The egress 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. 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 ([*40-42*](#_ENREF_40)) might be involved in MVM transport from the nucleus to the cell periphery ([*27*](#_ENREF_27)).

* Nüesch.. COPII-mediated, ERM-family, Radixin, Moesin ACTIVE!!

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.

***Polio-paper…***

…Documentation that such events are truly nonlytic, however, requires rigorous demonstration that no cell lysis occurred. However, it has been difficult to test this and other hypotheses concerning unconventional secretion because the use of cell populations makes it nearly impossible to exclude the possibility that lysis of a few cells is responsible for the release of cytoplasmic constituents. …

1. Martin-Serrano, J., and Neil, S. J. (2011) Host factors involved in retroviral budding and release, *Nature reviews. Microbiology* *9*, 519-531.

2. Morita, E., and Sundquist, W. I. (2004) Retrovirus budding, *Annual review of cell and developmental biology* *20*, 395-425.

3. Falanga, A., Cantisani, M., Pedone, C., and Galdiero, S. (2009) Membrane Fusion and Fission: Enveloped Viruses, *Protein Peptide Lett* *16*, 751-759.

4. Votteler, J., and Sundquist, W. I. (2013) Virus Budding and the ESCRT Pathway, *Cell Host Microbe* *14*, 232-241.

5. Calistri, A., Salata, C., Parolin, C., and Palu, G. (2009) Role of multivesicular bodies and their components in the egress of enveloped RNA viruses, *Rev Med Virol* *19*, 31-45.

6. Morita, E. (2012) Differential requirements of mammalian ESCRTs in multivesicular body formation, virus budding and cell division, *The FEBS journal* *279*, 1399-1406.

7. Bieniasz, P. D. (2006) Late budding domains and host proteins in enveloped virus release, *Virology* *344*, 55-63.

8. Martin-Serrano, J. (2007) The role of ubiquitin in retroviral egress, *Traffic* *8*, 1297-1303.

9. Tattersall, P. (1972) Replication of the parvovirus MVM. I. Dependence of virus multiplication and plaque formation on cell growth, *Journal of virology* *10*, 586-590.

10. Cotmore, S. F., and Tattersall, P. (1987) The autonomously replicating parvoviruses of vertebrates, *Advances in virus research* *33*, 91-174.

11. Daeffler, L., Horlein, R., Rommelaere, J., and Nuesch, J. P. F. (2003) Modulation of minute virus of mice cytotoxic activities through site-directed mutagenesis within the NS coding region, *Journal of virology* *77*, 12466-12478.

12. Maul, G. G. (1976) Fibrils attached to the nuclear pore prevent egress of SV40 particles from the infected nucleus, *The Journal of cell biology* *70*, 714-719.

13. Tucker, S. P., and Compans, R. W. (1993) Virus infection of polarized epithelial cells, *Advances in virus research* *42*, 187-247.

14. Mohl, B. P., and Roy, P. (2014) Bluetongue virus capsid assembly and maturation, *Viruses* *6*, 3250-3270.

15. Wirblich, C., Bhattacharya, B., and Roy, P. (2005) Nonstructural protein 3 of bluetongue virus assists virus release by recruiting ESCRT-I protein Tsg101, *Journal of virology* *80*, 460-473.

16. Feng, Z., Hensley, L., McKnight, K. L., Hu, F., Madden, V., Ping, L., Jeong, S. H., Walker, C., Lanford, R. E., and Lemon, S. M. (2013) A pathogenic picornavirus acquires an envelope by hijacking cellular membranes, *Nature* *496*, 367-371.

17. Bird, S. W., Maynard, N. D., Covert, M. W., and Kirkegaard, K. (2014) Nonlytic viral spread enhanced by autophagy components, *Proceedings of the National Academy of Sciences of the United States of America* *111*, 13081-13086.

18. Tucker, S. P., Thornton, C. L., Wimmer, E., and Compans, R. W. (1993) Vectorial release of poliovirus from polarized human intestinal epithelial cells, *Journal of virology* *67*, 4274-4282.

19. Clayson, E. T., Brando, L. V., and Compans, R. W. (1989) Release of simian virus 40 virions from epithelial cells is polarized and occurs without cell lysis, *Journal of virology* *63*, 2278-2288.

20. Jourdan, N., Maurice, M., Delautier, D., Quero, A. M., Servin, A. L., and Trugnan, G. (1997) Rotavirus is released from the apical surface of cultured human intestinal cells through nonconventional vesicular transport that bypasses the Golgi apparatus, *Journal of virology* *71*, 8268-8278.

21. Pintel, D., Dadachanji, D., Astell, C. R., and Ward, D. C. (1983) The genome of minute virus of mice, an autonomous parvovirus, encodes two overlapping transcription units, *Nucleic acids research* *11*, 1019-1038.

22. Caillet-Fauquet, P., Perros, M., Brandenburger, A., Spegelaere, P., and Rommelaere, J. (1990) Programmed killing of human cells by means of an inducible clone of parvoviral genes encoding non-structural proteins, *The EMBO journal* *9*, 2989-2995.

