Role of Varicella Zoster virus ORF9p in the secondary egress : importance of its interaction with the cellular Adaptin Protein-1.

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ORF9p (homologous to HSV-1 VP22) is a VZV tegument protein essential for the viral replication. During the lytic cycle it is the mostly expressed gene. We have recently demonstrated that it is a substrate of the viral kinase ORF47p and that its ORF47p-dependent phosphorylation is important for the secondary envelopment process. We also have identified an acidic cluster (AC) within the protein that is important for its correct localization in the infected cells and for the interaction with ORF47p. The recombinant VZV expressing ORF9p-ΔAC presents an accumulation of capsids in the perinuclear space. ORF9p seems then to play an important role in several steps of the egress process. In this context, we sought to identify cellular partners of ORF9p that might be important for these functions. Via a two-hybrid screen we identified AM1P1 (μ subunit of the cellular apdatin-1 complex) as potentially interacting with ORF9p.

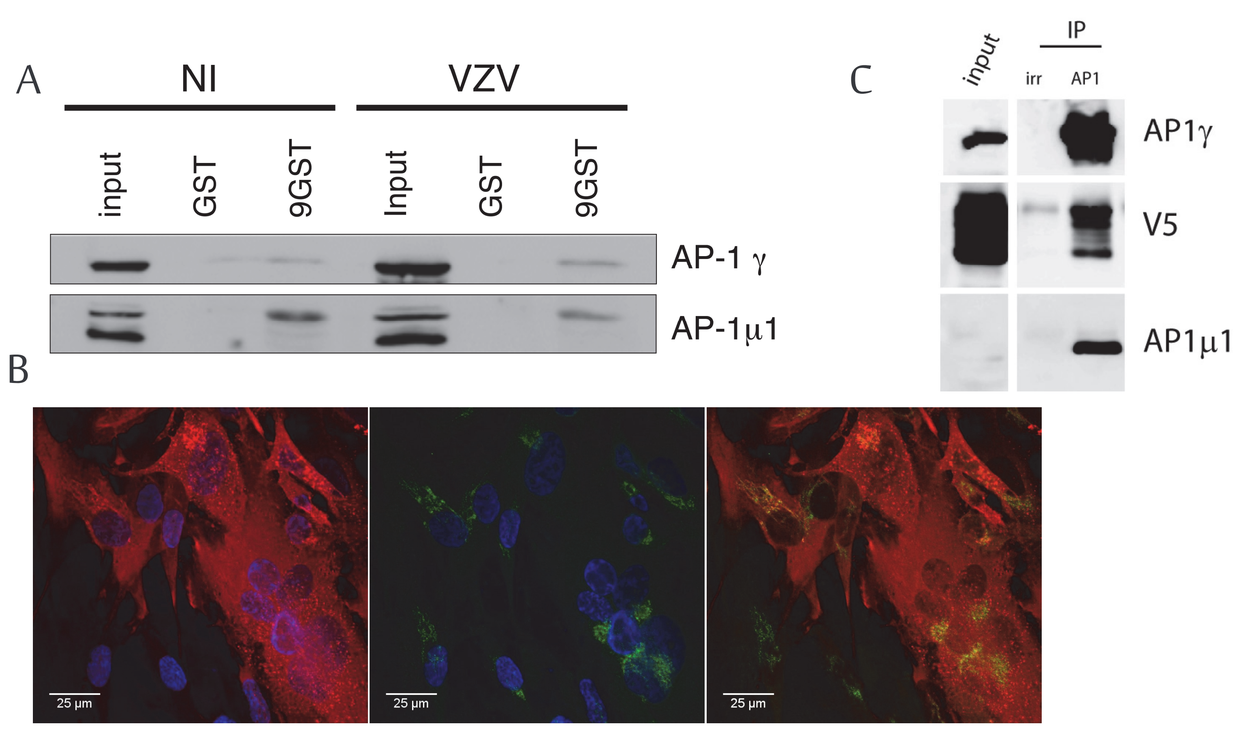

Figure 1: ORF9p interacts with the AP-1 complex

