The egress (and maturation..) of enveloped viruses, particularly RNA viruses (retroviruses..), is well characterized ([1-4](#_ENREF_1)). Nascent capsids become engulfed in a plasma membrane-derived lipid envelope by hijacking the multivesicular body (MVB) biogenesis machinery ([5](#_ENREF_5)). Current models propose a sequential, virus-induced recruitment of class E vacuolar protein sorting (VPS) proteins which act in three complexes, referred to as ESCRT I-III (endosomal sorting complex required for transport) ([6](#_ENREF_6)). Retroviruses directly interact with the ESCRT components via proline-rich late domains (L-domains) which are present in their structural proteins ([7](#_ENREF_7)). ESCRT components or viral sequences in the proximity of the L-domains become ubiquitinated by ubiquitin binding proteins, such as tumor-susceptibility gene 101 (TSG101) or Nedd4-like ubiquitin ligases. Ubiquitination results in the biogenesis and fission of MVBs and thus, budding of the virions ([8](#_ENREF_8)).

In contrast, the release of non-enveloped viruses from mammalian host-cells is generally associated with cellular lysis, thus considered a passive process ([9-13](#_ENREF_9)). However, there is rising evidence that an active egress of non-enveloped viruses precedes virus-induced cell lysis. For instance bluetongue virus (BTV), ~~a member of the genus~~ *~~Orbivirus~~* ~~within the~~ *~~Reoviridae~~* ~~family~~, has been demonstrated to usurp the ESCRT machinery for egress ([14](#_ENREF_14)). Comparable to enveloped viruses, BTV interacts with TSG101 via its L-domain in order to exploit the cellular MVB sorting pathway ([15](#_ENREF_15)). Similarly, Hepatitis A virus (HAV) uses host membrane hijacking to egress in a TSG101-independent manner. Thus, HAV release involves ESCRT-associated proteins but seems to be independent on the early ESCRT complexes required for initial cargo recruitment ([16](#_ENREF_16)). Poliovirus, another picornavirus, constitutes a further example for non-lytic egress of viruses lacking an envelope. Drug-induced stimulation of the autophagy pathway increased non-lytic spread of the virus ([17](#_ENREF_17)). Additionally, progeny virions were shown to accumulate unilaterally on the apical surface of polarized and productively infected epithelial cells ([18](#_ENREF_18)). Equally, simian vacuolating virus 40 (SV40) was almost exclusively recovered from the apical culture fluid of polarized epithelial cells. Moreover, the egress of SV40 occurred prior to cell lysis. The appearance of SV40 in smooth membrane reticular structures argues for a vesicle-associated release of SV40 virions ([19](#_ENREF_19)). Finally, simian rotavirus (RRV) was demonstrated to egress from the apical pole of epithelial cells before any cell lysis was detected. Electron microscopy studies and specific inhibition of vesicular transport pathways indicate a vesicle-associated release of progeny virions that is irrespective of the golgi-dependent secretory pathway ([20](#_ENREF_20)).

Recent studies show increasing evidence for an active egress of parvoviruses (PV), a group of small, non-enveloped viruses. Rodent PVs, including minute virus of mice (MVM), belong to the genus *Protoparvovirus* within the subfamily *Parvovirinae*, whose members infect a wide variety of vertebrates. These autonomous PVs display a T=1 icosahedral capsid containing a single-stranded DNA genome of about 5 kb ([10](#_ENREF_10)). The compact coding sequence contains two large overlapping open reading frames (ORFs) under the control of an early and a late promoter, termed P4 and P38, respectively. P4 controls the expression of the viral non-structural proteins NS1 and NS2, while P38 drives the expression of the viral capsid proteins VP1 and VP2, as well as the non-structural protein SAT ([21](#_ENREF_21)). Productive PV infection causes dramatic morphological and physiological changes of their host cells, culminating in cell death and lysis ([22](#_ENREF_22), [23](#_ENREF_23)). PV cytotoxicity is mainly associated with the large non-structural protein NS1, an 83-kDa multifunctional protein ([24](#_ENREF_24)). NS1 is involved in various essential processes during the PV life cycle ([25](#_ENREF_25)).

