

ESCRTs breach the nuclear border

Brant M Webster and C Patrick Lusk*

Yale School of Medicine; New Haven, CT USA

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Abbreviations: ERAD, ER-Associated Degradation; ESCRT, Endosomal Sorting Complexes Required for Transport; INM, Inner Nuclear Membrane; LEM, Lap2, Emerin, MAN1; NPC, Nuclear Pore Complex; NE, Nuclear Envelope; MVB, Multivesicular Body; NLS, Nuclear Localization Signal; ONM, Outer Nuclear Membrane; SINC, Storage of Improperly assembled Nuclear pore Complexes.

© Brant M Webster and C Patrick Lusk

*Correspondence to: C Patrick Lusk; Email: patrick.lusk@yale.edu

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The endosomal sorting complexes required for transport (ESCRT) are best known for their role in sorting ubiquitylated membrane proteins into endosomes. The most ancient component of the ESCRT machinery is ESCRT-III, which is capable of oligomerizing into a helical filament that drives the invagination and scission of membranes aided by the AAA ATPase, Vps4, in several additional subcellular contexts. Our recent study broadens the work of ESCRT-III by identifying its role in a quality control pathway at the nuclear envelope (NE) that ensures the normal biogenesis of nuclear pore complexes (NPCs). Here, we will elaborate on how we envision this mechanism to progress and incorporate ESCRT-III into an emerging model of nuclear pore formation. Moreover, we speculate there are additional roles for the ESCRT-III machinery at the NE that broadly function to ensure its integrity and the maintenance of the nuclear compartment.

Introduction

A defining feature of eukaryotic cells is the compartmentalization of cellular processes into organelles. This is thought to have enabled the increased sophistication and complexity of modern eukaryotes, but how this elaborate endomembrane system came into being remains a central question for biologists. The evolution of the nucleus in particular poses a significant challenge, but autogenous as opposed to exogenous (i.e. endosymbiosis) models are favored.¹ This is due, in part, to recent discoveries of membrane sculpting proteins that are shared between the nucleus and other elements of the endomembrane system - relationships that support that

their evolution was intrinsically interwoven. One of the best examples of this connection was the discovery that the scaffold proteins that build the NPC are structurally similar to vesicle coat complexes like COPI, COPII and clathrin.^{2,3} This discovery suggested the existence of an ancient “proto” coatomer that features heavily in both outside-in¹ and the more recent inside-out⁴ evolutionary theories. Our work expands on the functional and physical relationships between the nucleus and the rest of the endomembrane system by uncovering a new (but likely old) function for the ESCRT-III membrane-bending module at the NE.⁵

The ESCRTs – membrane scaffolding and fission machines

The modern eukaryotic ESCRT-III works with the AAA-ATPase, Vps4, and is best understood in its role in generating multi-vesicular bodies (MVBs) during the endocytosis of ubiquitylated plasma membrane receptors.⁶ In this pathway, membrane proteins destined for degradation are internalized by endocytosis and delivered to early endosomes. An ordered recruitment of the other ESCRT subunits including ESCRT-0, I and II, contribute to the sorting and concentration of the membrane proteins into invaginations of the endosomal membrane, ultimately capturing them in an intraluminal vesicle. The precise mechanisms at each of these steps remain to be fully understood but it is clear that ESCRT-III and Vps4 are late-acting factors that drive membrane scission in order to complete the formation of the intraluminal vesicle (Fig. 1A).⁷ Several models of the ESCRT-III/Vps4 mechanism of membrane fission have been proposed (reviewed extensively elsewhere⁶); a unifying theme in these models is that they rely on ESCRT-III’s

