1. SUMMARY

Bunyaviruses are enveloped arthropod-borne viruses containing a tripartite single-stranded RNA genome of negative polarity. We have studied the strategy of gene expression of one member of this large family, namely Uukuniemi virus, the prototype of the genus Uukuvirus. Three mRNA species, which do not bind to oligo(dT)-cellulose, are found associated with polysomes of infected cells. Sucrose gradient and gel analyses, in vitro translation and molecular cloning have shown that: (i) The M virion RNA (vRNA) segment is transcribed into an mRNA of roughly the same size (Mr 1.1x10^6) and encodes a precursor (p110) to the two glycoproteins G1 and G2. (ii) Sequence analysis of the 3' end of the M vRNA, which is complementary to the 5' end of the M mRNA, indicates an open reading frame starting from an AUG codon 18 nucleotides from the end of the mRNA. (iii) The S vRNA segment (Mr 0.5x10^6) is transcribed into a full-length plus-strand RNA (antigenome) and two small mRNA species (Mr about 0.3x10^6) that encode a nonstructural (NS2) protein (Mr 30,000) and the nucleocapsid (N) protein (Mr 25,000). Whether the two mRNA species contain overlapping nucleotide sequences is not known. By elimination, the L mRNA, transcribed from the L vRNA segment (Mr 2.4x10^6), codes for the L protein (Mr 200,000) the putative RNA polymerase. The results obtained with Uukuniemi virus and other bunyaviruses indicate that the strategy of gene expression of these viruses in some aspects is different from that of other known RNA viruses.

2. INTRODUCTION

The Bunyaviridae family of arboviruses comprises more than 200 different viruses grouped into four genera, called Bunyavirus, Nairovirus, Phlebovirus and Uukuvirus (1). In addition, a large number of possible members are still unclassified. The bunyaviruses have been grouped together because of similar structural properties and mode of maturation (1, 2, 3). All characterized members have a lipoprotein envelope containing two glycoproteins, G1 and G2, and an internal, probably helical nucleoprotein.
consisting of three single-stranded RNA segments, designated L, M and S, of negative polarity to which multiple copies of the N protein and a few copies of the L protein are associated. The size of the structural proteins and the RNA segments varies between viruses of different genera (2, 3). Both the nucleo-proteins (4, 5, 6) and the protein-free RNA segments (7, 8) have a circular structure due to base-pairing of short inverted complementary sequences at the 3' and 5' ends of the RNA segments (7, 9, 10, 11). In viruses of the same genus, about 10-13 nucleotides at the ends of each RNA segment are conserved (12), suggesting some important role of the ends in replication.

As a model, we have studied one bunyavirus, Uukuniemi virus, the prototype of the Uukuvirus genus (1). This virus has two envelope glycoproteins, G1 (Mr 70,000) and G2 (Mr 65,000) (13, 14). The oligosaccharide sequences of the glycans attached to the proteins have been determined (14). The N protein (Mr 25,000) (13, 15) is associated with the RNA segments forming the nucleoproteins (4). The RNA-associated L protein (Mr about 200,000) may represent the RNA-dependent RNA polymerase detected in virions (16, 17). The three circular RNA segments (7) have Mr's of about 2.4x10^6 (L), 1.1x10^6 (M) and 0.5x10^6 (S) (ref. 18) and have a negative polarity (16, 18). In addition to the above mentioned structural proteins, a nonstructural protein (NS) (Mr 30,000) is found in infected cells (17).

A unique feature of the bunyavirus morphogenesis is that the virus particles are formed intracellularly by a budding process at smooth-surface vesicles in the Golgi area (2, 19, 20). Using Uukuniemi virus as a model we have shown by immunofluorescence and immunoelectron microscopy techniques that the site of maturation in fact is the Golgi complex (20). Virus particles are thought to be expelled from the cells in large vesicles, which fuse with the plasma membrane.

Here we summarize our results on the characterization of the mRNAs found in Uukuniemi virus-specific cells and present a model for the general strategy of gene expression of this virus. A part of these results has been published previously (17).