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STRUCTURE AND INTRACELLULAR ASSEMBLY OF THE TRANSMISSIBLE GASTROENTERITIS CORONAVIRUS

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1. ABSTRACT

Coronaviruses have been described as pleomorphic, round particles with a helical nucleocapsid as the unique internal structure under the virion envelope. Our studies on the organization of the transmissible gastroenteritis coronavirus (TGEV) have shown that the structure of these viruses is more complex. Different electron microscopy techniques, including cryomicroscopy of vitrified viruses, revealed the existence of an internal core, most probably icosahedral, in TGEV virions. Disruption of these cores induced the release of elongated ribonucleoprotein complexes. Ultrastructural analysis of freeze-substituted TGEV-infected swine testis (ST) cells showed characteristic intracellular budding profiles as well as two types of virions. While large virions with an electron-dense internal periphery are seen at perinuclear regions, smaller viral particles exhibiting compact internal cores of poligonal contours are more abundant in areas closer to the plasma membrane of the cell. These data strongly suggest that maturation events following the budding process are responsible for the formation of the internal core shell, the new structural element that we have recently described in extracellular infectious TGEV virions.

2. INTRODUCTION

The morphogenesis of coronaviruses has been defined as a complex process, that starts with the interaction of a viral nucleocapsid with membranes of the endoplasmic reticulum-Golgi intermediate compartment (ERGIC). This compartment is an specialized domain of the endoplasmic reticulum where the coronavirus M glycoprotein accumulates,

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defining the cellular location of viral assembly (Krijnse-Locker *et al.*, 1994). The assembled virions pass through the Golgi apparatus and finally use the constitutive secretory pathway to exit the cell. Our recent description of a new structural element in coronavirus particles, the internal core (Risco *et al.*, 1996), introduced a new component in the sequence of assembly. After visualizing the different structural elements that form the infectious extracellular virion, we have looked for them inside the infected cells. The use of cryomethods at the EM level is being decisive for the detailed characterization of the coronavirus structure and assembly.

3. MATERIALS AND METHODS

3.1. Electron Microscopy Characterization of Purified Extracellular Virions and Viral Cores

TGEV virions (PUR46-MAD strain) were purified from infected cultures of swine testis (ST) cells (Jiménez *et al.*, 1986). Negative staining of both intact and detergent-treated virions and viral cores was done with 2 % uranyl acetate or 2 % sodium phosphotungstate according to standard procedures (Risco *et al.*, 1996). Cryoelectron microscopy of vitrified, unstained hydrated virions was performed as described (Booy, 1993). Immunogold labeling with specific monoclonal antibodies (MAbs) (Gebauer *et al.*, 1991) and a 5 - nm colloidal gold conjugate was performed following previously stablished procedures (Risco *et al.*, 1995a).

3.2. Structural Analysis of TGEV-Infected Cells

TGEV-infected ST cells were fixed at different post-infection times before cryoprotection and rapid freezing in liquid propane. Vitrified samples were then submitted to freeze-substitution, embedding, ultrathin sectioning and immunogold labeling as described (Grief *et al.*, 1994; Risco *et al.*, 1995b). Samples were studied in a JEOL 1200 EX II electron microscope.

4. RESULTS

Our structural studies, based of different electron microscopy methods (Risco *et al.*, 1996) have revealed the existence of an internal core in TGEV virions. In particular, cryomicroscopy of vitrified viruses provided a direct view of the different viral components (Figure 1A-C). Since these specimens are hydrated and no chemical fixation or staining was used, the information obtained is very close to the native state of the structure. Purification of the viral cores was done after finding an adequate treatment with detergents, that releases the internal core after removing the envelopes (Figure 1D-F). The cores released by this treatment contained both M and N proteins, as well as RNA (Risco *et al.*, 1996). Disruption of the viral cores induced the release of an internal component that heavily reacted with anti-N MAbs and that, once separated from the core, exhibited an elongated structure covered by N protein molecules, according to immunogold detection (Figure 2).

The search for the generation of all these viral structural elements has been done in ultra-thin sections of infected cells processed by freeze-substitution. Using this method a considerable improvement of fine structural details (when compared with conventional

