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Review

African swine fever virus-cell interactions: From virus entry to cell survival

Covadonga Alonso*, Inmaculada Galindo, Miguel Angel Cuesta-Geijo, Marta Cabezas, Bruno Hernaez[1], Raquel Muñoz-Moreno

Dpto. de Biotecnología, INIA, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Ctra. de la Coruña Km 7.5, 28040 Madrid, Spain

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ABSTRACT

Viruses have adapted to evolve complex and dynamic interactions with their host cell. The viral entry mechanism determines viral tropism and pathogenesis. The entry of African swine fever virus (ASFV) is dynamin-dependent and clathrin-mediated, but other pathways have been described such as macropinocytosis. During endocytosis, ASFV viral particles undergo disassembly in various compartments that the virus passes through en route to the site of replication. This disassembly relies on the acid pH of late endosomes and on microtubule cytoskeleton transport. ASFV interacts with several regulatory pathways to establish an optimal environment for replication. Examples of these pathways include small GTPases, actin-related signaling, and lipid signaling. Cellular cholesterol, the entire cholesterol biosynthesis pathway, and phosphoinositides are central molecular networks required for successful infection. Here we report new data on the conformation of the viral replication site or viral factory and the remodeling of the subcellular structures. We review the virus-induced regulation of ER stress, apoptosis and autophagy as key mechanisms of cell survival and determinants of infection outcome. Finally, future challenges for the development of new preventive strategies against this virus are proposed on the basis of current knowledge about ASFV-host interactions.

Contents

* Corresponding author. Tel.: +34 91 3476896; fax: +34 91 3478771.
E-mail addresses: calonso@inia.es (C. Alonso), galindo@inia.es (I. Galindo), cuesta.miguel@inia.es (M.A. Cuesta-Geijo), marta.cabezas@inia.es (M. Cabezas), bhernaez@cbm.uam.es, hernaez@inia.es (B. Hernaez), munoz.raquel@inia.es (R. Muñoz-Moreno).
[1] Present address: Centro de Biología Molecular Severo Ochoa, CSIC-UAM (CBMSO), C\ Nicolas Cabrera 1, Cantoblanco, 28049 Madrid, Spain.

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1. Entry of African swine fever virus

The entry of a virus into host cells is not only the first step that initiates infection, but also a key determinant of viral tropism and pathogenesis. For an intracellular pathogen, the crucial issue is not merely the crossing of the cytoplasmic membrane since the entry pathway determines whether a productive infection takes place or not. There is also a substantial degree of complexity associated with the entry pathways of large DNA viruses. ASFV interaction with cellular receptor/s promotes subsequent entry steps involving the activation of signaling and endocytosis. However, early studies on ASFV entry in Vero cells and porcine macrophages characterized this event as a low pH- and temperature-dependent process consistent with saturable and specific receptor-mediated endocytosis (Alcami et al., 1989a,b; Alcami et al., 1990; Valdeira and Geraldes, 1985). An interesting observation was that the virus entered the macrophages of another species (rabbit), thus leading to an abortive infection when using a different mechanism mediated by non-saturable or non-specific receptors. These data are consistent with clathrin-mediated entry. In fact, early electron microscopy observations found ASFV particles frequently adsorbed to invaginations similar to clathrin-coated pits (Alcami et al., 1989a).

1.1. ASFV entry is dynamin-dependent and clathrin-mediated

Clathrin-mediated endocytosis is regulated by a network of proteins and lipids that are recruited in a dynamic temporal sequence. These molecules take part in membrane bending and elongation, and final fission of the endocytic vesicle (Fig. 1) (Merrifield et al., 2005; Taylor et al., 2011). The cell invaginates the plasma membrane, thus giving rise to a small intracellular vesicle composed by a clathrin coat with adaptor proteins, Epsin15 (Ede1), and dynamin. The latter recruits BAR domain proteins, which in turn recruit actin-related signaling molecules (Merrifield et al., 2002; Traub, 2009). Dynamin and actin nucleation at the base and the neck of the vesicle would propel the membrane inward and promote scission of the clathrin-coated pit (Taylor et al., 2012). Epidermal growth factor receptor (EGFR) and transferrin are characteristic proteins that are internalized through this endocytic pathway.

Biochemical and molecular analysis of ASFV entry, using the specific dynamin inhibitor dynasore, but also a dominant-negative mutant of dynamin-2, have revealed that viral endocytosis depends on dynamin GTPase, which participates in vesicle fission from the plasma membrane (Hernaez and Alonso, 2010). Clathrin-assembly inhibitors, such as chlorpromazine, and also knock-out of clathrin-adaptor Epsin15 by expression of a dominant-negative mutant, profoundly affect virus infectivity and subsequent virus production. This was shown using a highly adapted virus isolate (BV71V), a low passage one in Vero cells, and also in the WSL cell line, derived from wild boar lung cells (Hernaez and Alonso, 2010). Moreover, at very early post-infection times, virions colocalize with clathrin-heavy-chain antibodies on the cell surface. Jointly, these findings led to the conclusion that ASFV entry involves dynamin-dependent and clathrin-mediated endocytosis (Hernaez and Alonso, 2010). In addition, this entry mechanism requires cholesterol (Bernardes et al., 1998) as it is sensitive to membrane cholesterol depletion by cyclodextrin. Conversely, it is insensitive to nystatin, a drug that disorganizes cholesterol in lipid rafts without reducing cholesterol levels (Hernaez and Alonso, 2010). These data are not consistent with a caveolae-dependent pathway for entry, which is another dynamin-dependent endocytic route. Other information about the relevance of the cholesterol biosynthesis pathway for virus entry is discussed below.

Although it is tempting to exclude clathrin-mediated endocytosis because of the large size of ASFV particles (200 nm), there is increasing scientific evidence that the direct participation of actin in membrane dynamics during clathrin-mediated endocytosis promotes the efficient internalization of large viruses, such as vesicular stomatitis virus (70 × 200 nm) (Cureton et al., 2009, 2010), and even bacteria (Pizarro-Cerda et al., 2010; Veiga and Cossart, 2005) and fungi (Moreno-Ruiz et al., 2009). This may be the case of ASFV.

1.2. Entry by macropinocytosis. The role of actin

Recent studies on ASFV entry, using BA71V or E70 isolates either in Vero or IPAM cells, have demonstrated the activation of the small Rho-GTPase Rac1 immediately after infection (Quetglas et al., 2012; Sanchez et al., 2012). Rac1 has been implicated in the modulation of actin dynamics and in the stabilization of microtubules by acetylation. Disruption of actin cytoskeleton with cytochalasin D alters infectivity (Sanchez et al., 2012), in contrast with others reporting scarce effects on infectivity using jasplakinolide and latrunculin A (Hernaez and Alonso, 2010). Field emission scanning electron microscopy has revealed that actin is involved in