Study of the role of the complex formed by the Varicella Zoster virus ORF9p and the cellular Adaptin Protein-1 in the secondary egress

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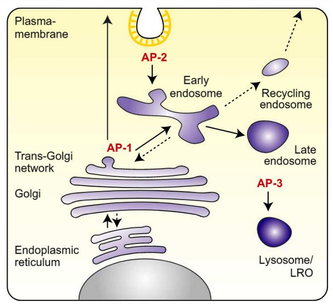
ORF9p (homologous to HSV-1 VP22) is a VZV tegument protein essential for the viral replication. We have shown that ORF9p co-immunoprecipitates with the cellular clathrine Adaptor Protein-1 (AP-1) complex and have identified the leucine 231 as important for this interaction. The mutation of this residue strongly impairs the virus replication in MRC-5 cells and in a 3D-skin model. These observations suggest that the interaction between ORF9p and AP-1 is important for VZV replication and envelopment. In this context, the role of the ORF9p/AP-1 complex in the secondary envelopment has been further investigated.

A Yeast-two-Hybrid experiment using ORF9p as a bait screened against the human ORFeome 5.1 has identified AP-1µ, a subunit of the cellular AP-1 complex as a putative partner of ORF9p. AP-1 complex mediates the transport of cargo molecules between TGN and endosomes and might thus be important for the transport of the viral components between the cellular compartments.



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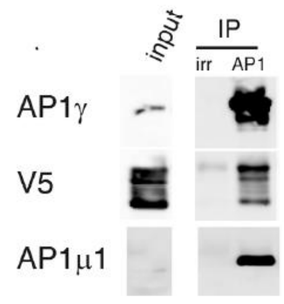


ORF9p is part of the AP-1 complex in infected cells

The gamma subunit of the AP-1 complex was immunoprecipitated from cellular extracts of WT VZV-infected MeWo cells and the presence of ORF9p, AP1γ and AP1µ1 subunits was verified by western blotting.

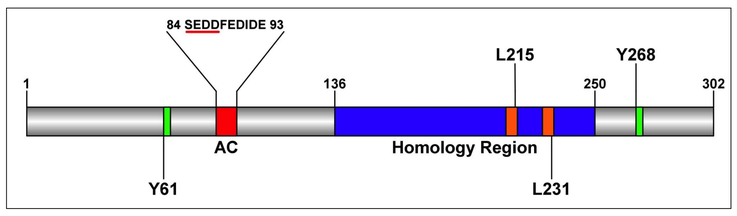
2

Tyr-based motifs, Di-leucine motifs and acidic clusters are known to be important for the interaction with AP-1

Tyr-based motifs: NPXY or YXXΦ (Φ=I;L;V)

Di-Leu motifs: DXXLL or [D/E]XXXL[L/I]

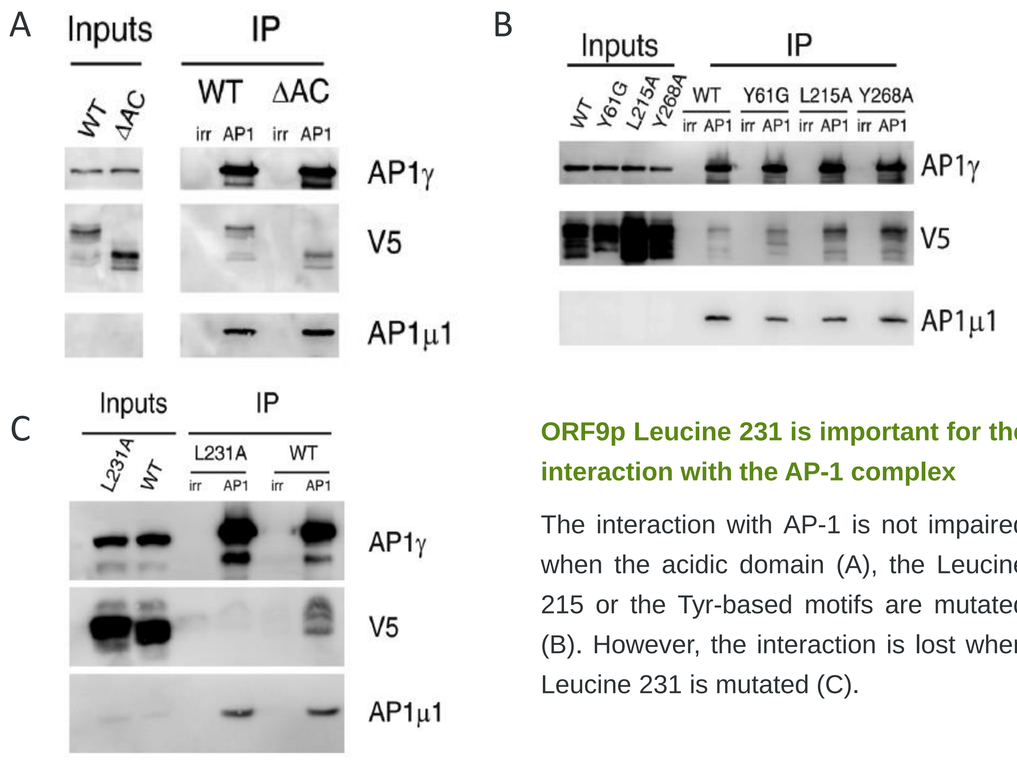
Acidic clusters



Such motifs are present in ORF9p's sequence

ORF9p harbors two tyrosine-based motifs (in green), two di-leucine motifs (in orange) and an acidic cluster (in red).

