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Border safety: quality control at the nuclear envelope

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Abstract

The unique biochemical identity of the nuclear envelope confers its capacity to establish a barrier that protects the nuclear compartment and directly contributes to nuclear function. Recent work uncovered quality control mechanisms employing the ESCRT machinery and a new arm of ERAD to counteract the unfolding, damage or misassembly of nuclear envelope proteins and ensure the integrity of the nuclear envelope membranes. Moreover, cells have the capacity to recognize and triage defective nuclear pore complexes in order to prevent their inheritance and preserve the longevity of progeny. These mechanisms serve to highlight the diverse strategies used by cells to maintain nuclear compartmentalization; we suggest they mitigate the progression and severity of diseases associated with nuclear envelope malfunction like the laminopathies.

Keywords

nuclear envelope; nuclear pore complex; inner nuclear membrane; compartmentalization; ESCRT; ERAD; quality control

The nuclear envelope as a nuclear subcompartment

As the defining organelle of eukaryotes, the nucleus serves as an excellent model to identify mechanistic paradigms that generate and maintain organelle identity and function throughout the lifetime of a cell. Two concentric membranes delimit the nucleus, the “inner” and “outer” nuclear membrane (INM and ONM), which together comprise the nuclear envelope (NE). The NE is contiguous with the endoplasmic reticulum (ER) but is enriched in a subset of proteins (and likely lipids) that biochemically and functionally distinguish it from ER. This specialization is contributed predominantly by integral membrane proteins that localize to the INM (Box 1; Fig. 1), and by nuclear pore complexes (NPCs), which mediate the bidirectional exchange of molecules across the NE (Box 2). The nuclear lamina, which is an interwoven network of the intermediate-filament like lamin proteins that underlies the INM is also generally considered to be part of the NE, although it is not universal to all eukaryotes (Box 1). Over the last few decades major efforts by the field have illuminated mechanisms that lead to the formation and function of the NE. These include studies aimed at elucidating how the NE is broken down and reformed during mitosis[1,2],

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how NPCs are assembled[3] and mediate nuclear transport[4], and how integral INM proteins are targeted to[5], and function, at the INM[6]. It is becoming clear, however, that there is a disruption in NE organization and function as cells age and in human diseases caused by NE malfunction[6,7]. These observations suggest that a cell's capacity to maintain NE function in the context of genetic or physical perturbation will be critical for its ultimate viability and lifespan. With this in mind, recent work is revealing quality control (QC) mechanisms that safeguard the integrity and function of the NE, while also protecting cell viability in the face of NE malfunction.

The compartmentalization of QC

The best understood QC mechanisms are those that contribute to protein homeostasis (proteostasis) by stimulating the refolding or degradation of misfolded or damaged proteins [8,9]. The gradual loss of these mechanisms is thought to contribute to the age-related decline of cellular function, and to the accumulation of toxic misfolded protein aggregates characteristic of the pathology of neurodegenerative disorders like Alzheimer's or Huntington's disease[10]. Interestingly, toxic protein species often accumulate in cellular organelles like the nucleus[11], which suggests that QC mechanisms might act locally to protect the functionality of cellular compartments. Indeed, there is evidence that the cytosol[12,13], plasma membrane[14], mitochondria [15], nucleus[16] and ER[17,18] all have dedicated protein QC machineries capable of recognizing and eliminating misfolded or damaged proteins.

These organelle protein QC pathways generally converge on either the ubiquitin-proteasome system or autophagy machineries to ultimately clear defective proteins from the cell. It is worth considering that there is also a spatial segregation of these degradation mechanisms as the autophagy machinery is thought to be excluded from the nuclear compartment[19], whereas some proteasome substrates are degraded in the nucleus[10] - a reflection of the steady state nuclear localization of the proteasome (often at the nuclear periphery[20,21]) of many cells[22]. This segregation of degradation machineries has clear implications for how misfolded or damaged NE proteins and insoluble protein aggregates (typically dealt with by autophagy) are cleared from the nucleus. For example, the accumulation of proteasomal subunits at the nuclear periphery makes the prediction that a ubiquitin-proteasome-dependent protein degradation mechanism might occur specifically at the INM; recent work supports that this is indeed the case[23,24].