Besides having the possibility to passively egress the host cell by NS1-induced cellular lysis, the latest data of several research groups suggest an active, pre-lytic egress for MVM ([26-28](#_ENREF_26)). In order to actively egress the host cell, progeny particles of karyophilic viruses need to cross considerable cellular barriers. Apart from the plasma membrane, the nuclear envelope constitutes a second barrier to MVM. Although the mechanism for nuclear export and subsequent release of MVM virions remains elusive, several important viral and cellular effectors involved in PV egress have been identified and characterized. MVM is exported from the host’s nucleus by a Crm1 dependent mechanism. Stable interaction of NS2 with Crm1 was successfully demonstrated ([29](#_ENREF_29), [30](#_ENREF_30)). Classical nuclear export signals (NES) exhibit low affinity for Crm1 to prevent from the formation of the Crm1/cargo complex in the cytoplasm where RanGTP is absent ([31](#_ENREF_31)). Surprisingly, the NES of NS2 belongs to the supraphysiological NES which tightly bind to Crm1 regardless of the presence of RanGTP. Therefore, NS2 competitively inhibits Crm1 function by sequestering endogenous nuclear export receptors ([32](#_ENREF_32)). MVM mutant genomic clones generating NS2 proteins harbouring either regular NES, or substitutions which abrogated Crm1 interaction were shown to be compromised in viral nuclear export and productive infection. NS2 was demonstrated to have a critical role in MVM replication depending on the infected cell type. Following infection or transfection of restrictive murine cells with NS2-null mutants, little amount of mutant dsDNA replicative from (RF), and no detectable accumulation of progeny unit-length ssDNA genomes was detectable. However, the restricted replication of MVM genomes was less evident in permissive human cells ([33](#_ENREF_33)). Contrarily, NS2-Crm1- mutants produced dsDNA dRF and mRF levels comparable to those of the wild type at both early and late times post-transfection. Interestingly, the amount of accumulated viral progeny ssDNA drastically decreased in restrictive murine cells at proceeding times post-transfection. This observation suggests that the interaction of NS2 with Crm1 is dispensable for MVM dsDNA replication. However, it is strictly required for the production of progeny ssDNA, particularly in restrictive murine cells ([34](#_ENREF_34)). As expected, NS2-Crm1- mutants showed nuclear accumulation of export deficient NS2 in transfected cells. Surprisingly, the nuclear retention of mutant NS2 proteins came along with a substantial accumulation of progeny virions in the nucleus of infected cells, suggesting a NS2-dependent export of progeny virions. Additionally, an indirect involvement of NS2 in viral egress was demonstrated using the closely related H1-PV. An in-frame deletion of 38 amino acids within the common coding sequence of NS1 and NS2 was demonstrated to beneficially influence virus infectivity *in vitro*, indicated by a lower particle-to-infectivity (P/I) ratio. The increase in infectivity, which resulted from an accelerated egress of the mutant progeny virions, positively affected tumor growth suppression *in vivo* ([35](#_ENREF_35)). However, approaches to demonstrate a direct interaction between NS2 and the viral capsid and/or individual structural proteins *in vitro* have not yet been successful.

The differences in nuclear export observed during productive MVM infection in either permissive human cells or restrictive murine cells may result from the cell-type-specific use of alternative strategies for nuclear export. It became apparent when different cell types were treated with the antifungal antibiotic leptomycin B (LMB), a drug which inhibits Crm1-dependent nuclear export ([36](#_ENREF_36)). LMB treatment of susceptible murine cells resulted in a significant but not complete inhibition of nuclear export of MVM progeny virions. In contrast, even high doses of LMB did not inhibit nuclear export of MVM in transformed human cells, indicating that Crm1 is not essentially involved in the nuclear export of MVM in these cells ([28](#_ENREF_28)). The observed differences may result from a cell-type dependent phosphorylation status of MVM. Generally, MVM capsids derived from permissive human cells displayed prominent phosphorylation compared to the decent phosphorylation status of capsids isolated from restrictive murine fibroblasts ([37](#_ENREF_37)). Significantly, the three distal serine residues at position 2, 6, and 10 of the unordered N-VP2 terminus showed high phosphorylation levels in permissive cells. Site-directed mutagenesis verified an important role of these phosphorylations in the Crm1-independent nuclear export of MVM in permissive human cells. When the N-terminal phosphorylations were diminished, progeny virions were predominantly retained in the nucleus and the corresponding mutants displayed a small plaque phenotype, indicating the importance of those phosphorylations in viral spread ([28](#_ENREF_28)).

