Virus Research 194 (2014) 16–36

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Membrane proteins of arterivirus particles: Structure, topology, processing and function

Michael Veit[a,*], Anna Karolina Matczuk[a], Balaji Chandrasekhar Sinhadri[a], Eberhard Krause[b], Bastian Thaa[a,1]

[a] Institut für Virologie, Veterinärmedizin, Freie Universität Berlin, Germany
[b] Leibniz Institute of Molecular Pharmacology (FMP), Berlin, Germany

ARTICLE INFO

Article history:
Available online 30 September 2014

Keywords:
Arterivirus
Equine arteritis virus
Porcine reproductive and respiratory syndrome virus
Signal peptide
Glycosylation
Membrane topology

ABSTRACT

Arteriviruses, such as equine arteritis virus (EAV) and porcine reproductive and respiratory syndrome virus (PRRSV), are important pathogens in veterinary medicine. Despite their limited genome size, arterivirus particles contain a multitude of membrane proteins, the Gp5/M and the Gp2/3/4 complex, the small and hydrophobic E protein and the ORF5a protein. Their function during virus entry and budding is understood only incompletely.

We summarize current knowledge of their primary structure, membrane topology, (co-translational) processing and intracellular targeting to membranes of the exocytic pathway, which are the budding site. We profoundly describe experimental data that led to widely believed conceptions about the function of these proteins and also report new results about processing steps for each glycoprotein. Further, we depict the location and characteristics of epitopes in the membrane proteins since the late appearance of neutralizing antibodies may lead to persistence, a characteristic hallmark of arterivirus infection. Some molecular features of the arteriviral proteins are rare or even unique from a cell biological point of view, particularly the prevention of signal peptide cleavage by co-translational glycosylation, discovered in EAV-Gp3, and the efficient use of overlapping sequons for glycosylation. This article reviews the molecular mechanisms of these cellular processes. Based on this, we present hypotheses on the structure and variability of arteriviral membrane proteins and their role during virus entry and budding.

1. Introduction to arteriviruses

Arteriviridae is a family of enveloped, positive-stranded RNA viruses. Despite their importance in veterinary medicine, the arteriviruses are only poorly characterized in molecular terms. The prototype arterivirus is equine arteritis virus (EAV), which can cause substantial disease in horses; further arteriviruses are porcine reproductive and respiratory syndrome virus (PRRSV), the most important pathogen in the pig industry worldwide, the murine lactate dehydrogenase-elevating virus (LDV) and simian haemorrhagic fever virus (SHFV). To date, no arterivirus affecting humans has been encountered (for recent review, see Balasuriya et al., 2013; Meulenberg, 2000; Snijder et al., 2013).

Arterivirus infection may be subclinical (especially in the case of LDV), but can lead to severe symptoms, most prominently lesions of arteries (arteritis, hence the name of the virus family), oedema, respiratory symptoms/pneumonia as well as abortion in pregnant animals, with devastating implications in animal breeding (for review, see Cho and Dee, 2006; Nodelijk, 2002; Rossow, 1998). The primary target cells for arteriviruses are macrophages, but highly pathogenic PRRSV isolates may have an expanded tropism

Abbreviations: BHK, baby hamster kidney cells; BiP, binding immunoglobulin protein; BM2, M2 protein of influenza B; CD, cluster of differentiation; CHO, Chinese hamster ovary cells; DTT, dithiothreitol; EAV, equine arteritis virus; Endo H, endoglycosidase H; ER, endoplasmic reticulum; ERGIC, ER–Golgi intermediate compartment; ESCRT, endosomal sorting complexes required for transport; GFP, green fluorescent protein; Gp, glycoprotein; GPI, glycosylphosphatidylinositol; HIV, human immunodeficiency virus; HR, hydrophobic region; LC–MS/MS, liquid chromatography–tandem mass spectrometry; LDV, murine lactate dehydrogenase-elevating virus; MHC, major histocompatibility complex; ORF, open reading frame; OST, oligosaccharyltransferase; PAMs, porcine alveolar macrophages; PFU, plaque-forming unit; PI-PLC, phosphatidylinositol-specific phospholipase C; PNGase F, peptide:N-glycosidase F; PRRSV, porcine reproductive and respiratory syndrome virus; SARS, severe acute respiratory syndrome; SHFV, simian haemorrhagic fever virus; SPase, signal peptidase; SP, signal peptide; SRP, signal recognition particle; TMR, transmembrane region; VLP, virus-like particle; YFP, yellow fluorescent protein.