Protein QC at the INM

There are several reasons why the INM might require a dedicated protein QC machinery. Since there is no protein synthesis in the nucleus, proteins at the INM are isolated from other protein QC pathways that act early, during, or just after translation to ensure proper folding and targeting[25–27]. Further, in addition to 'normal' stresses encountered by all proteins (thermal, oxidative etc.), we suggest that the nucleus, as it is such a large and 'stiff' organelle[28], is also uniquely subjected to both internal (mediated by connections to the cytoskeleton) and external mechanical stress that could lead to unfolding or damage of INM proteins, including lamins, which play a key role in determining the nucleus' mechanical

properties[28]. As the NE is continuous with the ER, it would seem plausible that ER protein QC mechanisms like ER associated degradation (ERAD;[18]) might also extend to the INM proteome. The key components of ERAD are evolutionarily conserved but are best characterized in yeast and include two E3 ubiquitin ligases, Hrd1 and Doa10, thought to be responsible for the ubiquitylation of all known ERAD substrates, thus targeting them for proteasomal degradation[29].

Many clues from work carried out over the last fifteen years support that ERAD might function to degrade unfolded INM proteins, including pioneering work establishing that Doa10 (but not Hrd1) is capable of accessing and acting at the INM[30]. Moreover, ERAD components are known to be involved in the turnover of several NE proteins[31–33]. The most direct evidence for ERAD at the INM was provided by recent work testing the stability of the product of a *SEC61* allele (*sec61-2*), which encodes an ER translocon component that can be conditionally unfolded after synthesis and membrane integration at high temperature[23]. By appending an INM-targeting sequence to the *sec61-2* protein, its unfolding could be specifically triggered at the INM. Remarkably, the degradation of the INM-localized *sec61-2* was dependent on the function of two relatively uncharacterized E3 ligases, Asi1 and Asi3(Fig. 1A). Unlike Doa10 and Hrd1, Asi1 and Asi3 accumulate at the INM at steady state[34,35], supporting the existence of a dedicated INM-specific arm of ERAD (Fig. 1A).

Protecting the identity of the INM

Beyond the removal of unfolded proteins in specific sub-compartments like the INM, organelles might have the capacity to specifically recognize and degrade inappropriately localized proteins. One compelling example is the degradation of mistargeted tail-anchored ER proteins to mitochondria, mediated in part by a mitochondrial AAA ATPase[36]. Thus, in addition to serving as recipients of targeted proteins after synthesis, organelles may have a greater capacity than anticipated in controlling their local proteome by distinguishing ‘self’ from ‘non-self.’ The INM might be particularly vulnerable to the mistargeting of membrane proteins because of its continuity with the ER, the nearly exclusive site of membrane protein integration. Consistent with this idea, Sec61, a component of the ER translocon, can access the INM[30], suggesting that many ER proteins might be capable of traversing the nuclear pore membrane. It is plausible that the mistargeting or inappropriate retention of membrane proteins might either displace native INM proteins, or drive dominant negative deleterious effects on nuclear functions, requiring mechanisms to protect the INM proteome.

The concept that the INM has the capacity to differentiate between properly targeted and mistargeted proteins is supported by a genetic screen used to identify putative targets of Asi-mediated ERAD, several of which turned out to be components of the vacuole membrane[24]. As the screen was carried out by assessing the fluorescence of a C-terminal tandem fluorescent timer protein appended to virtually every yeast open reading frame, it was possible that the addition of a tag affected the vacuolar localization of these proteins. This assumption was correct, as the over expression or C-terminal tagging of a subset of vacuolar membrane proteins resulted in their accumulation at the NE and ER[24]. These

results support a model in which the Asi complex surveys the INM proteome and has the capacity to distinguish ‘self’ and ‘non-self’ (Fig. 1A).

It will be exciting to understand whether the mechanism that underlies the molecular triaging of the Asi complex reflects fundamental principles that can be extended to other examples of organelle proteome surveillance[36]. Since the composition of the INM is ultimately governed by the NPC, which imposes a diffusion barrier to membrane proteins with extraluminal domains greater than ~90 kD and facilitates the active-transport of others[5,37], such a model makes the testable prediction that mutations that make the NPC more permissive to INM protein accumulation[30,38–40] could provide genetic backgrounds where the Asi complex (or mammalian equivalent, yet to be identified) is uniquely required for cell viability.

