Using CRISPR/Cas9 mutagenesis to uncover cellular key players during herpesvirus nuclear egress.

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Herpesviruses, as e.g. human herpes simplex and porcine pseudorabies virus (PrV), are highly complex DNA viruses causing disease in humans or animals. Their replication involves cytosolic and nuclear compartments. Nucleocapsids are assembled in the nucleus while further maturation to infectious particles proceeds in the cytoplasm. To overcome the nuclear barrier nucleocapsids engage a novel vesicle-mediated transport by budding through the inner nuclear membrane (INM) into the perinuclear space (PNS) followed by fusion of the INM derived viral (primary) envelope with the outer nuclear membrane (ONM). Viral proteins mediate budding at and from the INM, but fusion with the ONM seems to be executed by cellular components.

To shed light on this putative cellular machinery, we targeted proteins suspected to be involved in fusion processes of nuclear membranes. CRISPR/Cas9 mutagenesis for gene knockout but also dominant-negative expression constructs were used to generate cells with defects in Torsins A and B, LULL1, or LAP1. Mutant cells lines were infected with PrV and tested for infectious progeny. Only TorA/B double knockout cells revealed an accumulation of primary enveloped virions in the PNS pointing to a role of torsins in nuclear egress.

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