DOI: 10.1017/S1431927605505324
Microsc Microanal 11(Suppl 2), 2005

Ultrastructural and confocal microscopy analysis of foot-and-mouth virus (FMDV) structural and non-structural proteins on replication complexes in cultured cells.

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Picornavirus infection of cells induces a rapid proliferation of cytoplasmic single and double membrane vesicles. These membranes associate into structures called replication complexes (RC) [1-3]. Visualizing mature FMDV particles has been difficult due to their fragility below pH 7. However, high-pressure freeze-substitution studies have produced clear images of mature FMDV particles in cytoplasm [4]. Using a different approach, IBRS2 cells were infected with FMDV O1 Campos at a multiplicity of 5 and fixed at 4 hrs post infection (pi) with a solution containing 4% paraformaldehyde and 5% glutaraldehyde in a 0.2M cacodylate buffer (pH 8.0). Hexagonal particles, 28-32 nm in diameter, were attached to the external surface of single membrane RC vesicles (Fig.1). Examination of serial sections from infected cells revealed that numerous particles were present on individual membranes at 4 hrs pi. For confocal analysis, BKLF cells, were grown on glass cover slips, were infected at moi 10 and were fixed with 4% paraformaldehyde. The distribution of FMDV's non-structural proteins 2B, 2C, 3A and 3C was determined at 2, 3, and 4 hrs pi by incubation with monoclonal and monospecific polyclonal antisera followed by anti-species antiserum labeled with Alexa 488 or Alexa 594. Confocal microscopy localized early binding of 2B, 2C, and 3A to small, uniformly distributed cytoplasmic vesicles that coalesced into a larger complex by 4 hrs (Fig. 2). 3C, on the other hand, showed a more diffuse distribution that did not coalesce (Data not shown). FMDV structural protein VP1, identified by Mabs co-localizes with 2B and 3A at 4 hrs pi (Fig. 3). These results suggest that FMDV non-structural proteins are present with the RC and suggest that assembly of mature virions takes place at or near the surface of the RC vesicles.

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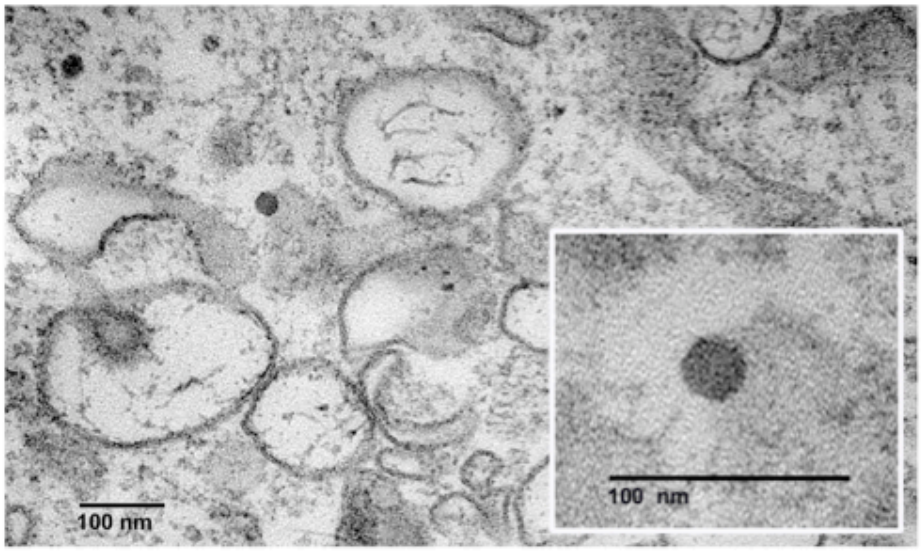

Figure 1

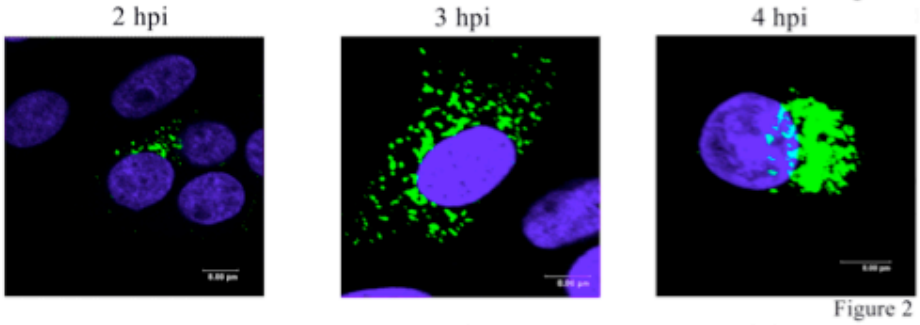

Figure 2

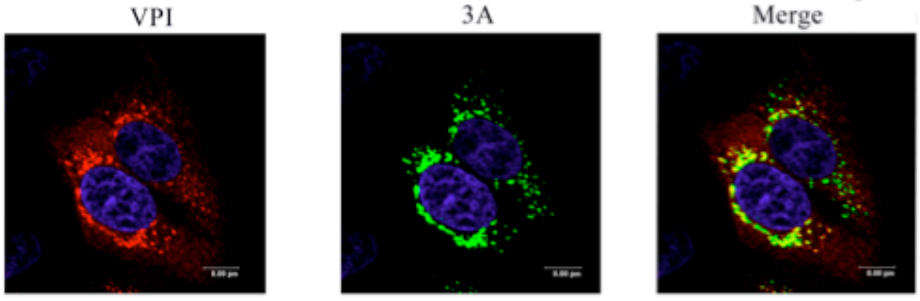

Figure 3

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