* Corresponding author at: Michael Veit, Institut für Virologie, Veterinärmedizin, Freie Universität Berlin, Robert-von-Ostertag-Straße 7–13, 14163 Berlin, Germany. Tel.: +49 30 83851891; fax: +49 30 838451847.
E-mail address: mveit@zedat.fu-berlin.de (M. Veit).
[1] Present address: Karolinska Institutet, Institutionen för mikrobiologi, tumör- och cellbiologi (MTC), Nobels väg 16, SE-171 77 Stockholm, Sweden.

http://dx.doi.org/10.1016/j.virusres.2014.09.010
0168-1702/

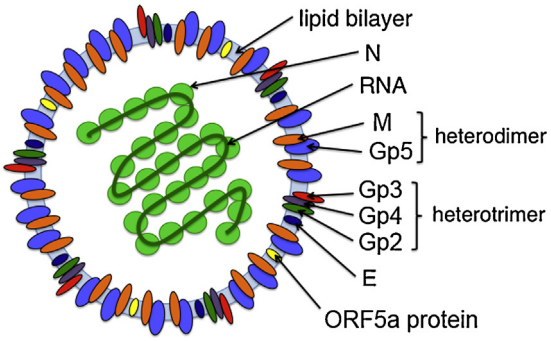

Fig. 1. Scheme of an arterivirus particle. The membrane contains the major Gp5/M complex (blue and orange), the minor glycoprotein complex Gp2/3/4 (green, red and purple, respectively), the small hydrophobic E protein (dark blue) and the ORF5a protein (yellow).

to include epithelial cells (Zhou and Yang, 2010). Transmission is mainly via the respiratory route; sexual transmission occurs as well (Cho and Dee, 2006; Nodelijk, 2002; Rossow, 1998).

One common, relevant trait of arterivirus infection is persistence. After acute infection, the virus is often not eliminated entirely, but continues to replicate at low levels in lymphoid tissues (PRRSV) or in the reproductive tract (EAV). It is generally assumed that the host's immune system is incapable of setting up a robust immune response against the virus. This is evidenced by the finding that neutralizing antibodies against PRRSV and LDV are generated only late after infection, and that their titres stay low. Several hypotheses about the mechanistic basis for persistence have been put forward (for review, see Chand et al., 2012; Darwich et al., 2010; Kimman et al., 2009; Mateu and Diaz, 2008; Murtaugh and Genzow, 2011).

This article will focus on the biochemical processing of the structural proteins of arteriviruses, with emphasis on EAV and PRRSV. We believe that understanding the biochemical properties of the viral proteins is highly relevant, not least in order to develop vaccines and treatments for the arteriviral diseases in a knowledge-based manner. In addition, some molecular features of the arteriviral proteins are very interesting from a cell biological point of view since they have been found to be unusual or even unique. For background information on the cellular biology of synthesis and processing of membrane proteins and the molecular features of the enzymes and proteins involved, the reader is referred to *Addendum*. Upon their first appearance in the main text, terms related to these topics are highlighted in italics and bold to refer the reader to *Addendum*.

1.1. The structural proteins of arteriviruses: overview

Due to similarities in genome organization and replication strategy, *Arteriviridae* are grouped in the order *Nidovirales* together with *Coronaviridae*, *Roniviridae* and *Mesoniviridae*. The biochemical properties of the structural proteins are, however, markedly different in *Arteriviridae*; yet, all *Nidovirales* share the property of possessing a nested genome, where the structural proteins are expressed from a set of 3′ co-terminal subgenomic mRNAs (Fang and Snijder, 2010; Gorbalenya et al., 2006). The open reading frames encoding the structural proteins are partially overlapping. Accordingly, one needs to keep in mind that a given amino acid context in a structural protein may actually be a by-product of evolutionary pressure on a different protein, encoded in part by the same RNA sequence. Also, this feature restricts the possibility to address virus protein mutations in recombinant viruses without affecting the overlapping ORFs.

