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Protocols

Localisation of Theiler's murine encephalomyelitis virus protein 2C to the Golgi apparatus using antibodies generated against a peptide region

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ABSTRACT

The picornavirus 2C protein is highly conserved and indispensible for virus replication. Polyclonal antibodies against Theiler's murine encephalomyelitis virus (TMEV) 2C protein were generated by immunisation of rabbits with a peptide comprising amino acids 31–210 of the protein. Antibodies were used to investigate the localisation of 2C in infected cells by indirect immunofluorescence and confocal microscopy. Analysis of infected cells revealed that the distribution of 2C changed during infection. Early on, the protein was localised in the perinuclear region with punctate staining in the cytoplasm and at later stages, it was concentrated in a large structure in close proximity to the nucleus and occupying almost 50% of the cell size. Dual label immunofluorescence using wheat germ agglutinin (WGA) and anti-TMEV 2C antibodies suggested that 2C, and therefore virus replication, is targeted to the Golgi apparatus. At late stages of infection Golgi staining was dispersed, indicating potential reorganisation of membranes. Infection was accompanied by "rounding up" of the cells and a redistribution of actin around the putative replication complex. The results suggest that TMEV behaves similarly to FMDV which also forms replication complexes in the perinuclear region.

1. Introduction

Picornaviruses include significant human and animal pathogens, notable members of the family being poliovirus (PV), human rhinovirus (HRV), hepatitis A virus (HAV) and Foot-and-Mouth-disease virus (FMDV). Current disease treatment and control strategies are limited by an incomplete understanding of the interactions between the non-structural, replicative proteins and host cell components. Picornavirus genomes consist of positive-sense, single-stranded RNA encoding the viral proteins in a single, long, open reading frame which is cleaved by viral proteases during translation. Three discrete domains are contained within the polyprotein: P1, encoding the four structural polypeptides of the capsid and the P2/P3 domains which encode several non-structural proteins involved in viral replication. The central P2 domain contains a viral peptide 2A and two proteins with important roles in replication, namely 2B and 2C, while the RNA-dependent RNA polymerase (3D), the viral protease (3C), as well as proteins 3A and 3B are located in the P3 domain (for a review see Bedard and Semler, 2004).

Picornavirus infection has long been associated with extensive rearrangement of intracellular membranes culminating in the proliferation of cytoplasmic vesicles that support virus replication (Barco and Carrasco, 1995; Bienz et al., 1983, 1987; Caliguiri and Tamm, 1970; Dales et al., 1965). During this process the P2 proteins 2B, 2C and 2BC localise to membranes of the endoplasmic reticulum and Golgi apparatus, and their role in the structural changes observed is well documented (Aldabe and Carrasco, 1995; Barton and Flanegan, 1997; Bienz et al., 1990, 1992; Cho et al., 1994; Doedens and Kirkegaard, 1995; Doedens et al., 1997; Moffat et al., 2005; Rust et al., 2001; Schlegel et al., 1996; Sandoval and Carrasco, 1997; Suhy et al., 2000; Teterina et al., 1997a).

The 2C protein is highly conserved among picornaviruses and extensive studies have revealed that it has multiple functions during the viral life cycle. Using computer-assisted analysis of its sequence it has been proposed that, upon folding, 2C comprises three domains and that separate regions of the protein are responsible for the variety of functions in which it engages (Teterina et al., 1997b). For example, the highly conserved central domain contains nucleoside triphosphate-binding motifs (Gorbalenya et al., 1990) and the protein is associated with NTP binding and hydrolytic activities (Mirzayan and Wimmer, 1994; Rodriguez and Carrasco, 1993). N- and C-terminal domains of the protein are involved in membrane binding (Echeverri and Dasgupta, 1995; Echeverri et al., 1998; Teterina et al., 1997b), and interaction with viral RNA (Banerjee et al., 1997; Barton and Flanegan, 1997; Rodríguez and Carrasco, 1995). A cysteine-rich, zinc-binding motif involved in virus replication has also been identified (Pfister et al., 2000). Other

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