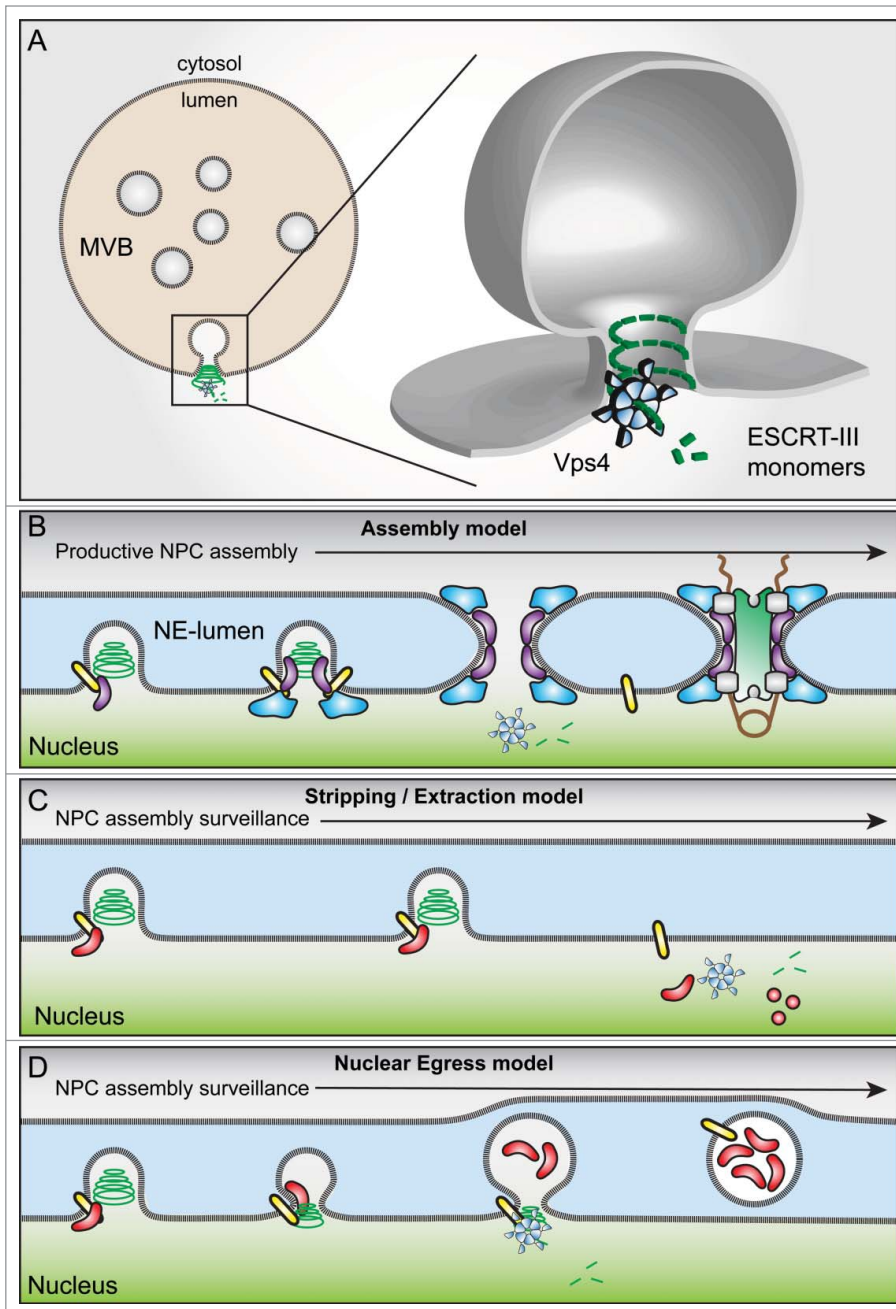


Figure 1. Models of ESCRT-III function. **(A)** The canonical membrane scission model of ESCRT-III at endosomes/MVBs. The ESCRT-III spiral (green) lines the neck of an intraluminal invagination (zoomed in view on right) of the endosomal membrane. Vps4 contributes to membrane scission and the formation of an intraluminal vesicle by disassembling the ESCRT-III filament into monomers. **(B)** A model of NPC assembly where ESCRT-III is recruited to an assembly site by binding to Heh2 (yellow) and the inner ring complex (purple). In this model, ESCRT-III contributes to the invagination of the INM, which would generate membrane curvature permissive for recognition and recruitment of the outer ring complex (blue), which would presumably stabilize the neck of the invagination, preventing membrane scission and promoting INM-ONM fusion. Other nups are recruited to make a functional transport channel. **(C)** The strip/extraction model of NPC assembly surveillance where defective NPC assembly intermediates (red) are stripped from the membrane through the action of the AAA-ATPase, Vps4. **(D)** The nuclear egress model of NPC assembly surveillance. Here, a defective nup protomer (red) inhibits further assembly. In this context, ESCRT-III promotes membrane invagination and scission to form an intraluminal vesicle that encapsulates the defective intermediate.

remarkable capacity to polymerize into a spiral that lines the neck of a membrane invagination (Fig. 1A). One compelling analogy for how the Vps4-mediated ESCRT-III disassembly might promote membrane scission is through a mechanism that resembles a purse-string cinching closed an open purse.⁸