Protecting the INM lipidome

Interestingly, in addition to its role in protein QC, ERAD also controls the levels of enzymes that synthesize sterols in both yeast and mammalian cells[18]. This raises the possibility that a major function of the Asi pathway is to directly regulate the ergosterol biosynthesis pathway, perhaps by spatially segregating ergosterol synthesis away from the INM[23]. Indeed, Erg11, a critical enzyme in the ergosterol synthesis pathway, localizes to the ER and enriches at the NE when its turnover is attenuated in *asi1* cells[23]. Since ergosterol is known to alter the fluidity of membranes, one model would be that membrane fluidity is critical for NE function. This idea echoes several studies that have implicated a distinct spatial segregation of lipids within the NE critical for enabling NE dynamics[41], including the insertion of the centrosome in yeast[42,43], the biogenesis of NPCs[44–46] and changes in membrane composition and shape during mitosis[47–50]. Thus, it is exciting to consider the Asi complex as a critical component of a QC machinery crucial for the maintenance of the NE subcompartment by protecting not only its unique proteome, but perhaps a unique lipidome as well. Much like the challenges inherent in identifying the full INM proteome due to its intimate connection with the ER[51], identifying a lipidome of the INM will prove even more daunting. We suggest that this will be a critical necessity, however, as it will provide an invaluable source of insights into the mechanisms underlying NE dynamics.

Maintaining the NE barrier with ESCRTs

Changes in the biophysical properties of the NE membranes likely enable dynamic membrane remodeling events essential for NE function. For example, unlike the majority of other organelles, which are inherited intact by daughter cells during mitosis, the NE (including NPCs) are completely broken down and reformed with every cell division in some eukaryotes [1,2]. During interphase, the NE is remodeled through the biogenesis of new NPCs, and, at least in some cells, the budding of vesicles carrying ‘mega’ ribonucleoprotein particles through the NE[52]. We suggest that under circumstances in which these ‘normal’ membrane remodeling events are not performed correctly they pose a threat to nuclear compartmentalization, and therefore these events might be under surveillance of QC mechanisms that would function to ensure NPC function and/or NE integrity. Consistent with this idea, the depletion of nuclear basket nups triggers an Aurora-

B mediated inhibition of cytokinetic abscission, thus intimately linking the biogenesis of NPCs to cell cycle progression[53]. Interestingly, this abscission checkpoint relies on the function of the Endosomal Sorting Complexes Required for Transport (ESCRT) machinery[54](Box 3), exemplifying one of several ways in which the ESCRTs are linked to NE function.

Beyond NPC assembly, the greater threat to nuclear compartmentalization during the terminal steps of NE reformation after mitosis is the establishment of a continuous NE. During NE reformation, ER tubules attach to the chromatin surface, flatten into a sheet and spread laterally to encapsulate the genome[1,2]. These ER membrane sheets converge into annuli, which ultimately need to be fused to seal the NE[1]; two exciting studies implicate the ESCRT-III machinery and the AAA ATPase VPS4 (Box 3) as mediators of this critical process [55,56](Fig. 1C). Moreover, ESCRT-III also recruits spastin to sites where spindle microtubules (MTs) are attached to chromatin[56]. Since spastin cleaves MTs, its recruitment provides a compelling mechanism that links MT-clearance to NE sealing (Fig. 1C). The use of the ESCRT-III machinery to close NE holes is appealing as the membrane topology at these sites would be identical to what is observed in other ESCRT-III-mediated membrane fission events[57](Fig. 1C; Box 3). However, it is unlikely that topology alone is sufficient to recruit ESCRT-III to the NE because protein adaptors are needed for its distribution at other subcellular locations[57].

Consistent with the idea that there might be NE-specific ESCRT-III adaptors, UFD1 promotes ESCRT-III recruitment to the nascent NE, perhaps through direct binding to the ESCRT-III subunit, CHMP2A[55]. A role for UFD1 makes sense as, along with its binding partners NPL4 and the AAA-ATPase Cdc48/p97, it has been previously implicated in sealing the NE, likely through the removal of the inhibitory influence of Aurora B kinase from the chromatin surface[58,59]. It might not act alone, however, as a less well characterized ESCRT-III-like component, CHMP7, also appears to be a key player in ESCRT-III NE recruitment[56]. Moreover, as neither UFD1 nor CHMP7 are components of the NE, it remains plausible that additional NE recruitment factors are yet to be identified. Because of their known roles in NE reassembly, key candidates are integral INM proteins[2] (Box 1).

