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THE STRUCTURE OF TOGAVIRUSES AND BUNYAVIRUSES

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ABSTRACT

This article reviews the properties of arthropod-borne and nonarbo togaviruses and of members of the Bunyaviridae family. Averaged information is given on the morphology and substructure of the virion, its physical properties, its chemical composition and the function of its constituents. Special attention is given to distinctive traits which might be useful for virus classification.

KEY WORDS: TOGAVIRUSES; BUNYAVIRUSES; STRUCTURE; CLASSIFICATION

INTRODUCTION

The criteria by which the vast variety of arboviruses are subdivided have just been summarized by Dr. Porterfield. My task in this workshop on »Structure and Classification of Arboviruses» is to survey concisely the structure of toga- and bunyaviruses, which together make up more than 60 % of about 360 arboviruses now registered in the Catalogue (53) and its supplements. Articles reviewing the structure and replication of the arthropod-borne togaviruses, which are sometimes referred to as »classical arboviruses», have been published recently (42, 36); not long ago, however, it was realized that arthropod-transmission is not an essential property of *Togaviridae* since several viruses with a conventional mode of transmission (such as the rubella, equine arteritis — EAV —, lactic dehydrogenase — LDV —, hog cholera and bovine diarrhoea viruses) were shown to possess togavirus characteristics (for reviews see 25, 26 — only more recent publications will be quoted here). The frontier between arthropod-borne and nonarbo togaviruses even subdivides a genus: most flaviviruses are arthropod-borne, while Modoc and bat salivary gland virus, for instance, are not (13). With about 140 members bunyaviruses (43) are probably the most numerous and at the same time the least known group of animal viruses; lack of arthropod-transmission has not been demonstrated so far.

TOGAVIRUSES

Togaviruses are defined as enveloped, single-stranded RNA viruses with nucleocapsids of cubic symmetry. On the basis of antigenic relationships two arthropod-borne genuses (alphaviruses and flaviviruses) and one nonarbo genus (pestiviruses, 25) can be distinguished. When introducing the molecular weights of the structural polypeptides as an additional taxonomic criterion, LDV and EAV would form a fourth cluster within the family *Togaviridae* (34, Brinton-Darnell and Plagemann, submitted for publication; Zeegers, van der Zeijst and Horzinek, manuscript in preparation); they will be referred to as lactiviruses for the sake of convenience.

Although considered as medium-sized by most authors, togaviruses are the smallest lipid-containing animal viruses known. Alphaviruses are about 60 nm in diameter, flaviviruses appear smaller (about 45 nm). This difference in size is also reflected in the sedimentation coefficients,

which range between 240 and 300 S for alphaviruses and 170 to 220 S for members of the flavigenus. The buoyant density depends on the substance used for building the gradient and varies between 1.18 (sucrose) and 1.24 g/cm^3 (CsCl) for alpha- and flaviviruses; unusually low densities (< 1.15 g/cm^3) have been recorded for some nonarbo members, e.g. LDV (17, 28).

In the electron microscope togavirions appear as spherical particles consisting of an envelope and an isometric core. In thin sections the envelope shows unit membrane characteristics. Negatively stained particles of the alpha- and flaviviruses and of rubella virus possess ill-defined surface projections which are probably composed of the viral glycoproteins and carry the pH-dependent hemagglutinating activity found in these togaviruses. Pesti- and lactiviruses are devoid of hemagglutinating activity and have been described as smooth-surfaced by most authors. For lactiviruses (EAV: 29; LDV: Horzinek & van Wielink, unpublished observations), however, there are indications that fragile surface projections do exist and that bald particles are in fact produced during purification, such as has been observed, for example, with oncornaviruses (37) and coronaviruses (38). Exposure of togaviruses to organic solvents or detergents inactivates their infectivity, the latter treatment leading to removal of the envelope and liberation of the nucleocapsid.

