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Filovirus proteins for antiviral drug discovery: Structure/function of proteins involved in assembly and budding

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ABSTRACT:

There are no approved medications for the treatment of Marburg or Ebola virus infection. In two previous articles (Martin *et al.*, 2016, Martin *et al.*, 2017), we reviewed surface glycoprotein and replication proteins structure/function relationship to decipher the molecular mechanisms of filovirus life cycle and identify antiviral strategies. In the present article, we recapitulate knowledge about the viral proteins involved in filovirus assembly and budding. First we describe the structural data available for viral proteins associated with virus assembly and virion egress and then, we integrate the structural features of these proteins in the functional context of the viral replication cycle. Finally, we summarize recent advances in the development of innovative antiviral strategies to target filovirus assembly and egress. The development of such prophylactic or post-exposure treatments could help controlling future filovirus outbreaks.

1. Introduction

This review is the third of a series of three reviews that aim to recapitulate the structure/function relationship of filovirus proteins. We have previously published two papers reviewing aspects of the filovirus entry (Martin et al., 2016) and replication cycle (Martin et al., 2017), with the intent of identifying potential targets for antiviral intervention. This third article is now dealing with filovirus assembly and budding. After a short outline on taxonomy and genomic organization, we first described structures of proteins involved in filovirus assembly and budding. Then, we depicted an overview of molecular mechanisms driving nucleocapsid formation, virion assembly and egress. We finally proposed different strategies that might be interesting for the development of filovirus antiviral therapies.

2. Background: taxonomic classification and genomic organization

This viral family belongs to the order *Mononegavirales*, which includes enveloped, single strand, negative polarity RNA-containing viruses. Filoviruses are classified in three genera: *Marburgvirus*, *Ebolavirus* and *Cuevavirus* (Bukreyev et al., 2014). The genus *Ebolavirus* includes five species: *Zaire ebolavirus* (Zaire virus – EBOV), *Sudan ebolavirus* (Sudan virus – SUDV), *Taï Forest ebolavirus* (Taï Forest virus – TAFV), *Bundibugyo ebolavirus* (Bundibugyo virus – BDBV) and *Reston ebolavirus* (Reston virus – RESTV). The genus *Marburgvirus*, represented by the single species, *Marburg marburgvirus*, contains two separate viruses, Marburg virus (MARV) and Ravn virus (RAVV). The genus *Cuevavirus* also contains a single species, *Lloviu cuevavirus,* represented by Lloviu virus (LLOV). Noteworthy, sequencing and partial characterization of LLOV have been performed in the absence of replicating virus isolates (Negredo et al., 2011). Unlike RESTV and most likely LLOV, other four ebolavirus species and marburgviruses are highly pathogenic for humans.

All filoviruses share a common genomic organization. Their genomes encode seven structural proteins: the nucleoprotein NP, the viral proteins VP35, VP40, VP30 and VP24, the surface glycoprotein GP, and the polymerase L (Feldmann et al., 1992; Sanchez et al., 1993; Negredo et al., 2011). GP is responsible for virus attachment to the target cells and drives the membrane fusion process, leading to the release of the ribonucleoprotein (RNP) complexes in the cytosol (reviewed in Martin et al., 2016). Viral RNP contains the RNA genome, which is tightly encapsidated by NP, and is associated with polymerase L and its co-factors VP35 and VP30. Recently, it has been shown that VP24 also constitutes viral RNP. Genome replication and transcription occurs in the host cell cytoplasm where inclusion bodies are formed later post

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infection (reviewed in Martin et al., 2017). VP24 is required for viral nucleocapsids maturation and silencing of VP24 expression prevents the release of the virions (Bamberg et al., 2005; Mateo et al., 2011). VP40 orchestrates the virus assembly process by recruiting newly formed nucleocapsids to the membrane, where viral particles acquire their envelope and are released by budding at the cell surface.

Assembly and budding are the final steps in filovirus intracellular cycle and, therefore, represent good targets for the development of innovative antiviral therapies. In this third review, we focus on these last steps of filovirus life cycle. First, we resume the knowledge about the different viral proteins involved in assembly and budding. We then describe how they regulate each other and how viruses interact with the cellular machinery to drive newly formed nucleocapsids from perinuclear inclusion bodies to the plasma membrane where budding occurs. Finally, we summarize recent advances in the development of innovative antiviral strategies to target filovirus assembly and budding.

3. Structure/function of filovirus assembly proteins

Assembly and release of filoviral particles is a complex process requiring interactions between different viral and host cell proteins, and which altogether divert the host cell trafficking machinery to trigger virion budding (see 4.).