While there are many ESCRT-III components, Snf7, Vps2, Vps20 and Vps24 are typically referred to as the “core” subunits in yeast. All of these ESCRT-III components share a helical hairpin structure with a C-terminal tail that folds back to “autoinhibit” their assembly.⁶ Binding partners like the ESCRT-II, Vps25, are thought to release this autoinhibition and activate polymerization. Whether ESCRT-III polymerization contributes strictly to a fission mechanism or whether it is also capable of driving membrane invaginations by forming a membrane scaffold remains to be fully appreciated. For example, *in vitro* studies modeling MVB biogenesis favor that upstream ESCRTs help form the membrane invagination with ESCRT-III being sufficient to drive scission,^{9,10} however, it is clear from *in vivo* studies that the polymerization of ESCRT-III can lead to the formation of a scaffold that pushes membranes away from the cytosol in much the same way that one could imagine NPC assembly progressing^{11,12} (Fig. 1B). Perhaps most interestingly, there appears to be plasticity in the diameter of the ESCRT-III filament. Indeed, ~4 nm Snf7¹³ and ~5–6 nm diameter CHMP4B¹¹ have been observed while an ESCRT-III filament at the midbody has an outer diameter of ~17 nm.¹⁴ When Vps4 is inhibited, ESCRT-III filaments can form conical membrane-associated spirals of ~110 nm,¹² similar to the upper size limit of plasma membrane holes that can be repaired by ESCRT-III.¹⁵ These data suggest that ESCRT-III might form unique filaments that are context and function-specific. Since ESCRT-III polymers are often formed through the heterodimerization of 2 ESCRT-III subunits, one possibility is that unique membrane scaffolds are formed by different combinations of the many ESCRT-III. A key question will be to understand how binding to nups, which are themselves

membrane scaffolds, might influence the properties and function of ESCRT-III.

Adaptors localize ESCRT-III to different subcellular locations

ESCRT-III functions in several subcellular contexts: it has been shown to contribute to membrane abscission during cytokinesis (likely its most ancient role), plasma membrane wound repair,^{15,16} ectosome shedding, exosome biogenesis, centrosome homeostasis, and is a key player in viral egress from the plasma membrane.⁶ ESCRT-III's role in multiple subcellular locations raises the question of how it reaches these distinct destinations and suggests the use of site-specific adaptor molecules. Consistent with this idea, while ESCRT-II subunits are thought to recruit ESCRT-III to endosomes, they are not required for other ESCRT-III functions.⁶ Instead, the ESCRT associated protein ALIX acts as an adaptor (often alongside the ESCRT-I, Tsg101) capable of directing ESCRT-III to different sites by binding to, for example, CEP55 at the midbody, Gag at an HIV bud site, and ALG-2 at a plasma membrane rupture.¹⁶ Interestingly, we were unable to implicate the yeast ALIX ortholog, Bro1, or an ESCRT-II component in the recruitment of ESCRT-III to the NE.⁵ Instead our data support that conserved integral inner nuclear membrane (INM) proteins of the Lap2-Emerin-MAN1 (LEM) family, the paralogs Heh1 and Heh2,¹⁷ bind to the ESCRT-III, Snf7.⁵ Beyond recruitment, the absence of either ESCRT-II or ALIX, both of which are capable of activating ESCRT-III polymerization, suggests the possibility that the Heh proteins could represent a new set of proteins capable of releasing ESCRT-III autoinhibition in order to facilitate ESCRT-III spiral formation; we are in the process of testing this idea.

The interaction between Heh2 and Snf7, and our visualization of this interaction at the NE by bifunctional complementation, supports that Snf7 has a nuclear pool.⁵ While there are no obvious canonical nuclear localization signals (NLSs) in Snf7, there are basic patches of amino acids that might be capable of binding nuclear transport receptors/karyopherins. Interestingly, the ESCRT-III component CHMP1 was characterized in

an early study to function in the nucleus by binding to chromatin and has a predicted bipartite NLS.¹⁸ In fact the "CHMP" moniker serves both "CHromatin Modifying Proteins"¹⁸ and the more common "CHarged Multivesicular body Protein," a representative example of the long recognized cross talk between the endocytic pathway and the nucleus, the significance of which remains to be fully appreciated.¹⁹ The greater challenge to how ESCRTs access the nucleus is Vps4, which functions as a hexamer of ~245 kD²⁰ that would be incapable of traversing the NPC diffusion barrier. It is thought, however, that the high local concentration of Vps4 binding sites on ESCRT-III in its polymerized form facilitates hexamer formation²¹ and that at steady state, Vps4 has a monomer and dimer pool^{22,23}; as a monomer, Vps4 would be capable of diffusing through the NPC. Alternatively, it may piggy-back on components of ESCRT-III. This might be more likely when one considers that a model for Vps4 recruitment to the membrane is to first bind ESCRT-III in the cytoplasm before it is targeted to its site of action.²¹