(A) MeWo cells non infected or infected with the VZV-ORF9-V5 for 24 hours, were harvested in lysis buffer and added to purified GST or ORF9-GST, performing a GST pull-down. The pulled-down proteins were resolved by SDSPAGE and immunoblotted using antibodies against the γ and μ subunits of the adaptin complex. (B) Immunofluorescence on VZV-infected MRC5 cells against ORF9p (red channel) and gamma AP1 (green channel), nuclei are labeled with hoechst. (C) MeWo cells infected with the VZV-ORF9-V5 for 48 hours, were harvested in lysis buffer and cell extracts were incubated with beads coated with anti- γ -adaptin antibody. The immunoprecipitated proteins were resolved by SDS-PAGE and immunoblotted using antibodies against V5 tag, AP1- γ and AP-1μ1 .

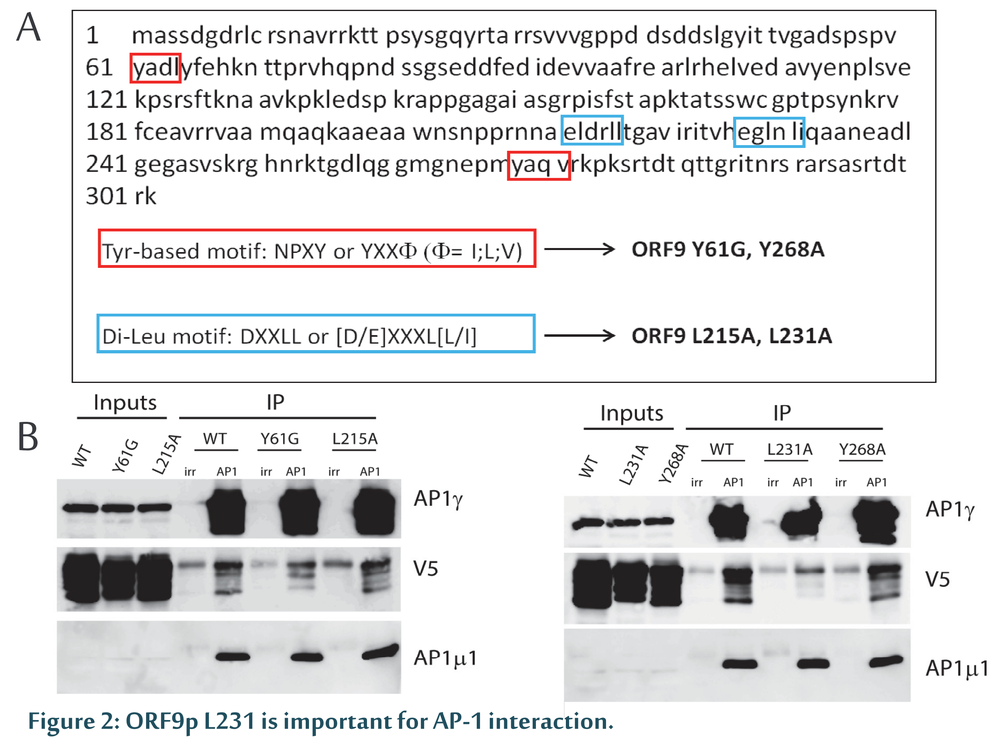

Figure 2: ORF9p L231 is important for AP-1 interaction.

(A)The AP1 complex is known to interact with its cargo via tyrosine-based or dileucine motifs. An *in silico* analysis of ORF9p reveals the presence of 4 potential AP-1 interaction domains. Via homologous recombination in bacteria , these sites were independently mutated within a BAC containing the entire VZV pOka genome. The transfection of the BAC into MeWo cells led to the generation of a productive infection, even though ORF9p L231A clearly has a growth defect. (B) The γ subunit of the AP-1 complex was immunoprecipitated from total extracts of MeWo cells infected with either WT- or ORF9p-mutant VZV. The immunoprecipitates were resolved by SDS-PAGE and immunoblotted using antibodies against V5 tag, AP1- γ and AP-1μ1 .