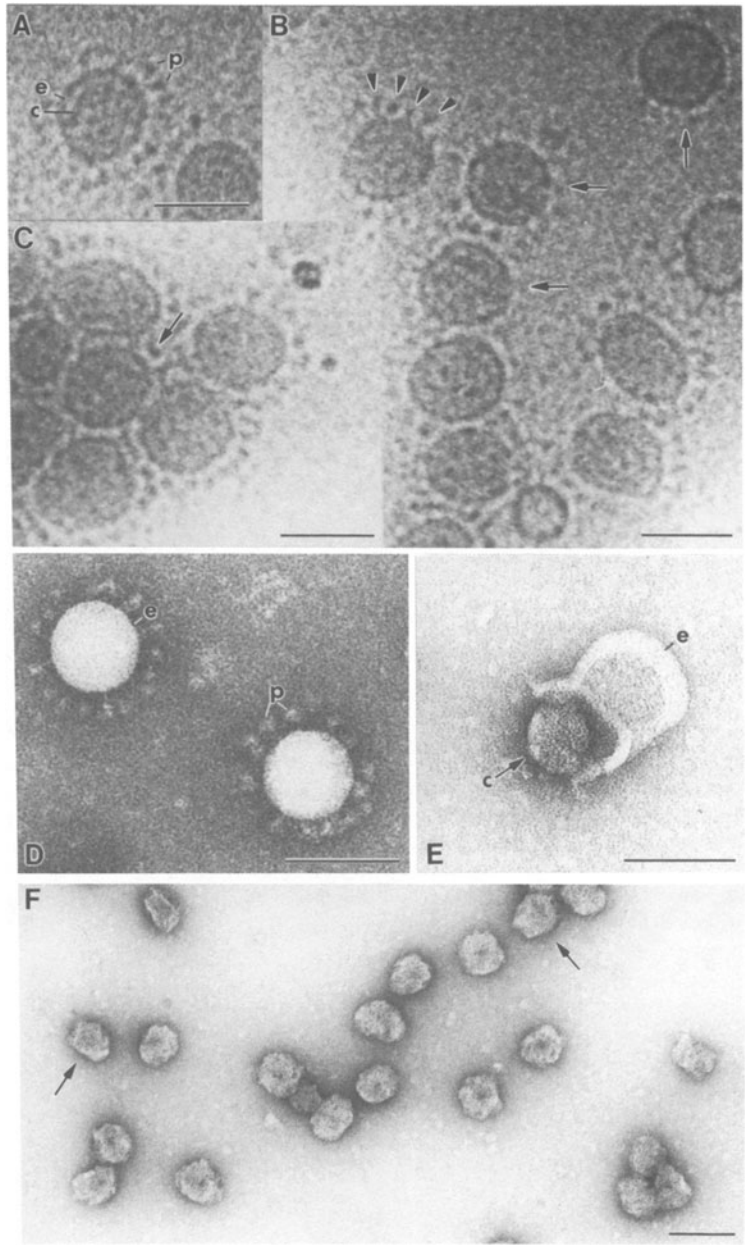

Figure 1. Electron microscopy visualization of intact and detergent-treated TGEV virions, as well as viral cores. A) to C) vitrified virions show well-extended peplomers (p) and a compact internal core (c), clearly separated from the viral envelope (e). D) and E) show intact and NP40-treated negative stained TGEV virions, respectively. The treatment with the detergent induces the release of the internal core (c) from the disrupted envelope (e). In F) a group of released viral cores is shown. The arrows point to particles that exhibit a clear polygonal contour. Bars: 100 nm.

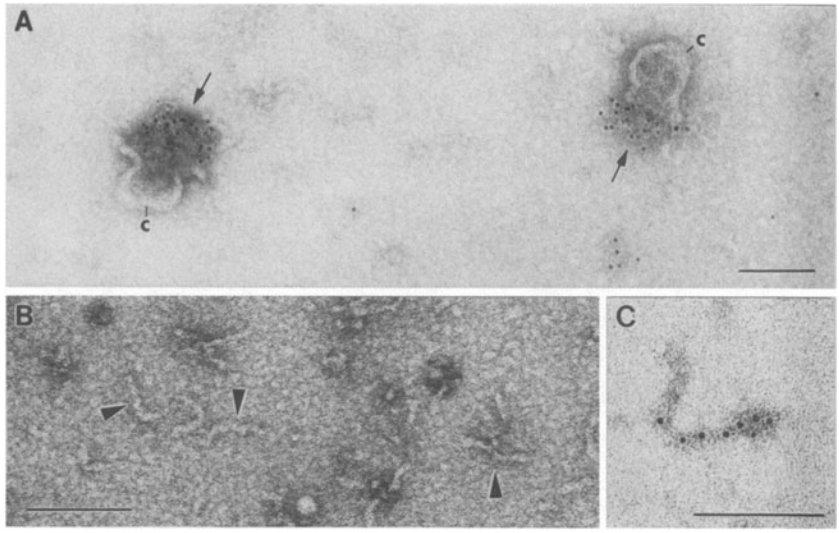

Figure 2. Release of N protein–containing aggregates from disrupted viral cores. A) Triton X-100-treated cores (c) release a material that strongly reacts with monoclonal antibodies against the nucleocapsid protein (arrows). B) The released material corresponds to elongated structures (arrowheads) of rather homogeneous length. These structures are covered by N protein molecules, according to immunogold detection with anti-N MAbs (C). Bars: 100 nm.

embedding procedures) has been obtained in ST cells. In freeze-substituted infected cells we have distinguished two types of viral particles. Large virions (apparently generated from budding profiles) as well as smaller viral particles with dense cores are clearly distinguished (Figure 3A). These smaller virions are similar in dimensions and internal organization to the extracellular purified TGEV viruses characterized in the first part of this study. Furthermore, and in spite of their great difference in volume and organization, both types of virions react with anti-TGEV antibodies (Figure 3B).