The size differences noted for the whole virions are reflected by the nucleocapsid diameter values found for alphaviruses, lactiviruses and rubella virus on one hand (30—40 nm) and for flavi- and pestiviruses on the other (20—30 nm). However, acid pH or RNase may cause contraction of the nucleocapsid, as has been demonstrated in the case of Semliki Forest virus (see 5); consequently size values obtained by using unbuffered uranyl acetate as a stain or capsids which have not been prepared under RNase-free conditions may be too small. Despite the size difference between alpha- and flavivirus nucleocapsids they show identical sedimentation behaviour in density gradients (9); rubella virus cores, too, sediment at about 150 S (55, 56). Isolated nucleocapsids of lactiviruses have slightly higher sedimentation coefficients (LDV: 28, 34; EAV: Zeegers, van der Zeijst & Horzinek, manuscript in preparation). In the electron microscope, they show a substructure suggestive of cubic symmetry; in contrast to naked animal viruses, however, they are rather flexible, a property which has been interpreted in terms of the essential protein-RNA bonding necessary for the structural integrity of isolated alphavirus nucleocapsids (5). Although most workers agree upon an icosahedral construction, the triangulation number has not been unequivocally determined by electron microscopy. Values of three (32 capsomers, 27), four (42 capsomers, 5) and nine (92 capsomers, 11) have been proposed; on the other hand, morphological variants of alphaviruses have been described (22); in Sindbis virus they were thought to contain nucleocapsids composed of subunits organized in different triangulation numbers (10). The number of protein molecules building the nucleocapsid has been estimated at 230 (30); 240 subunits would be required for a 42-capsomere capsid. Using detergent techniques, nucleocapsids have been prepared and/or demonstrated by electron microscopy for alpha-, flaviviruses (see 42), rubellavirus, pestiviruses (see 25) and lactiviruses (28, 29, 34; Brinton-Darnell and Plagemann, submitted for publication).

Electrophoretic analysis of purified labelled nucleocapsids showed one species of nonglycosylated polypeptide with molecular masses of 30—32 $\times$ 10^3 daltons for alphaviruses and 13—14 $\times$ 10^3 daltons for flaviviruses (see 42); for rubella virus values around 35,000 daltons (see 25) and for lactiviruses of about 15,000 daltons were reported (34; Brinton-Darnell and Plagemann, submitted for publication; Zeegers, van der Zeijst and Horzinek, manuscript in preparation). Antigenic analysis of alphaviruses has indicated that the nucleocapsid protein carries determinants with genus specificity (16).

The envelope of togaviruses is acquired from host cell membranes by a budding process in the final stage of maturation; differences have been noted for alpha-, flavi- and the nonarbo togaviruses with respect to the sites where extrusion occurs preferentially (see 26). The membrane of

the Semliki Forest virus has been subjected to intensive study, especially by our Finnish hosts; it was found to contain three virus-specific glycosylated proteins in about equimolar ratios, two of them with molecular masses of around 50,000 and a third one of about 10,000 daltons (21). For Sindbis virus, another alphavirus, only two glycoproteins have been found (49). They appear to occupy a superficial position in the alphavirus membrane as evidenced by enzymatic iodination (50) and protease digestion experiments (15). When the surface projections were cleaved off the membrane of Semliki Forest virus by thermolysin, two hydrophobic peptides were still left in the membrane and these are derived from the bigger glycoproteins (54). These and other studies (12, 20) led to the conclusion that the viral »spikes» penetrate the lipid bilayer rather than being attached to it (23). Crosslinking and detergent solubilization experiments have prompted the suggestion that the three glycoproteins of Semliki Forest virus form oligomeric structures in the viral membrane which may correspond to the »spikes» on the virion surface (19). The envelope of flaviviruses contains two proteins with molecular masses of 8—9 and 50—60 $\times 10^3$ daltons, respectively, of which only the latter is glycosylated (see 42). Difficulties have been encountered in the identification of nonarbo togavirus glycoproteins. In rubella virus, only one of them (molecular mass about 63 $\times 10^3$ daltons) gives a distinct maximum in polyacrylamide gels whereas the other(s) appear as a broad and variable peak in the region between 47 and 56 $\times 10^3$ daltons (31, 55). Similar observations have been made for lactiviruses (LDV: 34, Brinton-Darnell and Plagemann, submitted for publication; EAV: 29; Zeegers, van der Zeijst and Horzinek, manuscript in preparation). Since it is not known whether this behaviour is caused by heterogeneity in the carbohydrate moiety associated with the protein or to the presence of more than one glycoprotein difficulties may arise if molecular weights of virion polypeptides are used for taxonomical purposes (31). Any comparative approach must take into account anomalies of electrophoretic migration due, for instance, to the varying SDS-binding capacity of glycoproteins, the batch of SDS used etc. (45, 52). The envelope of togaviruses carries antigenic determinants responsible for the induction of antibodies which can be specifically detected by means of neutralization and hemagglutination inhibition (1).