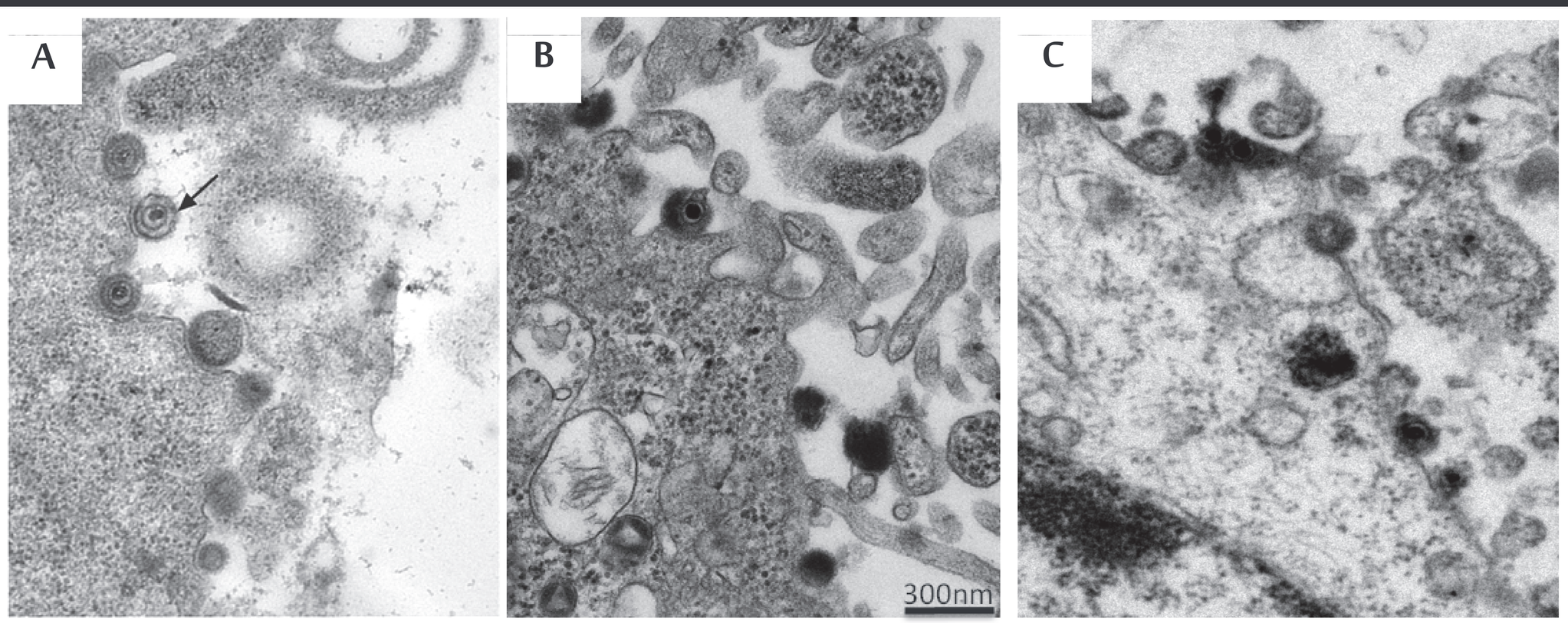

Figure 3: The mutation L231A dramatically impairs the formation and egress of viral particles. Surprisingly, the deletion of the 33 first amino acids leads to a similar phenotype.

MeWo cells were infected VZV-ORF9-V5 (A) , VZV-ORF9-L231A-V5 (B) or VZV-ORF9-33-302 (C); after 48 hours cells were fixed and analyzed by transmission electron microscopy (TEM).

CONCLUSIONS and PERSPECTIVES:

- ORF9p interacts with the AP1 complex
- VZV-ORF9 L231A presents a strong growth defect.whereas the mutation of Y61, L215 or Y268 has little impact on viral infectivity
- The interaction of ORF9p with AP1-complex is strongly impaired with the L231A mutant
- VZV ORF9p L231A and VZV ORF9p 33-302 display defect in the egress process.
- Is ORF9p palmitoylated? On Cysteine 10? Is this palmitoylation necessary for the interaction with the AP-1 complex?
- Is ORF9p important for the correct localization of glycoproteins at the site of secondary egress?
- Are these ORF9p mutant strains still able to infect dendritic and T cells ? To enter and exit latency? To induce PHN?

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