Recent work in budding yeast indicates that Heh1 and Heh2 (the LEM domain integral INM protein orthologues; Fig. 1), interact with ESCRT-III at the NE[60]. The data support a model in which ESCRT-III/Vps4 contribute to NPC biogenesis, perhaps by facilitating the clearance of defective NPC assembly intermediates from the NE (Fig. 1B). The ultimate illumination of the molecular mechanisms will require the examination of NE morphology at sites where ESCRT-III is recruited by the LEM-domain containing proteins. For example, it is possible that aberrant NPC assembly leads to the formation of a hole in the NE that necessitates an ESCRT-III-mediated membrane fusion event to seal the NE(Fig. 1C). Alternatively, defective NPC assembly intermediates might be stripped from the membrane by the AAA ATPase Vps4, which could remodel protein complexes through a mechanism analogous to other AAA ATPases[61](Fig. 1B). Last, an exciting possibility would invoke a model in which ESCRTs clear misassembled nups through a vesicular intermediate within the NE lumen[62](Fig. 1B) in a mechanism analogous to herpesvirus nuclear egress[63].

While the precise mechanisms are currently elusive, a unifying phenotype between yeast and mammalian cells is the loss of nuclear compartmentalization in the absence of ESCRT-III/Vps4 function[55,56,60]. Therefore, cumulatively, these ESCRT studies suggest that the most conserved aspect of ESCRT-III function at the NE is to protect nuclear compartmentalization throughout the cell cycle.

Protecting NPC inheritance by spatial quality control

While it is possible to envisage how ESCRTs could seal the NE or remove a defective NPC assembly intermediate, it is less obvious how cells are capable of coping with an NPC that loses function due to protein unfolding or damage, particularly since there are no known mechanisms to remove fully formed NPCs that span both the INM and ONM. Indeed, the scaffold components of the NPC (Box 2) are extremely stable[64,65], being replaced over month(s) timescales[66,67]. Moreover NPCs can accumulate oxidative damage that impedes their function in post-mitotic neurons[65] where some scaffold nups are lost over time[67], suggesting that NPC malfunction might be an input to aging. Consistent with this idea, work in budding yeast supports that the robustness of the nuclear transport system might directly influence replicative lifespan, suggesting NPC function is critical for ensuring longevity[68]. Since yeast undergo an asymmetric division in which the NE does not break down, it provides a valuable model to explore how cells assess and deal with malfunctioning proteins and complexes (including NPCs) and how these pathways impact aging[69].

Studies in budding yeast have been instrumental in identifying ‘spatial quality control’ mechanisms that lead to the sequestration of misfolded proteins into aggregates or inclusions[70]. These inclusions serve as holding tanks that mitigate the deleterious effects of defective proteins on cellular homeostasis, viability and lifespan. Often they are associated with organelles, for example, as its name suggests, the juxtannuclear (JUNQ;[71]) or intranuclear quality control (INQ;[72]) compartment is associated with the nucleus, whereas the insoluble protein deposit (IPOD) is proximal to the vacuole[71]. In some cases, organelle-binding serves as an effective strategy to restrict the inheritance of misfolded proteins to daughter cells[73,74]; other mechanisms invoke motor-driven transport along cytoskeletal elements[75]. The evidence that damaged NPCs might also be an input to aging suggests that there might be mechanisms to prevent their inheritance to daughters; thus in lieu of removal, the use of aggregation or clustering at the NE is a potential strategy.

