African horse sickness virus (AHSV) Seg-10 ORF2 potentially encodes a novel non-structural protein

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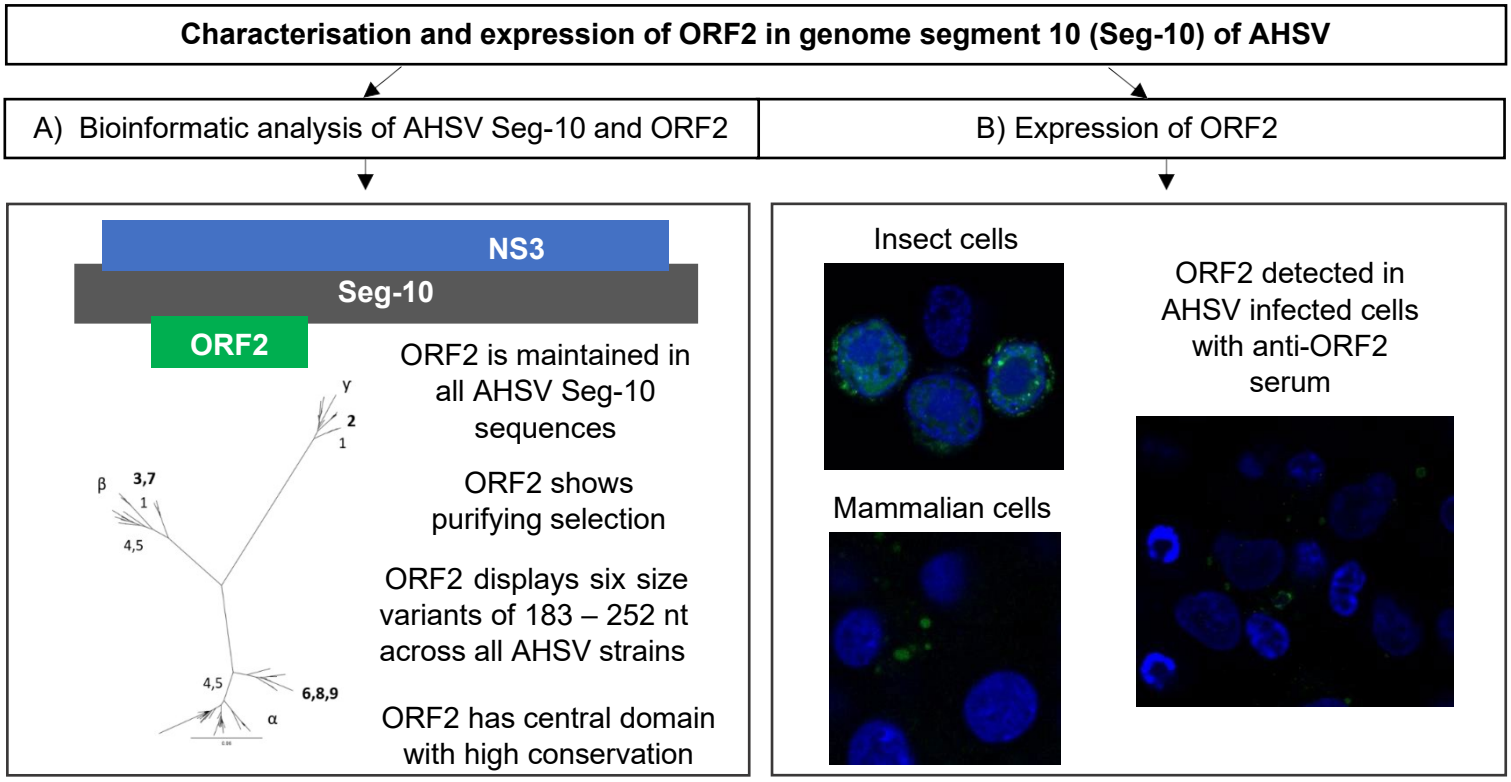

Figure 1: Investigation of the second open reading frame in AHSV genome segment 10 (Seg-10). A) Bioinformatic analysis of Seg-10 identified the conserved ORF2 in all strains of AHSV. B) Recombinant expression of the protein encoded by ORF2 in insect and mammalian cells. After production of an ORF2 specific antibody, the protein was detected during normal AHSV infection.

Introduction: Viral genome segments are often under strong selective pressure to enhance the coding capacity of the genome, allowing expression of multiple proteins from a single mRNA (Sealfon *et al*., 2015; Stewart *et al*,. 2015). A second additional open reading frame, ORF2, was identified in African horse sickness virus (AHSV) Seg-10 which encodes the non-structural protein NS3. The ORF2 conservation, size, nature, expression, function and potential subcellular localisation of the putative protein product AHSV is not known.

Methodology: A bioinformatic analysis of all available AHSV Seg-10 sequence data was done to investigate the two overlapping open reading frames and their relationship, to identify conservation selection pressures on ORF2, and to predict RNA secondary structure and/or RNA structural elements. The protein encoded by AHSV ORF2 was recombinantly expressed in insect and mammalian cells. Antibodies raised against ORF2 was used to investigate the presence and subcellular localisation of the protein during AHSV infection.

Results: ORF2 was maintained in over 400 AHSV Seg-10 sequences, and six size variants were identified ranging from 183 to 252 nucleotides. ORF2 clustered into 3 clades, and showed high conservation in the central region. AHSV Seg-10 ORF2 shows strong positive selection. Recombinant baculoviruses were confirmed to express ORF2 or ORF2-eGFP. Localisation of ORF2-eGFP was specific within the nucleus and cytoplasm of Sf9 cells. Bac-ORF2 was used for gel purification of the ORF2 protein and subsequent ORF2 antibody production. ORF2 transiently expressed in BSR-T7 cells initially localised in the cytoplasm and later moved to the nucleus, and ultimately caused cells to shrivel showing a cytotoxic effect. Using this antibody, ORF2 could be detected in AHSV infected cells, potentially identifying a novel AHSV non-structural protein.

Conclusion: This is the first detection of a putative protein product of Seg-10 ORF2 during normal AHSV infection, and paves the way for further functional analysis of this protein in the AHSV replication cycle.

References: Sealfon, R. S., M. F. Lin, I. Jungreis, M. Y. Wolf, M. Kellis et al., 2015 FRESCo: finding regions of excess synonymous constraint in diverse viruses. Genome Biol 16: 38.
Stewart, M., A. Hardy, G. Barry, R. M. Pinto, M. Caporale et al., 2015 Characterization of a second open reading frame in genome segment 10 of bluetongue virus. J Gen Virol 96: 3280-3293.

Keywords: African horse sickness virus, Seg-10, additional open reading frame, synonymous constrain regions, recombinant baculovirus expression.