MVM transport from the nucleus to the cell periphery is associated with the degradation of actin fibers and the formation of “actin-patches”. These alterations to the filamentous network were attributed to a virus-induced imbalance between the actin polymerization factor N-WASP (Wiscott-Aldrich syndrome protein) and gelsolin, a member of the actin-severing protein family ([38](#_ENREF_38)). Indeed, the MVM titer in the culture supernatant following MVM infection drastically declined when gelsolin function was diminished. During MVM infection, gelsolin activity is regulated by the CKIIα/NS1 complex which was demonstrated to be capable of phosphorylating gelsolin. Consequentially, inhibition of CKIIα correlated with prolonged persistence of actin fibers and delayed formation of the characteristic “actin patches” ([27](#_ENREF_27), [39](#_ENREF_39)). Several lines of evidence coincide with an active, vesicle-associated, gelsolin-dependent export of MVM. Progeny virions were shown to co-localize with exocytic, endosomal, and lysosomal markers in immunofluorescent experiments ([27](#_ENREF_27), [40](#_ENREF_40)). Cell fractionation experiments confirmed this hypothesis by demonstrating a co-migration of viral particles with cytosolic vesicles rather than free, vesicle-independent localization in the soluble cytosolic fraction. Moreover, dynamin was found to accumulate in the perinuclear region where it co-localized with de novo synthesized MVM capsids. A cooperative cross-talk between actin- and microtubule dependent transport ([41-43](#_ENREF_41)) might be involved in MVM transport from the nucleus to the cell periphery, resulting in the destruction of actin filaments and the stabilization of microtubules ([27](#_ENREF_27)).

The secretion pathway represents the proposed route for active egress of MVM. It is supposed that progeny virions become engulfed by COPII-vesicle formation in the perinuclear ER. In order to verify this hypothesis, cells lacking functional effectors of the secretory pathway were productively infected. Accordingly, a dramatic retention of virions in the perinuclear area was observed, accompanied by inhibited virion release into the medium. Contrarily, no significant co-localization between MVM progeny virions and representative markers of the recycling pathway or the Trans Golgi Network (TGN) were evident ([26](#_ENREF_26)). In addition, members of the ERM family, such as radixin and moesin, were shown to play a role in virus maturation and spreading capacity, as judged by their impact on MVM plaque morphology ([44](#_ENREF_44)). Indeed, dominant negative radixin or moesin mutants failed in wrapping progeny virions into transport vesicles, resulting in a marked reduction of egressed virions in the culture supernatant. As a consequence, corresponding markers for alternative export routes, e.g. direct transport from the TGN to the PM or through recycling endosomes, exhibited increased co-localization with progeny virions. Finally, active egress promotes cellular lysis as demonstrated by the prolonged viability of cells in which vesicular transport was either inhibited or by-passing the Golgi apparatus. Besides, the involvement of progeny particles in cytolysis was demonstrated by the prolonged survival of murine cells transduced with a viral vector deficient for the production of progeny virion particles ([26](#_ENREF_26)).

Documentation of active egress requires accurate demonstration that no cell lysis occurred during the experiment. However, it is challenging to exclude the possibility that lysis of a few cells may contaminate the medium with ejected progeny virions which even could contribute to uncontrolled second rounds of infection. In tissue culture, passive egress considerably contributes to viral spread. However, its importance in animal infection might be extensively limited by viral clearance through components of the immune system. In addition, incoming particles need to be excluded from the analysis in order to separate them from progeny virions. Parvoviruses, particularly MVM, are highly robust and persist as intact particles mainly in the lysosomes of infected cells where they are slowly degraded ([45](#_ENREF_45)). Since the endosomal pathway is highly dynamic and partially overlaps with the progeny egress route, separation of incoming particles and progeny virions provides a major challenge.

The present investigation aims to characterize the final maturation steps leading to nuclear export and egress of MVM. Using anion exchange chromatography (AEX) in combination with quantitative PCR (qPCR) we demonstrate that two distinct populations of DNA containing progeny particles accumulate in the nucleus of infected murine cells. *De novo* synthesized capsids undergo a maturation step in the nucleus that involves displaying surface phosphorylation(s) and exposure of the N-terminus of the major virus protein VP2 (N-VP2). This maturation is required for the MVM progeny in order to leave the host’s nucleus and allows active egress of progeny virions prior to host cell lysis. However, comparison of immature progeny particles to mature virions indicated that the observed maturation step only marginally increases infectivity. When infected into susceptible cells, immature capsids were fully infectious, but showed aberrant cleavage of N-VP2 and slightly delayed genome delivery to the nucleus.

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