The Proteins and RNAs Specified by Clo Mor Virus, a Scottish Nairovirus

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SUMMARY

The proteins and RNAs of Clo Mor virus have been analysed. Virus-specific proteins in infected cells had previously been identified by isotopic labelling and radioimmunoprecipitation; these were three glycoproteins (mol. wt. 115K, 90K and 80K) and an unglycosylated nucleocapsid (N) protein (50K). We have performed pulse–chase experiments which indicated that the 115K protein is processed to give the 90K and 80K proteins, while a 45K protein was detected in released virions after prolonged chase. Translation in vitro of mRNA extracted from Clo Mor virus-infected cells resolved only the N protein. Three species of RNA were extracted from Clo Mor virus intracellular nucleocapsids and have been designated L (11000 to 13000 bases), M (6300 bases) and S (1900 bases). The processing of viral proteins and the sizes of RNAs are characteristic of the Nairovirus genus of the family Bunyaviridae.

The family Bunyaviridae contains more than 200 arthropod-borne viruses classified into four genera: Bunyavirus, Nairovirus, Phlebovirus and Uukuvirus (Bishop et al., 1980). Although viruses within the family share the common feature of a negative-strand RNA genome comprising three segments (designated L, M and S), there are differences in the sizes of the virion proteins and RNA segments which are characteristic for each of the four recognized genera (Bishop et al., 1980). With regard to the Nairovirus genus the L RNA segment is up to 60% larger than the L RNA segments of bunyaviruses, phleboviruses or uukuviruses, and the nairovirus nucleocapsid (N) protein is about twice the size of the N proteins of the other three genera (Clerx et al., 1981). The Nairovirus genus is divided into six serogroups (Crimean–Congo haemorrhagic fever, Nairobi sheep disease, Qalyub, Sakhalin, Dera Ghazi Khan and Hughes) on the basis of haemagglutination inhibition and neutralization tests (Casals & Tignor, 1980). Comparative analyses enumerating viral polypeptides and estimating the sizes of virion RNA species for representatives of these serogroups have been reported by Clerx et al. (1981), but detailed biochemical studies on the replication of nairoviruses are limited. Clerx & Bishop (1981) described the induction and processing of the proteins specified by Qalyub virus (Qalyub serogroup) and Cash (1985) reported on the polypeptides synthesized by Dugbe virus (Nairobi sheep disease serogroup).

Clo Mor virus was isolated from a pool of Ixodes uriae ticks collected at Clo Mor, Cape Wrath, Scotland, U.K. (Main et al., 1976). The virus was identified as a member of the Sakhalin serogroup by complement fixation tests (Main et al., 1976). Subsequently, we confirmed the assignment of Clo Mor virus to the Nairovirus genus by analysis of the viral proteins (Watret et al., 1985): in infected cells the N protein (mol. wt. 50K) and three glycoproteins (mol. wt. 115K, 90K and 80K) were identified, while in released virions the N protein, the 90K and 80K glycoproteins and an additional 45K glycoprotein were observed. In this communication we report on the processing of viral proteins and the translation in vitro of the N protein, and describe the RNAs extracted from viral nucleocapsids.

The maintenance of BSC-1 cells and the origin and preparation of the Clo Mor virus stock are described in Watret et al. (1985). The techniques of radiolabelling infected cells, immunoprecipitation, translation in vitro and gel electrophoresis of proteins and RNAs have been detailed previously (Watret & Elliott, 1985; Watret et al., 1985). Immune ascitic fluid was
Fig. 1. Pulse-chase experiments to examine the processing of Clo Mor virus proteins. Cells were pulse-labelled with [35S]methionine and ‘chased’ with medium containing excess unlabelled methionine. Lane 1, uninfected BSC-1 cells; lanes 2 to 5, cell lysates of Clo Mor virus-infected cells; lanes 6 to 9, immunoprecipitates of lanes 2 to 5 respectively; lanes 10 to 12, viral proteins in the pelleted supernatant fluids of the cells in lanes 3 to 5. Lanes 2 and 6, ‘pulse’ sample; lanes 3, 7 and 10, chase for 2 h; lanes 4, 8, and 11, chase for 4 h; lanes 5, 9, and 12, chase for 8 h. The samples were run on the same 15% polyacrylamide gel, but lanes 1 to 5 were exposed for 3 days and lanes 6 to 12 were exposed for 2 weeks. Immunoprecipitation and PAGE were performed as described by Watret et al. (1985).

Prepared by injecting BALB/c mice intraperitoneally with a lysate of Clo Mor virus-infected 3T12 cells in Freund’s complete adjuvant weekly for 3 weeks; ascites were induced by injection of mouse myeloma cells at week 3, and ascitic fluid was harvested at regular intervals over the following 14 days.

