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African swine fever virus p10 protein exhibits nuclear import capacity and accumulates in the nucleus during viral infection

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Abstract

African swine fever virus (ASFV), a large enveloped DNA-containing virus, infects domestic and wild pigs, and multiplies in soft ticks, causing an economically relevant hemorrhagic disease. Evaluation of the nuclear import ability of ASFV p10 protein was the major purpose of the present work. Two approaches were used to determine if p10 protein is imported into the nucleus by an active process: a yeast-based nuclear import assay and the determination of the subcellular localization of p10 protein in mammalian cells by fluorescence microscopy. The results obtained clearly demonstrate that p10 protein is actively imported into the nucleus, both in yeast and mammalian cells. Experiments aiming at identifying the critical residues responsible for the nuclear import of ASFV p10 protein indicate that the amino acids comprised between the positions 71 and 77 are important, although not sufficient, for the protein active nuclear import. In ASFV-infected cells, the p10 protein strongly accumulates in the nucleus at late times post-infection, indicating that p10 protein may accomplish an important function inside the nucleus during the late phase of the viral replication cycle.

Keywords: African swine fever virus; Nuclear import; p10 protein

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1. Introduction

African swine fever virus (ASFV), the only member of the family *Asfarviridae* has been described as a missing evolutionary link between the families *Poxviridae* and *Iridoviridae*, since ASFV shares the genomic organization and the striking icosahedral symmetry of those families, respectively (Salas et al., 1999). The early descriptions of ASFV infections of domestic pigs were of an acute hemorrhagic fever, which caused approximately 100% mortality. In the last years, an increased number of isolates were identified, where the virus pathogenesis may range from rapidly fatal to subclinical, chronic or no explicit symptoms of disease (Leitão et al., 2001; Dixon et al., 2004).

The virus particle possesses a complex structure composed of several concentric domains with an overall icosahedral shape and an average diameter of 200 nm (Carrascosa et al., 1984; Andrés et al., 1997, 1998). The viral core is composed of a DNA-containing nucleoid surrounded by a thick protein layer, the core shell, and is wrapped by a lipid envelope and an icosahedral capsid (Carrascosa et al., 1984; Andrés et al., 1997). Extracellular ASFV particles usually possess an additional membrane acquired by budding through the host cell plasma membrane (Breese and Pan, 1978). The viral genome is a single molecule of double-stranded DNA ranging in size from 170 to 190 kbp with terminal inverted repetitions and terminal cross-links, which encodes approximately 50 structural proteins (Yáñez et al., 1995). Some of the ASFV proteins have been shown to exhibit DNA-binding activity like the structural p10 protein which is localized in the viral nucleoid (Muñoz et al., 1993; Andrés et al., 2002).

Although ASFV has been considered for many years as a virus that replicates exclusively in the cytoplasm of infected cells, the observation that the virus does not replicate in enucleated Vero cells nor is its DNA synthesized (Ortin and Viñuela, 1977) raised the possibility that the nucleus is involved in viral DNA replication. More recently, it has been reported that the replication of viral DNA is initiated in the nucleus, a stage which is followed by a longer cytoplasmic phase (García-Beato et al., 1992; Rojo et al., 1999). The mechanisms of ASFV viral genome nuclear import and export are still unknown. However, as described for other viral genomes, this nucleus-cytoplasm transport is most likely mediated by viral proteins associated with the ASFV DNA (Whittaker and Helenius, 1998).

The active and bidirectional transport of proteins across the nuclear pore complex (NPC) is a tightly regulated process that plays a vital role in eukaryotic cells. Small molecules like metabolites, ions and small proteins can diffuse through the 9-nm NPC channel in either direction, between the nucleus and cytoplasm, which sets an upper limit for free diffusion to ~45–60 kDa. However, particles as large as 36 nm in diameter can be transported through the NPC by a selective and energy-dependent mechanism that acts to translocate macromolecules into and out of the nucleus. The active nucleus-cytoplasm transport is mediated by shuttling receptors that interact with localization signals on cargo molecules, RanGTP and proteins of the NPC (Görlich and Kutay, 1999; Macara, 2001).

The active import of proteins across the nuclear envelope requires the presence of specific targeting sequences within the protein, named nuclear localization signals (NLSs), even when the proteins are small enough to diffuse through the NPC (Görlich et al., 1996). There are at least three types of NLSs, which are characteristically rich in the basic amino acids lysine and arginine (Dingwall and Laskey, 1991). The first type, referred as classical NLS, is composed of a basic amino acid stretch $(K/R)_{4–6}$ preceded by a glycine, proline or an acidic amino acid residue, similar to the NLS of simian virus 40 (SV40) large T antigen (Kalderón et al., 1984). The second type is a bipartite sequence composed of two basic amino acid stretches separated by an intervening nonconserved 10–12 amino acid spacer: $(K/R)_2X_{10–12}(K/R)_3$, an example of this being the *Xenopus Laevis* nucleoplasmin (Dingwall et al., 1988). The third type is a less well-conserved sequence with few basic residues such as that of the adenovirus E1A protein (KRPRP) (Lyons et al., 1987). However, many other NLSs have been identified that differ from these sequences with respect to size and/or the content in basic amino acids (Makkerh et al., 1996; Michael et al., 1997). In addition to linear NLSs, discontinuous epitopes that come together upon folding into tertiary structure have been described to contribute to the nuclear import of histone proteins (Baake et al., 2001). Therefore, it is