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Localization of ASFV DNA replication and morphological study of subnuclear compartments during viral infection

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African Swine Fever Virus (ASFV) is one of the most threatening agents of domestic pig diseases, without a vaccine or treatment, being its control exclusively based on compulsive sanitary measures. Until now, ASFV was thought to perform its viral life cycle within the cytoplasm although recent evidences indicate the presence of viral DNA material inside the host cell nucleus [1-3] promoting ASFV reclassification into the Nucleocytoplasmic Large DNA Viruses group (NCLDV) [4]. So far, no studies have been performed regarding ASFV genome replication phenomena or the host nuclear compartments morphology during cellular infection. Therefore, we aim to unveil the spatiotemporal localization of ASFV DNA replication and the distribution patterns of three subnuclear domains (PML, speckles and coiled bodies), in order to improve knowledge on host nucleus-viral interactions.

For the viral DNA replication foci staining, Vero cells were synchronized at a G2/M stage with nocodazole (250 ng/ml, 24h). Following synchronization, cells were grown on glass coverslips ($5{,}0x10^4$ cells/cm^2) and infected with ASFV-Ba71V isolate (1h adsorption period with a multiplicity of infection of 5). Afterwards, at specific time-points of viral infection, BrdU (150 µM/ml) was added for a short pulse (30 min) and immediate fixation was performed. For BrdU and ASFV immunodetection, the following primary and secondary antibodies were used: sheep polyclonal anti-BrdU (GTX21893, Genetex, USA; 1:100), an in-house clarified swine anti-ASFV whole-serum (1:100); Alexa Fluor 594 donkey anti-sheep IgG (A-11016, Life Technologies, USA; 1:500) and a FITC rabbit anti-swine IgG (ab6773, Abcam, UK; 1:400). For PML, speckles and coiled nuclear bodies immunolabeling a rabbit polyclonal anti-PML (ab53773, Abcam; 1:100), a goat polyclonal anti-SC35 (sc-10252, Santa Cruz Biotech, USA; 1:50) and a rabbit popyclonal anti-coilin (sc-32860, Santa Cruz Biotech; 1:50) were used, while DyLight 594 donkey anti-rabbit IgG (ab98490, Abcam; 1:500) and Alexa Fluor 594 chicken anti-goat IgG (A-21468, Molecular Probes, 1:400) were used as the secondary antibodies. Microscopic analysis of cells was performed with a Leica epifluorescence microscope (model DM R HC, Germany). *De novo* synthesized viral DNA was identified in a scattered nuclear localization (discreet dots) during the initial period of infection (Fig. 1, row 1), whereas in a later phase of infection (8h pi), most of the BrdU signal accumulated in the cytoplasmic viral factory (Fig. 1, row 2). PML bodies and nuclear speckles presented a morphological enlargement and a decreasing number in ASFV infected Vero cells (Fig. 2, rows 1 and 2). In an opposite manner, the coiled bodies increased their number during infection (Fig.2, row 3).

Our results provide the first evidence that ASFV DNA replication also occurs inside the host nucleus. Nuclear discrete replication foci, at the initial onset of the viral infection, contrast to an accumulation of synthesized viral DNA inside the bigger cytoplasmic viral factory, suggesting that these two distinct patterns are related to the viral life cycle demands. The early PML reorganization can probably be related with cellular antiviral defense mechanisms, given that DNA damage response and p53 are activated during ASFV infection. The low number of nuclear speckles observed in ASFV-infected cells, associated with their enlargement, is most possibly related to the relocation of host splicing factors, since ASFV genome lacks intronic regions.

References

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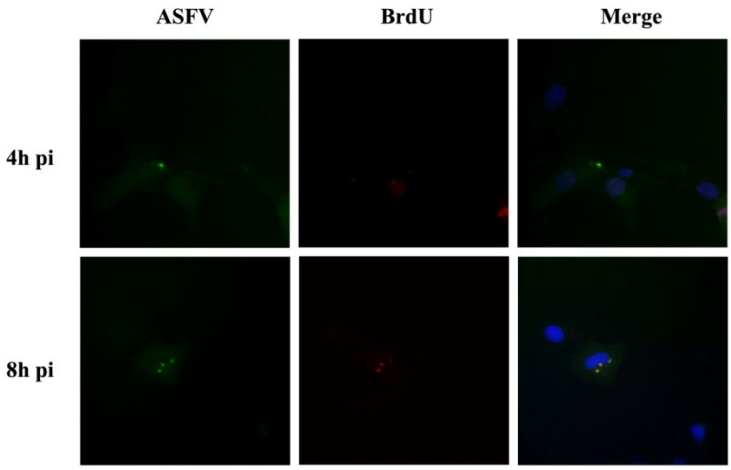

Figure 1. **Viral genome synthesis in host nucleus and cytoplasmic viral factories**. BrdU incorporation (red) revealed initial ASFV DNA replication foci inside the host nucleus followed by a cytoplasmic viral factory phase. ASFV-infected cells were stained in green. DAPI stained nuclear DNA (blue).

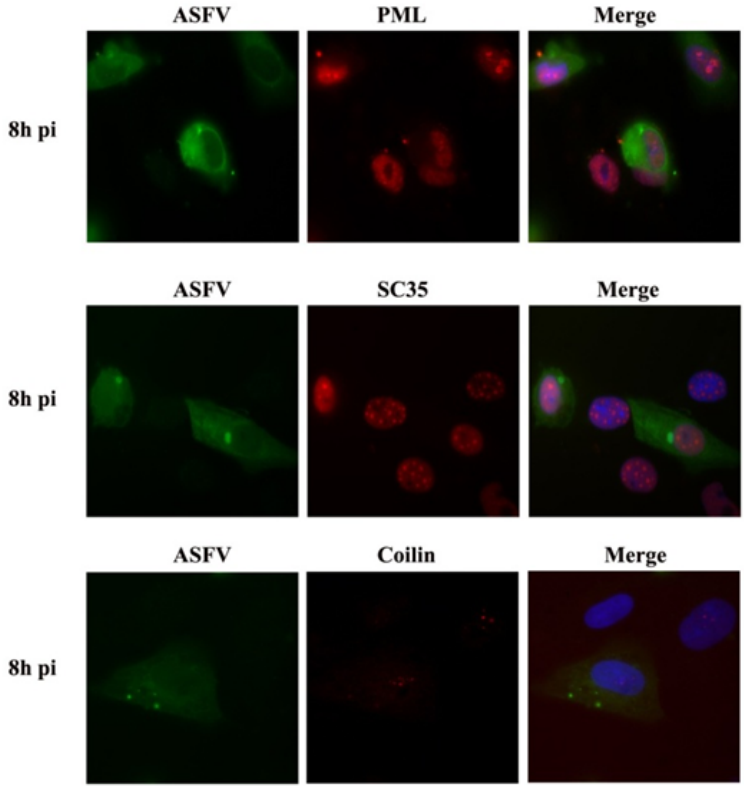

Figure 2. **PML, speckles and coiled nuclear bodies disruption during ASFV infection**. PML and nuclear speckles/SC35 (red) in ASFV-infected cells (green) showed spatial rearrangements with fewer organized compartments (row 1 and 2), while coiled bodies/coilin (red) revealed a multifocal relocation pattern during viral infection (row 3).

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