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Review

African swine fever virus organelle rearrangements

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ABSTRACT

Like most viruses African swine fever virus (ASFV) subsumes the host cell apparatus in order to facilitate its replication. ASFV replication is a highly orchestrated process with a least four stages of transcription, immediate-early, early, intermediate and late. As the infective cycle progresses through these stages most if not all of the organelles that comprise a nucleated cell are modified, adapted or in some cases destroyed. The entry of the virus is receptor-mediated, but the precise mechanism of endocytosis is a matter of keen, current debate. Once ASFV has exited from the endosomal–lysosomal complex the virus life-cycle enters into an intimate relationship with the microtubular network. Genome replication is believed to be initiated within the nucleus and ASFV infection completely reorders the structure of this organelle. The majority of replication and assembly occurs in discrete, perinuclear regions of the cell called virus factories and finally progeny virions are transported to the plasma membrane along microtubules where they bud out or are propelled away along actin projections to infect new cells. The generation of ASFV replication sites induces profound reorganisation of the organelles that comprise the secretory pathway and may contribute to the induction of cellular stress responses that ASFV modulates. The level of organisation and complexity of virus factories are not dissimilar to those seen in cellular organelles. Like their cellular counterparts the formation of virus factories, as well as virus entry and exit, are dependent on the various components of the cytoskeleton. This review will summarise these rearrangements, the viral proteins involved and their functional consequences.

Contents

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1. Incoming African swine fever virions are delivered to perinuclear sites along microtubules

1.1. Virus entry

Like all intracellular pathogens the first part of the host cell that African swine fever virus (ASFV) encounters is the plasma membrane. *In vivo* viral replication can be detected in a number of different cell types in the soft tick host (Kleiboeker et al., 1998), however virus replication is principally restricted to cells of the mononuclear phagocytic system in the domestic pig and bushpig (Oura et al., 1998a). *In vitro* ASFV can enter a wide range of cell types from a number of different species including those within which its replication is then restricted (Alcamí et al., 1990; Carrascosa et al., 1999). ASFV can enter Vero cells and porcine macrophages by receptor-mediated endocytosis (Alcamí et al., 1989, 1990), but can be also taken up non-specifically by rabbit macrophages but the latter infection is not productive (Alcamí et al., 1990). Successful infection of porcine macrophages is linked to the expression of the CD163 scavenger receptor (Sánchez-Torres et al., 2003) and antibodies to CD163 inhibit virion binding to macrophages. In addition to these routes, virus enters midgut digestive cells in ticks bound to red blood cells (Kleiboeker et al., 1998, 1999), and loss of the CD2v gene responsible for hemadsorption to red blood cells significantly reduces viral replication in ticks (Rowlands et al., 2009). Dissemination to other cell types of the tick is not dependent on CD2v as virus lacking the gene replicates to similar levels as virus expressing CD2v if it is injected across the gut wall (Rowlands et al., 2009). ASFV may also be able to directly infect neighbouring cells through virus-tipped actin projections that form late during infection (see Section 5), although this mechanism could also serve to embed virions on the surface of adsorbed red blood cells. Lastly, ASFV induces apoptosis in infected cells (Gómez-Villamandos et al., 1995; Oura et al., 1998b; Ramiro-Ibáñez et al., 1996) and it is possible that intracellular virus may be taken up as part of apoptotic cell bodies by phagocytic cells.

1.1.1. Virus structure

The structure and morphogenesis of the ASF virion will be discussed thoroughly in another chapter of this special issue (Salas and Andrés, 2012), but a brief description is merited here to aid the discussion on entry. Virions are icosahedral structures approximately 200 nm in diameter, the centre of the virus contains the genomic DNA which is then surrounded by matrix proteins which are primarily derived by processing of two viral polypeptides, pp220 and p62. The matrix is built up on the internal face of viral membranes (see Section 4), while the capsid is assembled on the outer face of the viral membranes (Andrés et al., 1997). A further membrane, the external envelope, is obtained when the virus buds out through the plasma membrane, however the importance of this envelope is unclear as it is not required for infectivity (Andrés et al., 2001). The p12 protein has been identified as the viral attachment protein (Carrascosa et al., 1991) and immunogold electron microscopy has localised this protein to the external envelope (Carrascosa et al., 1993). However, immunofluorescence microscopy showed that p12 is incorporated into virions within the virus factory which occurs before virus exit (Angulo et al., 1993) implying that p12 is part of the intracellular virus and this has recently been confirmed by immunoelectron microscopy (Salas and Andrés, 2012).