23. Herrero, Y. C. M., Cornelis, J. J., Herold-Mende, C., Rommelaere, J., Schlehofer, J. R., and Geletneky, K. (2004) Parvovirus H-1 infection of human glioma cells leads to complete viral replication and efficient cell killing, *International journal of cancer. Journal international du cancer* *109*, 76-84.

24. Corbau, R., Duverger, V., Rommelaere, L., and Nuesch, J. P. F. (2000) Regulation of MVM NS1 by protein kinase C: Impact of mutagenesis at consensus phosphorylation sites on replicative functions and cytopathic effects, *Virology* *278*, 151-167.

25. Vanacker, J. M., and Rommelaere, J. (1995) Nonstructural Proteins of Autonomous Parvoviruses - from Cellular Effects to Molecular Mechanisms, *Semin Virol* *6*, 291-297.

26. Bar, S., Rommelaere, J., and Nuesch, J. P. F. (2013) Vesicular Transport of Progeny Parvovirus Particles through ER and Golgi Regulates Maturation and Cytolysis, *Plos Pathog* *9*.

27. Bar, S., Daeffler, L., Rommelaere, J., and Nuesch, J. P. F. (2008) Vesicular egress of non-enveloped lytic parvoviruses depends on gelsolin functioning, *Plos Pathog* *4*.

28. Maroto, B., Valle, N., Saffrich, R., and Almendral, J. M. (2004) Nuclear export of the nonenveloped parvovirus virion is directed by an unordered protein signal exposed on the capsid surface, *Journal of virology* *78*, 10685-10694.

29. Bodendorf, U., Cziepluch, C., Jauniaux, J. C., Rommelaere, J., and Salome, N. (1999) Nuclear export factor CRM1 interacts with nonstructural proteins NS2 from parvovirus minute virus of mice, *Journal of virology* *73*, 7769-7779.

30. Ohshima, T., Nakajima, T., Oishi, T., Imamoto, N., Yoneda, Y., Fukamizu, A., and Yagami, K. (1999) CRM1 mediates nuclear export of nonstructural protein 2 from parvovirus minute virus of mice, *Biochemical and biophysical research communications* *264*, 144-150.

31. Nachury, M. V., and Weis, K. (1999) The direction of transport through the nuclear pore can be inverted, *Proceedings of the National Academy of Sciences of the United States of America* *96*, 9622-9627.

32. Engelsma, D., Valle, N., Fish, A., Salome, N., Almendral, J. M., and Fornerod, M. (2008) A supraphysiological nuclear export signal is required for parvovirus nuclear export, *Mol Biol Cell* *19*, 2544-2552.

33. Naeger, L. K., Cater, J., and Pintel, D. J. (1990) The Small Nonstructural Protein (Ns2) of the Parvovirus Minute Virus of Mice Is Required for Efficient DNA-Replication and Infectious Virus Production in a Cell-Type-Specific Manner, *Journal of virology* *64*, 6166-6175.

34. Miller, C. L., and Pintel, D. J. (2002) Interaction between parvovirus NS2 protein and nuclear export factor Crm1 is important for viral egress from the nucleus of murine cells, *Journal of virology* *76*, 3257-3266.

35. Weiss, N., Stroh-Dege, A., Rommelaere, J., Dinsart, C., and Salome, N. (2012) An in-frame deletion in the NS protein-coding sequence of parvovirus H-1PV efficiently stimulates export and infectivity of progeny virions, *Journal of virology* *86*, 7554-7564.

36. Kudo, N., Wolff, B., Sekimoto, T., Schreiner, E. P., Yoneda, Y., Yanagida, M., Horinouchi, S., and Yoshida, M. (1998) Leptomycin B inhibition of signal-mediated nuclear export by direct binding to CRM1, *Exp Cell Res* *242*, 540-547.

37. Maroto, B., Ramirez, J. C., and Almendral, J. M. (2000) Phosphorylation status of the parvovirus minute virus of mice particle: mapping and biological relevance of the major phosphorylation sites, *Journal of virology* *74*, 10892-10902.

38. Nuesch, E. R., Lachmann, S., and Rommelaere, J. (2005) Selective alterations of the host cell architecture upon infection with parvovirus minute virus of mice, *Virology* *331*, 159-174.

39. Nuesch, J. P., and Rommelaere, J. (2006) NS1 interaction with CKII alpha: novel protein complex mediating parvovirus-induced cytotoxicity, *Journal of virology* *80*, 4729-4739.

40. Praefcke, G. J., and McMahon, H. T. (2004) The dynamin superfamily: universal membrane tubulation and fission molecules?, *Nature reviews. Molecular cell biology* *5*, 133-147.

41. Stamnes, M. (2002) Regulating the actin cytoskeleton during vesicular transport, *Current opinion in cell biology* *14*, 428-433.

42. Slepchenko, B. M., Semenova, I., Zaliapin, I., and Rodionov, V. (2007) Switching of membrane organelles between cytoskeletal transport systems is determined by regulation of the microtubule-based transport, *The Journal of cell biology* *179*, 635-641.