1. SUMMARY

Orf virus (ORFV) strain D1701-V, a parapoxvirus belonging to the family *Poxviridae* possesses strong immune-modulating properties, enhancing long-term immunity against different pathogens while providing usually short-term vector-specific protection. For these reasons ORFV attracted attention as a novel virus vector system that was recently successfully used for the generation of recombinant vaccines. Therefore, the identification and characterization of viral genes involved or potentially involved in host tropisms or immune modulation is of great interest. The examples of such are ORFV genes encoding proteins which contain ankyrin-repeats (AR).

The main objective of the present work was to perform initial characterization of ORFV genes *ORFV126*, *ORFV128* and *ORFV129*, which are present in the near-terminal right end of the viral genome and code for three of the five AR-containing proteins, formerly designated as ANK-1, ANK-2 and ANK-3, respectively. First, ANK proteins expressed in bacteria were purified and used for rabbit immunization. Rabbit polyclonal sera were subsequently used to analyze the expression of ANK products in ORFV-infected cells and their subcellular localization in two different mammalian cell lines. Finally, detailed mutagenesis study of ANK-1 and ANK-2 was performed and resulted in identification of specific subcellular targeting signals within these proteins.

ANK-1 protein contains up to ten potential ankyrin repeats and is expressed as intermediate or late ORFV gene. The present study shows that ANK-1 is targeted to mitochondria of ORFV-infected and in ANK-1 transiently expressing cells. Taking advantage of ANK-1-EGFP fusion proteins and confocal fluorescence microscopy mutational and deletion analyses, I have demonstrated the importance of two ankyrin repeats (AR8 and AR9), which may contain a novel class of mitochondria-targeting sequence (MTS) in the central to C-terminal part of this AR-containing protein. The fluorescent findings were corroborated by cell fractionation and Western blotting experiments. The presented results show for the first time the co-localization of poxviral AR protein with mitochondria and indicate that ankyrin repeats may define a novel class of mitochondria targeting sequences.

ANK-2 protein contains up to ten potential ankyrin repeats and is expressed as an early ORFV gene. In both transiently transfected and ORFV-infected Vero cells ANK-2 localizes to nucleus and co-localize with 2 subnuclear compartments - nucleoli and nuclear speckles (splicing factor compartments, SCFs). These data demonstrate the independence of

nuclear ANK-2 expression from the presence of other ORFV proteins and virus multiplication. Localization of ANK-2 was clearly different in mouse cells non-permissive for ORFV replication (NIH 3T3), in which fluorescence signal was predominantly found in the cytoplasm and only weak in the nucleus. This observation could suggest ANK-2 role as a host range factor of ORFV contributing, at least partly, to its tropism. Deletion sequence analysis demonstrated that a single region located between amino acids 433 and 474 at the C-terminus of ANK-2 is responsible for nuclear and nucleolar targeting. The sequence required for nucleolar accumulation overlaps the putative nuclear localization signal (NLS) and arginine-rich motif (ARM) of ANK-2, whereas nuclear speckles co-localization seems to be dependent on the integrity of ankyrin repeat domains.

ANK-3 protein contains up to nine potential ankyrin repeats and is expressed as an early ORFV gene. ANK-3 protein localizes to the cell nucleus in both transiently transfected and ORFV infected Vero cells, but was not found in nucleoli. Similar results were obtained when ANK-3 was expressed in NIH 3T3 cell line. Targeting of ANK-3 to the nucleus seems to be independent from the expression of other ORFV products and virus multiplication.

The presented results open the avenue for more detailed investigations on cellular binding partners and the function of ANK proteins in viral replication or virulence.