ESCRT-III prevents aberrant NPC assembly

Our discovery that ESCRT-III and Vps4 function at the NE was aided by an epistasis network that was founded on a genetic interaction between the nup gene *POM152* and *VPS4*, identified in fission yeast.²⁴ In the absence of ESCRT-III, Vps4, or Heh2, a fraction of newly synthesized nups and NPC assembly factors accumulate into a single plaque on the NE in replicatively-old mother cells.⁵ Unlike the NPC clustering observed in well described NPC assembly mutants,^{25–28} where virtually all nups/NPCs are found clustered at the NE, we observed inconsistent stoichiometries of individual nups in this cluster, which varied from cell to cell.⁵ Most strikingly, the cluster was not inherited by daughter cells, supporting our conclusion that this was a previously undiscovered quality control compartment, which we termed the Storage of Improperly assembled Nuclear pore Complexes, or SINC. Our data therefore implicated Heh2, ESCRT-III and Vps4 in

preventing aberrant NPC assembly. The details of precisely how ESCRT-III functions in NPC assembly remains to be fully elucidated but we proposed a mechanism in which it acts in a surveillance pathway that clears defective NPC assembly intermediates – we will consider how this might occur below and examine a model that also invokes a direct role for ESCRT-III in NPC biogenesis, which we could not definitively rule out in our paper.⁵

Surveillance of NPC assembly

NPC assembly (at least during interphase) likely begins with the recruitment of membrane-associated nups to the NE, and is followed by a step-wise recruitment of individual nup subcomplexes; a major challenge is the lack of understanding of the structural and biochemical intermediates along this pathway. In addition, at some point in assembly, membrane remodeling at the nascent NPC leads to the fusion of the INM and outer nuclear membrane (ONM) and the formation of a pore, but the molecular mechanism remains ill defined.

We suggest that the local recruitment of hundreds of nups coupled to a membrane fusion event leads to possible scenarios in which an individual NPC biogenesis event proceeds down a path that leads to an aberrant or dead-end product. Possibilities could include a relative change in nup stoichiometry between complexes, or perhaps a misfolded nup protomer that prevents any further assembly – like a "wrench in the gears." In a surveillance model, Heh2 and/or ESCRT-III would have the capacity to recognize these aberrant assembly intermediates to facilitate their removal. Since Heh2 is capable of binding nups, but is not a stable component of NPCs,²⁹ we think that it is the most likely candidate that might help biochemically distinguish between an NPC assembly intermediate and an NPC. The mechanism by which Heh2 might perform this function is not obvious, but we favor a model in which there is an upstream factor likely in the ubiquitin-proteasome system capable of recognizing and ubiquitylating aberrant NPC assembly intermediates. This makes the prediction that nups bound by Heh2 would be

ubiquitylated—a focus of our current research.

Potential models of aberrant assembly clearance

Our focus on ubiquitin as a potential signal in NPC assembly is supported by the data that a fraction of at least Nup85 is degraded by the proteasome when NPC assembly is perturbed genetically, and stabilized in the absence of Vps4.⁵ Thus, our data raise the critical question of how ESCRT-III/Vps4 act to remove aberrant NPC assembly intermediates from the NE; we are testing 2 possible models based on our understanding of ESCRT-III/Vps4 function (Fig. 1C and D). Both begin with the recruitment of ESCRT-III/Vps4 to an NPC assembly site by direct interactions with either Heh2 and/or nups. In the first scenario, Vps4 plays a role in stripping ESCRT-III from the membrane in complex with nups (Fig. 1C). We think that this is plausible since there is a growing consensus that Vps4 functions like other AAA ATPases such as p97/Cdc48, NSF, and ClpB, which are thought to remodel proteins by threading them through the central core of the oligomerized AAA ATPase.³⁰ A variant of this scenario includes the ER-Associated Degradation (ERAD) machinery as a contributor to the removal of defective NPC assembly intermediates.³¹ Consistent with this possibility, invaginations of the INM and intra-NE-luminal vesicles similar to what we observe in *vps4Δ* strains have also been seen in yeast strains expressing temperature sensitive alleles of *NPL4*,³² a co-factor of the Cdc48 complex; these data support a potential convergence of quality control pathways. Interestingly, recent work supports that an arm of ERAD exists at the INM.^{33,34}

