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Herpesvirus-induced nuclear envelope breakdown can substitute for primary envelope-mediated nuclear egress

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Herpesvirus nucleocapsids assemble in the nucleus but are translocated through the nuclear envelope for final maturation in the cytoplasm by primary envelopment at the inner nuclear membrane and subsequent fusion of the primary envelope with the outer nuclear membrane. In the absence of the conserved viral pUL34 and pUL31 proteins, nuclear egress is strongly impaired but not totally abolished. We used the residual infectivity of a pUL34-deleted mutant of the alphaherpesvirus pseudorabies virus (PrV) for reversion analysis by serial passaging in rabbit kidney (RK13) cells, until the mutant virus PrV-DeltaUL34Pass replicated with wild-type kinetics independent of the pUL34/pUL31 nuclear egress complex (NEC). This phenotype could be reproduced using pUL31-deleted PrV indicating an inherent genetic disposition. Ultrastructural analyses demonstrated that NEC-independent nuclear egress occurred by disruption of the nuclear envelope, thereby allowing release of nucleocapsids into the cytoplasm. Roscovitine, an inhibitor of cyclin-dependent kinases, prevented nuclear envelope breakdown and production of infectious progeny of the passaged mutants. Specific inhibition of CDK1 or CDK2 had no effect but inhibition of MEK1/2 significantly affected PrV-DeltaUL34Pass and PrV-DeltaUL31Pass replication indicating involvement of the Ras/Raf/MEK/ERK signalling network in herpesvirus-induced nuclear envelope breakdown. Sequencing of PrV-DeltaUL34Pass and PrV-DeltaUL31Pass uncovered several mutations specific for the passaged mutants compared to parental strains. Our data show that regulated nuclear egress of herpesviruses can be substituted by nuclear envelope breakdown which demonstrates that intranuclear nucleocapsids are fully competent for further maturation once they gain access to the cytoplasm.