Arteriviruses contain at least seven structural proteins (Fig. 1; Music and Gagnon, 2010). The nucleocapsid protein N builds the scaffold for the genomic RNA, and several membrane proteins are incorporated into the viral envelope: the glycoproteins Gp2, Gp3, Gp4 and Gp5 as well as the non-glycosylated membrane proteins M and E. Gp2/3/4 form a disulphide-linked heterotrimeric complex in virus particles, and Gp5 is disulphide-linked to M. N, M and Gp5 are major virion components, whereas E, Gp2, Gp3 and Gp4 are referred to as minor structural proteins (de Vries et al., 1992). A novel membrane-anchored protein encoded by an alternative open reading frame of the subgenomic mRNA encoding Gp5 was discovered recently. This ORF5a protein is incorporated into virus particles, but probably as a very minor component (Firth et al., 2011; Johnson et al., 2011). Yet, it is relevant for replication: PRRSV does not replicate in the absence of the ORF5a protein, whereas recombinant EAV lacking the ORF5a protein could be generated, but grew to low titres and displayed a tiny plaque size (Firth et al., 2011; Sun et al., 2013). Very recently, the non-structural protein nsp2 was shown to be incorporated into virions of a variety of PRRSV strains (Kappes et al., 2013). This protein is predicted to have several transmembrane-spanning regions and assists in the induction of double membrane vesicles, ER-derived membrane scaffolds forming the viral replication and transcription complex (Snijder et al., 2001). The role of nsp2 in virions is not yet understood.

From reverse genetics experiments with EAV and to some extent PRRSV, it is known that all structural proteins of either virus are essential for virus replication. If either Gp5 or M is deleted from the viral genome, no virus particles are released from infected cells. Thus, Gp5 and M are required for virus budding (which does not exclude additional functions, e.g. during virus entry). If expression of either Gp2 or Gp3 or Gp4 is abrogated, virus-like particles bud from cells, but the particles are not infectious, indicating that cell entry is disturbed in the absence of the minor glycoprotein complex (Wieringa et al., 2004; Wissink et al., 2005). Furthermore, the Gp2/3/4 complex governs cell tropism: When the ectodomains of EAV and PRRSV are swapped, the cell tropism of the resulting recombinant virus is altered, but not by exchanging the ectodomains of Gp5 and M (Dobbe et al., 2001; Lee and Yoo, 2006; Tian et al., 2012; Verheije et al., 2002). Interestingly, the Gp2/3/4 complex interacts either physically or functionally with E. Removal of E from the viral genome completely prevents incorporation of the Gp2/3/4 complex into virus particles, whereas in the absence of either Gp2, Gp3 or Gp4, the amount of E in virions is greatly reduced (Wieringa et al., 2004). Another peculiarity of the Gp2/3/4 complex is that Gp3 appears to be linked to the pre-formed Gp2/4 dimer only after virus budding (Wieringa et al., 2003b).

1.2. Virus particles, budding and cell entry of arteriviruses

Arterivirus particles are pleomorphic, but typically spherical or oval with a diameter of approx. 60 nm and a relatively smooth outer surface (for review, see Dokland, 2010; Snijder and Meulenberg, 1998). The nucleocapsid – comprising the viral RNA wrapped by the N protein – is arranged as a double-layered, spherical hollow core, separated from the lipid envelope by a gap of 2–3 nm as revealed by cryo-electron tomography (Spilman et al., 2009). The arterivirus particles are produced intracellularly; no budding is observed at the plasma membrane (Costers et al., 2006; Mardassi et al., 1994; Wada et al., 1995). According to various electron microscopy studies, virus particle generation proceeds by budding of pre-formed nucleocapsids into the lumen of the smooth endoplasmic reticulum (ER) and/or the Golgi apparatus (Pol et al., 1997; Snijder and Meulenberg, 1998; Wada et al., 1995; Wood et al., 1970). In analogy to the distantly related coronaviruses (Klumperman et al., 1994; Stertz et al., 2007), the ERGIC – the ER–Golgi intermediate compartment – could be the budding compartment, which has, however,