The invaginations/intraluminal vesicles observed in strains containing *vps4*, *npl4* and other alleles that impact NPC function, hints at another potential model where NPC assembly intermediates might be cleared through a vesicular intermediate in the NE lumen (Fig. 1D). Such a model is compelling since the formation of an intraluminal vesicle would be topologically identical to the MVB and would employ ESCRT-III's established mechanism in membrane scission. This

mechanism would also be analogous to herpesvirus/Epstein-Barr virus and mega-RNP nuclear egress³⁵; genetic and biochemical evidence supports a role for ESCRT-III/Vps4 in Epstein-Barr virus nuclear egress.³⁶ Interestingly, it has been proposed that a nuclear egress mechanism might be an appealing way to clear large toxic nuclear protein aggregates too big to diffuse through the NPC,³⁷ precisely what we expect to accumulate upon NPC misassembly.

Does ESCRT-III directly contribute to NPC biogenesis?

A compelling argument can also be made for ESCRT-III function in the early steps of NPC assembly through the generation of an INM invagination (Fig. 1B). Indeed, there is mounting genetic and phenotypic evidence that supports a model in which pore formation could occur through an inside-out mechanism. For example, several studies suggest the involvement of integral INM proteins in helping to form an early NPC assembly intermediate.^{29,38} Moreover, NLSs are found on nups important for early assembly events.^{39–42} Lastly, biochemical intermediates and invaginations of the INM have been observed after the genetic imposition of assembly blocks.^{43–45} While proteins of the reticulon family^{27,28} and nups capable of deforming membranes^{46,47} might generate positive membrane curvature that could help form these invaginations, it has been appropriately argued that the generation of negative curvature might be more effective in promoting the formation of a nuclear pore,⁴⁸ precisely the kind of curvature that would be compatible with an ESCRT-III mediated mechanism.

That ESCRT-III might be involved with forming INM invaginations during nuclear pore formation is an intriguing possibility and suggests (as might be expected) that surveillance and NPC assembly could be linked. In such a scenario, ESCRT-III polymerization (recruited and activated to an NPC assembly site by Heh2) helps form an INM invagination. The 'positive' curvature at the interface of the INM and the invagination (Fig. 1B) would be recognized by the amphipathic helices in the scaffold nups

to facilitate building the outer ring complex.^{49,50} In this "normal" scenario the proper assembly of the NPC scaffold would stabilize the neck of the ESCRT-III-driven invagination, which could prevent membrane scission and might facilitate fusion with the ONM. In contrast, when NPC assembly goes awry, the scaffold is not built correctly and is incapable of stabilizing the invagination. Moreover, fusion with the ONM might be inhibited or delayed leading to persistent ESCRT-III/Vps4 action, which would ultimately drive membrane scission and the formation of a vesicle encapsulating the aborted NPC assembly intermediate in the NE lumen (Fig. 1D). While this model clearly requires extensive experimental evidence to prove, it makes the prediction that luminal vesicles would be more prevalent under conditions in which INM-ONM fusion is attenuated. One could interpret the luminal vesicles observed in *apq12*,⁴⁵ *acc1*,⁵¹ *gle2*,⁵² and *nup116*⁵³ strains in such a framework. This model would also support that membrane fusion is a commitment step in NPC formation, and might explain why malformed NPCs accumulate in the SINC, instead of being removed and degraded.

Outlook

There is little doubt that the precise mechanism by which ESCRT-III and Vps4 function in NPC biogenesis remains to be fully understood, and we look forward to contributing to this ongoing endeavor in the near future. NPC biogenesis is one of several membrane remodeling events that occurs at the NE, which might be aided by the membrane sculpting capacity of ESCRT-III. The insertion of the yeast centrosome equivalent (the spindle pole body; SPB) into the NE requires similar factors as in NPC biogenesis, suggesting overlap in their underlying mechanisms.^{54,55} Moreover, in fission yeast, the SPB is extruded after mitosis through a mechanism that remains ill defined. Indeed, defects in either insertion or extrusion mechanisms might underlie the observed over amplification of SPB components in *vps4Δ* in *S. pombe*.²⁴ In multicellular eukaryotes, massive membrane remodeling occurs during mitotic NE-disassembly and post-mitotic NE

reformation; we wonder how ESCRT-III will function in these contexts. Moreover, the capacity of ESCRT-III to repair plasma membrane wounds^{15,16} raises the possibility that it might also be capable of repairing the transient NE-ruptures that have been observed in several cancer cell lines^{56,57} and in cells containing laminopathy mutations,⁵⁸ a model consistent with the growing role of ESCRT-III in membrane homeostasis and quality control.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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