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Mapping of sequences in Pseudorabies Virus pUL34 required for formation and function of the nuclear egress complex

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During herpesvirus replication capsids are assembled in the nucleus while further maturation takes place in the cytosol. Translocation of capsids through the nuclear envelope occurs by budding at the inner nuclear membrane (INM), resulting in a primary enveloped particle in the nuclear cleft followed by fusion of the primary envelope with the outer nuclear membrane. This process is controlled by the viral nuclear egress complex consisting of the type II membrane protein designated as pUL34 in the alphaherpesviruses Pseudorabies Virus (PrV) and Herpes Simplex Virus and its nuclear interaction partner pUL31. Both proteins are conserved throughout the herpesviruses and necessary for efficient nuclear egress.
To map regions within PrV pUL34 required for nuclear membrane targeting and pUL31 interaction, we constructed a series of deletion/substitution mutants. We showed that 85 C-terminal amino acids (aa), including the membrane anchor, could be functionally substituted by the C-terminal transmembrane domain of cellular lamin associated polypeptide (Lap)2ß. However, deletion of the 90 C-terminal aa abrogated function despite continuing pUL31 recruitment delineating the functionally important region to five aa. This region comprises the sequence "RQR", a motif which has been suggested to mediate INM targeting (Meyer et al., 2002). Site-specific mutation to RQG indicated that this motif more likely acts as Golgi retrieval signal since nuclear targeting still occurred. Deletion/substitution mutants in the N-terminus of pUL34demonstrated that only few aa can be deleted without loss of function. Mutagenesis of three conserved cysteines was tolerated, whereas alteration of a conserved "EY" sequence led to a non functional protein.

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