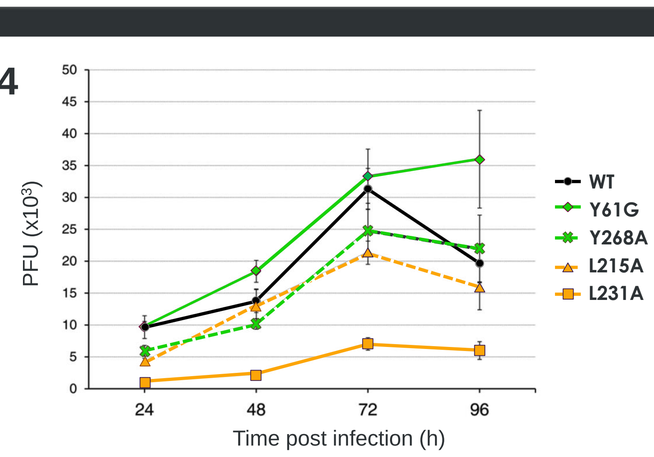
3



ORF9p Leucine 231 is important for the interaction with the AP-1 complex

The interaction with AP-1 is not impaired when the acidic domain (A), the Leucine 215 or the Tyr-based motifs are mutated (B). However, the interaction is lost when Leucine 231 is mutated (C).

4

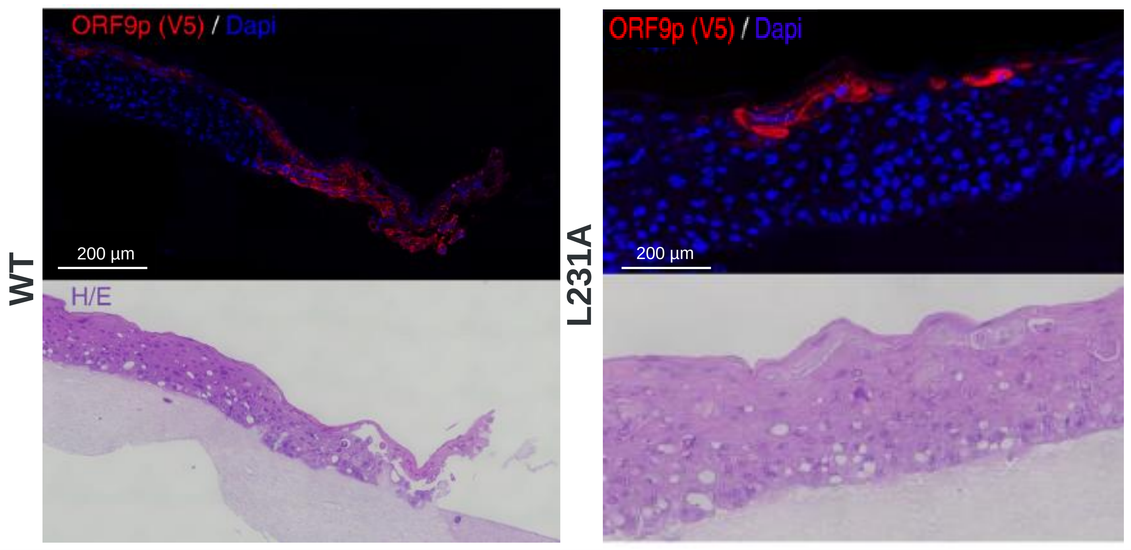


ORF9p leucine 231 is important for viral replication

Growth curves of ORF9p mutant strains compared to ORF9p WT VZV. The graph shows the result of one representative experiment out of three; error bars represent the standard error of the mean (SEM).

5 ORF9p Leucine 231 is important for viral infectivity in a 3D-skin model

Human primary keratinocytes were allowed to divide and differentiate at the air-liquid interface on top of a collagen matrix during four days. ORF9-WT-V5 and ORF9-L2131A-V5 VZV infected MRC5 were then layered on the epithelial cells and skin-rafts were processed 6 days later. One raft for each infection was embeded in paraffin and series of successive sections were analyzed.



6 The two di-leucine motifs of ORF9p are conserved among alphaherpesviruses

The primary sequence of 27 homologous VP22 were aligned with Vector NTi program Invitrogen. Only the region containing the two di-leucine motifs is shown. Yellow: identical residues; Blue: conserved residues.

The di-leucine motifs are conserved in 11 and 20 herpesviruses respectively.

CONCLUSIONS & PERSPECTIVES:

- ❑ ORF9p interacts with the cellular AP-1 complex and the leucine 231 is important for this interaction
- ❑ VZV-ORF9-L231A presents a strong growth defect compared to the wild type and the mutants of the other potential interaction motifs
- ❑ VZV-ORF9-L231A's growth defect is confirmed *in vivo* in a 3D-skin model
- ❑ The two di-Leucine motifs are conserved among the Alphaherpesviruses, suggesting that ORF9p/AP-1 interaction might be conserved
- ❑ Are there other viral proteins in the AP-1/ORF9p complex? Is ORF9p important for their incorporation in this complex?
- ❑ How is the AP-1/ORF9p complex involved in VZV secondary egress?
- ❑ Do the ORF9p homologs interact with the AP-1 complex?

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