The polypeptides synthesized in Clo Mor virus-infected BSC-1 cells and the polypeptides found in virus particles released from infected BSC-1 cells were identified by immunoprecipitation of [35S]methionine- and [3H]mannose-labelled preparations (data not shown; Watret, 1985). To study further the pattern of Clo Mor virus-specific protein synthesis, a pulse-chase experiment was performed (Fig. 1). BSC-1 cells were infected with Clo Mor virus (5 p.f.u./cell) and at 23 h post-infection were pulse-labelled with [35S]methionine for 1 h, and then incubated for various periods of time in medium containing 1% excess methionine. Cell lysates were prepared for immunoprecipitation, and the virus in the cell supernatant was recovered by centrifugation and also analysed by polyacrylamide gel electrophoresis. In the ‘pulse’ sample, only the N protein and the 115K glycoprotein were detected (lane 6). During the subsequent chase with excess methionine, the amount of N protein detected did not vary (lanes 7 to 9). However, the 115K glycoprotein band decreased in intensity, accompanied by the appearance of the 90K and 80K glycoprotein bands. After 8 h chase, the virion-specific 45K glycoprotein was detected in the pelleted supernatant fluid (lane 12). Note that only the N protein was
Fig. 2. Translation *in vitro* of mRNA extracted from Clo Mor virus-infected BSC-1 cells (lanes 1 to 4) and uninfected BSC-1 cells (lanes 5 to 7). Translation reactions were performed as previously described (Watret & Elliott, 1985). *In vitro* reactions were supplemented with microsomal membranes (MM) and immunoprecipitated (IP) with hyperimmune ascitic fluid as indicated. The *in vitro* equivalent of the N protein (50K) is indicated.

Fig. 3. Agarose gel electrophoresis of glyoxylated RNAs. Lane 1, 28S and 18S ribosomal RNAs; lane 2, Clo Mor virus nucleocapsid RNAs; lane 3, St. Abb's Head uukuvirus nucleocapsid RNAs; lane 4, tobacco mosaic virus RNA (6395 bases). Three RNA species were detected in the Clo Mor virus nucleocapsid preparation, and are designated L, M and S.

detected in the whole cell extracts (lanes 2 to 5) and little shut-off of host protein synthesis occurred.

By analogy with other nairoviruses the 45K glycoprotein of Clo Mor virus will be designated G2. However, the assignment of G1 is unclear because both the 90K and 80K glycoproteins are found in virus particles (Watret *et al.*, 1985). The relationship between these glycoproteins requires further investigation, e.g. by peptide mapping. The demonstration that the 115K intracellular glycoprotein has a precursor-product relationship with the 90K and 80K proteins is in agreement with the results of Clerx & Bishop (1981) using Qalyub virus, but these data are in contrast to those of Cash (1985) who was unable to demonstrate processing of an analogous glycoprotein in Dugbe virus-infected cells. It is possible that the 45K protein arises by a maturation cleavage of either the 90K or 80K polypeptides; the presence of the uncleaved precursor in our preparations of virus particles may account for the detection of the two high mol. wt. glycoproteins.

Messenger RNA was prepared from infected BSC-1 cells by detergent lysis and centrifugation through a CsCl cushion (Leppert *et al.*, 1979; Watret & Elliott, 1985). The mRNA pellet was used to programme a rabbit reticulocyte *in vitro* translation system, and the products were
analysed either directly or after immunoprecipitation, by polyacrylamide gel electrophoresis (Fig. 2). An immunoprecipitable protein of mol. wt. 50K was produced in the reactions programmed with the infected-cell mRNA preparation (lanes 1 to 4) and is thought to be the N protein. A minor protein (mol. wt. 45K) was also specifically immunoprecipitated from the infected-cell mRNA-programmed reactions, though no differences in the polypeptide profiles of the total reaction products of the infected and mock-infected mRNA reactions were seen in this region of the gel (lanes 1, 3, 5 and 7). The inclusion of microsomal membranes into the in vitro translation reactions, which would enable processing of glycoproteins to occur (Blobel & Dobberstein, 1975), did not alter the migration of the immunoprecipitable proteins specified by the infected cell mRNA preparation (lanes 3 and 4).

It is difficult to explain the origin of the 45K protein. Because detection of this protein was independent of the inclusion of microsomal membranes in the in vitro reaction, we do not consider the 45K protein to be related to any of the reported glycoproteins. It is perhaps an early translation termination product or breakdown product of the N protein; however, insufficient amounts of the 45K protein were obtained to test this experimentally.

Intracellular nucleocapsids were purified from infected cells by CsCl gradient centrifugation (Leppert et al., 1979; Watret & Elliott, 1985), and the RNA extracted for analysis. The RNA was treated with glyoxal (McMaster & Carmichael, 1977) and fractionated by agarose gel electrophoresis (Fig. 3). Tobacco mosaic virus RNA, ribosomal RNA extracted from BHK cells and St. Abb's Head uukuvirus nucleocapsid RNA were also run to act as markers (Watret & Elliott, 1985). Three RNA bands were observed in the Clo Mor virus nucleocapsid RNA preparation (lane 2), and were designated L, M and S. The S RNA segment was similar in size to the uukuvirus S RNA, 1900 bases; the M and L RNA segments were larger than the uukuvirus counterparts. The M RNA was estimated to be 6300 bases, and the L RNA, which was outside the range of the markers, was estimated to be 11000 to 13000 bases. The sizes of the Clo Mor virus RNA species further confirm this virus to be a member of the Nairovirus genus.

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REFERENCES


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