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Dynamics of herpesvirus replication revealed by live-cell imaging

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Morphogenesis of herpesviruses occurs in different cellular compartments. Capsid assembly in the nucleus is followed by tegumentation and envelopment in the cytosol. To gain access to the different compartments, viral (sub)assemblies have to move within the infected cell. However, little is known about the dynamics of intracellular movement and the cellular and viral proteins involved. In particular nuclear egress, i.e. the transfer of nucleocapsids from the nucleus to the cytosol via primary envelopment-deenvelopment, a process unique in cell biology, is not well understood. To analyze intracellular dynamics of herpesvirus infection in real-time, we used the well characterized porcine alphaherpesvirus Pseudorabies virus (PrV) and respective deletion mutants. Viral proteins were tagged with fluorescent proteins to allow confocal live-cell imaging after infection. We also isolated cell lines expressing recombinant fluorescent cellular proteins to identify and distinguish between different cellular compartments. Our studies demonstrate in real-time the movement of viral capsids within nucleus and cytosol, and allow the tracing of single viral particles as they migrate within infected cells.