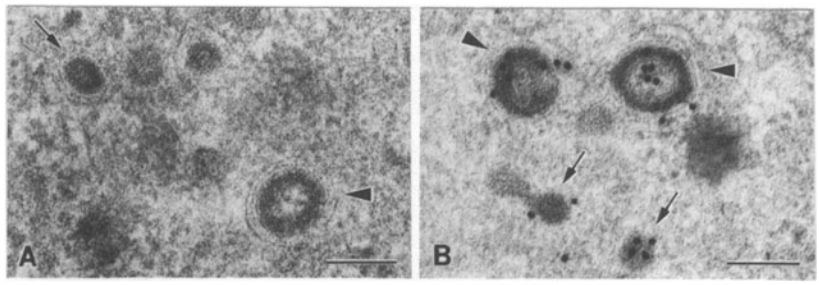

Figure 3. Ultra-thin sections of freeze-substituted TGEV-infected ST-cells. A) Small (arrow) and large (arrowhead) virions are distinguished. They both react with anti-TGEV antibodies, as shown by immunogold labeling (B). Bars: 100 nm.

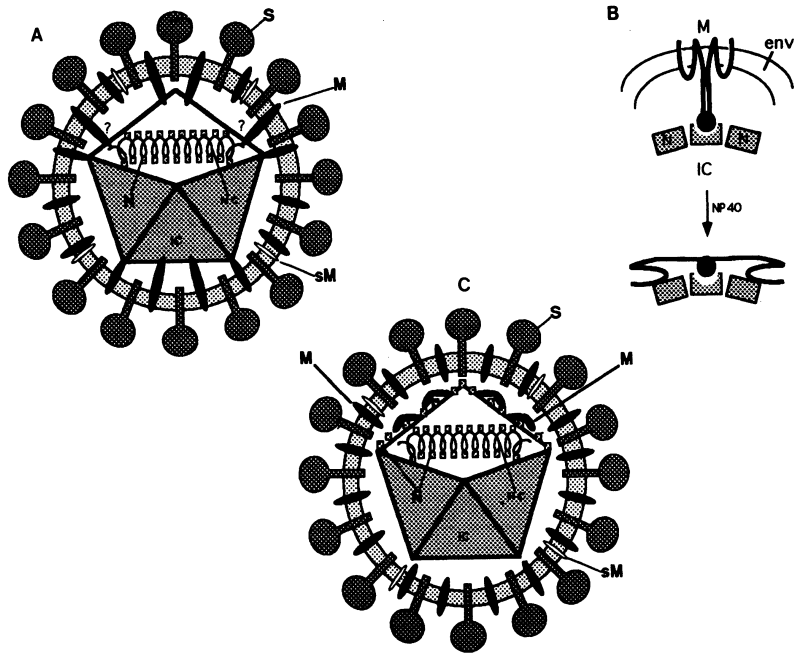

Figure 4. Structural models of TGEV showing two different possibilities to explain the association of M protein molecules to the surface of the purified viral core. A) Internal domains of envelope M protein molecules could interact with the viral core, remaining associated to it once the lipids are removed by the detergent (B). C) Hypothetically, two different populations of M protein molecules (that could exhibit or not different modifications) could separate during viral assembly. IC: internal core; NC: nucleocapsid; env: viral envelope.

5. DISCUSSION

The new model for the organization of coronaviruses (Figure 4), supported by our structural evidences has raised a number of questions concerning the organization of the different structural proteins within the viral particle.The envelope M protein, for example, was consistently found on the surface of purified viral cores. It is well documented that subviral particles prepared by NP-40 disruption of purified MHV, HEV, IBV, or BCV, still contained M protein associated with the nucleocapsids (Rottier, 1995). The significance of these interactions remains to be assessed. Envelope M protein molecules could interact with the internal core through an intravirion domain (Figure 4A), but a separation of two populations of M protein molecules (envelope and core-associated molecules) during viral assembly cannot be discarded (Figure 4B). High-resolution studies are necessary to define these aspects. Our structural analysis of TGEV-infected cells strongly suggest that assembly starts with the formation of large viral particles that undergo a dramatic reorganization to render smaller particles with assembled icosahedral cores. Studies on the cellular and viral factors involved in the sequence of assembly are now in progress. For this purpose, EM cryomethods, that provide an adequate preservation of fine structural details, are of key importance for the definition of the coronavirus morphogenesis.

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