nascent polypeptides during translation from a polycistronic messenger (complementary to M RNA). However, their onset of synthesis does not appear to be synchronous and, hence, unsuspected complexity may exist in the translation of these two proteins. For example, the possibility should be considered of internal initiation of translation
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on a polycistronic messenger, as reported for flaviviruses (Westaway, 1977; Svitkin et al., 1978), or of overlapping genes within the same RNA segment, as reported for influenza virus (Inglis et al., 1980).

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REFERENCES


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