The egress of enveloped viruses 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 four complexes, referred to as endosomal sorting complex required for transport (ESCRT 0-III) ([6](#_ENREF_6)). Direct interaction of retroviruses with ESCRT components via viral late domains (L-domains) results in the ubiquitination of either interaction partner ([7](#_ENREF_7)). Ubiquitination initiates the biogenesis and fission of MVBs and thus, budding of the virions ([8](#_ENREF_8)).

The egress of non-enveloped viruses is less well understood. In general, release of non-enveloped viruses is associated with cellular lysis, thus considered a passive process ([9-13](#_ENREF_9)). However, there is rising evidence that an active egress precedes virus-induced cell lysis and subsequent passive release. For instance, bluetongue virus (BTV) has been demonstrated to usurp the ESCRT machinery for egress by means of its L-domains ([14](#_ENREF_14), [15](#_ENREF_15)). Similarly, Hepatitis A virus (HAV) release involves ESCRT-associated proteins ([16](#_ENREF_16)). Furthermore, drug-induced stimulation of the autophagy pathway increased non-lytic spread of poliovirus and progeny virions were shown to accumulate unilaterally on the apical surface of polarized and productively infected epithelial cells ([17](#_ENREF_17), [18](#_ENREF_18)). Equally, simian vacuolating virus 40 (SV40) and simian rotavirus (RRV) were almost exclusively recovered from the apical culture fluid of polarized epithelial cells prior to cell lysis. Electron microscopy studies and specific inhibition of vesicular transport pathways indicate a vesicle-associated release of progeny virions ([19](#_ENREF_19), [20](#_ENREF_20)).

Recent studies show increasing evidence for an active egress of parvoviruses (PV), a group of small, non-enveloped viruses. Autonomous rodent PVs, including minute virus of mice (MVM), 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)).

Besides having the possibility to passively egress the host cell by NS1-induced cellular lysis, the latest data suggest an active, pre-lytic egress for MVM ([25-27](#_ENREF_25)). In order to actively egress the host cell, progeny particles of karyophilic viruses need to cross considerable cellular barriers, such as the nuclear envelope and the plasma membrane. 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. MVM is exported from the nucleus by a Crm1 dependent mechanism. Stable interaction of NS2 with Crm1 was successfully demonstrated ([28](#_ENREF_28), [29](#_ENREF_29)). 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 ([30](#_ENREF_30)). 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 ([31](#_ENREF_31)). 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. 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. 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.

Productive MVM infections in either permissive human cells or restrictive murine cells disclosed a cell 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 ([32](#_ENREF_32)). LMB treatment of susceptible murine cells resulted in a significant inhibition of nuclear export whereas transformed human cells were much less affected even at high doses of LMB. This indicates that Crm1 is not essentially involved in the nuclear export of MVM in the latter cell line ([27](#_ENREF_27)). The observed differences may result from a cell-type dependent phosphorylation status of MVM. Generally, MVM capsids derived from permissive human cells displayed more prominent phosphorylation compared to that of capsids isolated from restrictive murine fibroblasts ([33](#_ENREF_33)). Site-directed mutagenesis of the three distal serine residues at position 2, 6, and 10 of the unordered N-terminus of VP2 (N-VP2) revealed an important role of these phosphorylations in the Crm1-independent nuclear export of MVM in permissive human cells. When the N-terminal phosphorylations were mutated, 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 ([27](#_ENREF_27)).

MVM transport from the nucleus to the cell periphery is associated with the degradation of actin fibers. 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 ([34](#_ENREF_34)). Indeed, the MVM titer in the medium 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 ([26](#_ENREF_26), [35](#_ENREF_35)). Several lines of evidence indicate 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 ([26](#_ENREF_26), [36](#_ENREF_36)). Cell fractionation experiments confirmed this observation by demonstrating a co-migration of viral particles with cytosolic vesicles rather than free, vesicle-independent localization in the soluble cytosolic fraction. A cooperative cross-talk between actin- and microtubule dependent transport might be involved in MVM transport from the nucleus to the cell periphery ([37-39](#_ENREF_37)).

The secretory pathway has been proposed as the route for active egress of MVM. Progeny virions would become engulfed by COPII-vesicle formation in the perinuclear ER where they accumulated with dynamin. Accordingly, a dramatic retention of virions in the perinuclear area and inhibition of virion release into the medium was observed in cells lacking functional effectors of the secretory pathway ([25](#_ENREF_25)). 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 ([40](#_ENREF_40)). Consequentially, dominant negative radixin or moesin mutants failed in wrapping progeny virions into transport vesicles, resulting in a marked reduction of egressed virions in the medium. 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 ([25](#_ENREF_25)).

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 passively release progeny virions, which could additionally 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 largely limited by clearance of virus-infected cells by components of the immune system. Parvoviruses, particularly MVM, are highly robust and persist as intact particles mainly in the lysosomes of infected cells where they are slowly degraded ([41](#_ENREF_41)). Since the endosomal pathway is highly dynamic and partially overlaps with the egress route of progeny viruses, the discrimination of incoming from progeny virions represents 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 co-exist in the nucleus of infected murine cells. *De novo* synthesized capsids undergo a maturation step in the nucleus that involves surface phosphorylation(s) and exposure of N-VP2. Only fully infectious virions were able to exit the nuclei and egress from the cells prior to cell lysis, confirming an active egress of parvovirus MVM.

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