The RNA of togaviruses is a molecule of about 4 $\times 10^6$ daltons which is in one piece and is infectious when extracted and assayed appropriately. It shows properties indicating a significant secondary structure which is necessary for infectivity. Poly(A) sequences have been demonstrated in the genomes of several alphavirus members (see 14, 18), which, together with the absence of a virion-associated polymerase, (4) suggest that togaviral RNA is plus-stranded (3) and might serve as the initial messenger molecule during infection. The smaller RNA molecules (»26 S» or interjacent RNA) found by several investigators were proved to represent a unique fraction of the viral genome containing about 1/3 of the base sequences (51). High molecular weight virion RNA containing hidden breaks (2) could be transformed into this species by treatments known to break hydrogen bonds.

BUNYAVIRUSES

The second taxonomic subset of arboviruses which I was asked to review are the *Bunyaviridae,* for whom a family status has recently been proposed (35, 43). The family consists of the genus *Bunyavirus* (formerly Bunyamwera supergroup) with 11 serological groups on one hand and on the other some 55 additional viruses morphologically similar to Bunyamwera virus — of which the best studied so far is Uukuniemi virus. Uukuniemi virus and the related viruses are regarded as »other possible members» of the genus *Bunyavirus.*

Bunyaviruses are enveloped RNA viruses, with single-stranded segmented genomes enclosed in nucleocapsids of helical symmetry. They are of roughly spherical shape and measure about 100 nm in diameter (24, 32, 35); smaller values have

been reported for Batai and Inkoo virions (6). Sedimentation coefficients are about 450 S and buoyant densities in sucrose between 1.17 and 1.18 g/cm^3 (1.20 in CsCl) have been reported (8, 33, 41, 44). Negatively stained bunyavirions show surface projections which may be either indistinct and filamentous (6) or pronounced and arranged in a regular lattice (Uukuniemi virus: 46). In thin sections the envelope layer shows unit-membrane characteristics (32), which are usually less distinct as compared with the membranes of the host cell (35). Of the two to four structural polypeptides which have been found in various members of the family, the largest one has a molecular weight of $\geqslant$ 75,000 (33, 44) and is associated with the viral envelope (41); a further membrane polypeptide of intermediate size (30,000 to 45,000 daltons: 33, 44) is probably present. It has been shown that these proteins are glycosylated and that they are responsible for the induction of neutralizing and hemagglutination-inhibiting antibodies. Association of hemagglutinating activity with low density material from detergent-disrupted virions has been demonstrated (47).

The same technique has been employed for the liberation of the viral nucleocapsid, which was shown to have a helical structure by workers from this country (6, 41, 47). For Lumbo virus (48) and Uukuniemi virus (39) the ribonucleoprotein strands were shown to consist of closed circles — a unique observation for RNA viruses. Uukuniemi nucleocapsids are of three size classes (sedimenting at 150 S, 110 S and 90 S, respectively); all of them contain the same ratio of core polypeptide (molecular weight 25,000) to RNA. Polyacrylamide-agarose gel electrophoresis showed that the largest structure also contained the largest RNA species (molecular weight 4.1×10^6) and the remaining RNAs (1.9×10^6; 0.88 and 0.78×10^6) were distributed accordingly (39). Whether the segmented single-stranded genome of bunyaviruses (8, 33, 40) itself consists of circular RNAs remains to be established. The polarity of bunyavirus RNA is a further problem to be solved; extraction of infectious RNA from the La Crosse and Restan viruses (33, 57) would indicate plus-strandedness, but these results are difficult to reconcile with the demonstration of a RNA-dependent RNA-polymerase found by other workers (7, Pettersson, personal communication).

From the compilation of data given in this review it is evident that many distinctive structural properties can be used to identify an unknown enveloped RNA virus as a member of the *Toga-* or *Bunyaviridae* families. Most new viruses have been classified on the basis of their relationship to established antigenic virus groups. If these are lacking (as, for instance, in the case of rubella virus), negative staining electron microscopy of native and detergent-treated purified virus preparations would be the first method to choose since information on size, surface and capsid substructure can be obtained simultaneously. Morphology would thereby provide a basis for comparative analysis using more refined biochemical techniques.

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