3.1. Features of the nucleocapsid-associated protein VP24

Among viruses of the order *Mononegavirales*, VP24 is unique to viruses of the family *Filoviridae* (Sanchez et al., 1993). Currently, it is accepted that VP24, previously thought to be a minor matrix protein, indeed functions as a nucleocapsid maturation factor and a transcription/replication modulator (Han et al., 2003; Noda et al., 2007; Watanabe et al., 2007; Mateo et al., 2011; Zhang et al., 2012; Watt et al., 2014). In the case of ebolaviruses, VP24 is also involved in an interferon signaling antagonism and plays a role in increasing virus pathogenicity during virus passaging in guinea pigs (Reid et al., 2006). In case of marburgviruses, VP24 hijacks antioxidative stress pathway by targeting nrf2-negative regulator keap1 (Page et al., 2014). The structures of MARV, SUDV and RESTV VP24 have been solved by X-ray crystallography (Zhang et al., 2012, 2014) (Fig. 1). Although VP24 amino acid sequences are barely conserved (30%), it is likely that its overall structural organization is conserved among filoviruses (Zhang et al., 2014). Its structure reveals a α/β single domain that is organized in a pyramidal shape with two neighboring concave pockets at the bottom of the pyramid that contains highly conserved residues (green and pink in Fig. 1). A first difference between MARV and SUDV concerns residues 201 to 217 that form a great β-sheet in MARV and a central small α-helix within a flexible loop encased between small β-strands in SUDV (Zhang et al., 2012, 2014). Additionally, it is thought that the N-terminal region (1–5) of VP24 regulates filovirus nucleocapsid assembly and oligomerization (Han et al., 2003; Bamberg et al., 2005; Noda et al., 2007) (see 4.1.). It is interesting to note that structural differences have also been detected within this N-terminal region. Indeed, in MARV, VP24 N-terminus is arranged as an extended flexible strand. Conversely, in ebolaviruses, it forms a rigid α-helix that extends from the apex of the VP24 pyramid shape. This apex binds to a conserved hydrophobic pocket along one of the pyramid faces. Finally, it should be noted that the proposed immunosuppressive domain of ebolavirus VP24, which is held in a short α-helix at the top of the pyramid (in blue in Fig. 1), is also found in the non-immunosuppressive MARV VP24 (not detailed in this review).

Additional investigations on structure comparison and structure/function characterization are needed to better understand how VP24 drives the filovirus assembly and budding. It would also be interesting to elucidate the structure/function bases of ebolavirus VP24 interferon activity inhibition to design potent innate immunity restorative molecules.

3.2. Structural insights on the matrix protein VP40

The viral protein VP40 is one of the most abundantly expressed viral proteins. This protein is considered as the “matrix” protein of filoviruses because it shares analogous features of other so called proteins in other RNA viruses (oligomerization and self-assembly, hydrophobicity and membrane binding, hijacking of cellular traffic) (Timmins et al., 2004). Most of the matrix proteins of other mononegaviruses are shorter and they do not show any significant homology with filovirus VP40, indicating major structural differences and thus a folding that is unique to filoviruses (Dessen et al., 2000). VP40 intracellular distribution varies during the viral life cycle (Kolesnikova et al., 2004a, b; Nanbo et al., 2013) because it orchestrates the distribution of other viral components and viral assembly (Mittler et al., 2013; Nanbo et al., 2013) (see 4.2.). Additionally, MARV VP40 carries an interferon antagonist domain, forming a ring-like structure (Timmins et al., 2003; Oda et al., 2015) that is absent in ebolaviruses.

Moreover, despite VP40 C-terminal domain is poorly conserved (16% identical) between ebolaviruses and marburgviruses, their VP40 show many structural and functional similarities (Bukreyev et al., 1993a, b; Licata et al., 2003; Timmins et al., 2003; Jasenosky and Kawaoka, 2004; Yamayoshi and Kawaoka, 2007; Liu et al., 2010). Indeed, the structural analysis of VP40 indicted that the protein is composed of two distinct β-sandwich domains (Dessen et al., 2000; Clifton et al., 2015; Oda et al., 2015) (Fig. 1). Conversely, the VP40 N-terminal domain shows a higher percentage of similarity (42% of sequence identity) with conserved fold, and is essential for oligomerization (Hoenen et al., 2010) and budding.

It is likely that VP40 can adopt different quaternary structures that confer its various roles during the viral cycle (Gomis-Rüth et al., 2003; Hoenen et al., 2010; Bornholdt et al., 2013; Radzimanowski et al., 2014). The predominantly dimeric form acts as a building block via the N-terminal residues 52–65 and 108–117 (Bornholdt et al., 2013). This dimer form allows contacts with VP40 filaments, which are linear oligomers that interact with the plasma membrane (see 4.2.1.). VP40 can also form octameric rings structures that can bind to RNA (Gomis-Rüth et al., 2003). This structural arrangement is crucial for transcription regulation (Hoenen et al., 2005; Bornholdt et al., 2013). The N-terminal domain of ebolavirus VP40 contains also conserved overlapping tetrapeptide motifs (PTAP and PPXY) that are called late domains (Irie et al., 2005). These domains drive the assembly and budding mechanism by recruiting host cell components that are involved in multi-vesicular body biogenesis (MVB) (Harty et al., 2000; Licata et al., 2003) (see 4.2.1.). In the case of MARV, active late domains are split between NP and VP40 (Dolnik et al., 2010).

VP40 C-terminal domain, which is less conserved, contains domains that regulate its intracellular trafficking and membrane binding (Scianimanico et al., 2000; Adu-Gyamfi et al., 2013; Soni et al., 2013) (Fig. 1). It carries hydrophobic residues for anchoring to the inner leaflet monolayer of the plasma membrane. Upon membrane binding, VP40 undergoes a conformational change that favors its polymerization through interaction between the N-terminus of different VP40 molecules (see 3.3.) (Ruigrok et al., 2000). Also, in VP40 C-terminal domain, there is a patch of cationic residues that should interact with the anionic polar head of phospholipids present at the plasma membrane. This interaction is strengthened by an N-terminal loop adjacent to the C-terminal anchor for efficient VP40-mediated filovirus assembly, budding and egress (Adu-Gyamfi et al., 2014).

VP40 is a phosphorylated protein, like NP and VP30. Several phosphorylated tyrosine residues have been identified in its N-terminal domain (Kolesnikova et al., 2012) and in EBOV late domain motif PPXY (Kolesnikova et al., 2012). Therefore, VP40 phosphorylation could play a critical regulatory role in viral budding; especially because it significantly affects RNP complex recruitment to the plasma membrane (Kolesnikova et al., 2012) (see 4.2.4.).

The multi-functionality could be regulated by VP40 C- and N-tails