1.1.2. Virus entry mechanisms

The mechanism of ASFV uptake *in vitro* has been the subject of two recent detailed studies which separately concluded that clathrin dependent endocytosis (Hernaez and Alonso, 2010) and macropinocytosis (Sánchez et al., 2012) were the primary route of virus entry. The authors of these two papers used different methodologies to determine what constituted a successful entry event and this may have contributed to the different conclusions from apparently similar experiments. Sánchez et al. defined entry as the presence of the major capsid protein p72 1 h post infection as detected by flow cytometry and reported the percentage of cells staining positive for p72. Using this assay they showed that 5-ethylisopropyl amiloride (EIPA), IPA-3 and cytochalasin D, which are drugs that inhibit Na^+/H^+, Pak1 phosphorylation and actin polymerisation respectively and have a downstream effect on macropinocytosis, inhibited virus entry. Sánchez et al. also showed that treatment with chlorpromazine, a drug that interferes with clathrin-mediated endocytosis, had no effect on ASFV entry, but did affect late protein synthesis. Sanchez et al. therefore concluded that macropinocytosis was the primary cell entry method for ASFV. Hernaez and Alonso used the expression of the early protein p30 6 h post infection as a definition of successful virus entry, detected this by flow cytometry and reported the percentage of cells staining positive for p30. Using this method Hernaez and Alonso reported that EIPA only had a moderate effect on ASFV entry and that the actin disrupters jasplakinolide and latrunculin A had none at all. Hernaez and Alonso also reported that chloropromazine significantly inhibited virus entry and so concluded that clathrin-mediated endocytosis was the primary mechanism for ASFV entry, consistent with early EM observations showing virus in coated pits (Alcamí et al., 1989, 1990; Valdeira and Geraldes, 1985). One of the criticisms by Sánchez et al. of the Hernaez and Alonso paper was the use of p30 expression as a read-out for virus entry, this is a reasonable criticism as expression of early genes is dependent on a number of distinct events post entry (see below). However, the method used by Sánchez et al. to determine virus entry did not define the threshold for a p73 positive cell (i.e., entry of one virions or ten virions) and it is unclear whether a cell infected with one virion would count the same as cell infected with one hundred. To complicate matters further a number of the experiments, such as those with chloropromazine, in the two papers directly contradict each other and other researchers using [^{3}H]labelled ASFV have shown that cytochalasin D had no effect on ASFV binding to, or up take within, Vero cells (Valdeira et al., 1998). Furthermore experiments in macrophages with wortmannin, another drug that can influence macropinocytosis, does not affect virus replication (Basta et al., 2010). Lastly it is worth considering that the different authors discussed above all used different virus preparation methods, Basta et al. used neat tissue culture supernatants, Sánchez et al. concentrated their virus by centrifugation, and Hernaez and Alonso used virus purified by sucrose sedimentation. Valdeira et al. used Percoll purification which has been shown to eliminate contamination with membranes and vesicles (Carrascosa et al., 1985).

1.2. Delivery to perinuclear sites requires microtubules

After entry virions locate to the endosomal–lysosomal complex from where they exit into the cytoplasm. How this is achieved is poorly understood, but is dependent on pH as treatment with lysosomotropic weak bases such as ammonium chloride or chloroquine prevent virion escape into the cytoplasm (Alcamí et al., 1989; Geraldes and Valdeira, 1985; Valdeira and Geraldes, 1985). Virions can be maintained in neutralised vacuoles for at least 72 h without any significant loss of infectivity, as wash out of chloroquine allows replication to progress normally (Geraldes and Valdeira, 1985; Valdeira and Geraldes, 1985). Exit from endosomes/lysosomes may also be dependent on proteolysis as treatment with phenylmethylsulphonyl fluoride produces a similar phenotype to that seen with lysosomotropic drug treatment (Valdeira et al., 1998). Electron micrographs hint at a fusion between one of the viral envelopes and the membrane of the cellular compartment (Valdeira et al., 1998) and this may be dependent on cholesterol as viral DNA fails