Consistent with the idea that NPCs might aggregate with malfunction and/or age, a cluster of NPCs has been observed in replicatively-old mothers, which has been termed the ‘NPC-cap’ [76]. While the cap is most apparent when episomal plasmids are attached to NPCs through the SAGA complex, morphologically it resembles the Storage of Improperly assembled NPCs compartment (SINC), which is a repository for misassembled NPCs at the NE[60] (Fig. 1D). As the nuclear transport defects in SINC-containing mother cells are reversed in daughters[60], these data cumulatively suggest that yeast have the capacity to segregate functional from non-functional NPCs. A potential handle on the mechanism that triages NPCs might lie with the central channel FG-nup, Nsp1. Indeed, the levels of Nsp1 and a related nup, Nup116, are reduced in old mother cells[68]; these results synergize with studies in which the controlled depletion of Nsp1 prevents the inheritance of NPCs to

daughters[77,78]. Therefore, Nsp1 is likely a critical node in a QC pathway that senses the functionality of NPCs and regulates their transmission to progeny, perhaps through the modulation of a bud neck diffusion barrier[76–80].

Concluding remarks

We have highlighted several contemporary studies that support the existence of QC mechanisms that ensure nuclear compartmentalization by preserving NPC function and the integrity of the NE (Fig. 1). These studies raise important questions for future work (see Outstanding Questions Box), many of which are focused on the mechanisms that enable the recognition of defective INM proteins, NPCs, or NE holes. Moreover, as many of these mechanisms have been characterized in yeast, it will be important to identify their mammalian counterparts. It should be noted, however, that the cellular machineries that function in these pathways are, in general, well conserved, supporting that while mechanistic nuances might exist in multicellular eukaryotes, the concept that the NE is protected by analogous QC mechanisms is a likely scenario. With this in mind, it will be interesting to explore these mechanisms in multicellular, post-mitotic systems that do not benefit from a mitotic breakdown event, which would release unfolded or damaged proteins from macromolecular assemblies like NPCs or the lamina to facilitate their clearance. The observation that there are differences in the turnover kinetics of scaffold nups in rat brain neurons[67] supports that there are QC mechanisms capable of removing nups from the NPC over timescales of months to years; how this occurs, and how to study these events, is a challenge for future work.

Interestingly, the loss of scaffold nups due to damage in old neurons[65,67] suggests that the ability to replace them might be attenuated with age, supporting the concept that a reduced QC capacity contributes to the pathology of proteotoxic diseases[10]. Therefore, we think that it is critical that QC mechanisms are incorporated into our description of the pathology of many diseases that show defects in NE function[81]. Many of the laminopathies, for example, result from the expression of pathogenic *LMNA* alleles that act in a dominant negative fashion, perhaps by forming protein aggregates at the NE or in the nucleus[82,83]. To clear nuclear aggregates in post-mitotic cells might necessitate QC mechanisms that invoke a budding mechanism through the NE, which was recently hypothesized[81], perhaps aided by ESCRT-III acting at the INM[60]. Alternatively, a mechanism analogous to piecemeal autophagy of the nucleus (PMN) in which nuclear-vacuolar/lysosomal contacts engulf portions of the nucleus[84] could be employed. While PMN has only been observed in yeast, lysosomes with nuclear contents abut the nucleus in cells expressing some laminopathy alleles[85], hinting at the potential conservation of this pathway. Moreover, the pharmacologically-induced upregulation of autophagy has been shown to mitigate the deleterious impact of mutant *LMNA* alleles on cardiomyopathy[86], supporting that treatment scenarios might also benefit from considering QC pathway targets.

In addition to the potential proteotoxicity of proteins encoded by *LMNA* mutations, a common feature of cells with a defective lamina is a disruption of NE integrity and the ensuing mixing of cytosolic and nuclear contents[7,28]. Remarkably, some of the NE ruptures observed in cells expressing laminopathy alleles[87] and also those seen in several

cancer cell lines[88], are transient and are thus being constantly repaired. It is easy to imagine how ESCRT-III's role in sealing the NE at the end of mitosis could be ported to one that is capable of repairing such rupture events; this hypothesis is supported by ESCRT-III's capacity to repair plasma-membrane wounds[89,90] and thus provides another exciting avenue of future experimental pursuit into mechanisms that protect the nuclear compartment.

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Box 1**The inner nuclear membrane proteome**

The INM is enriched with a subset of integral membrane proteins that are synthesized and inserted into the ER. The mechanism of membrane-protein targeting to the INM remains a topic of active investigation and this work has been recently reviewed[5]. While there appears to be differences in the requirements for energy and/or nuclear transport receptors for the targeting of individual INM proteins, there is nonetheless consensus that membrane proteins move along the continuous bilayer from the ER/ONM, through the nuclear pore membrane, to the INM where they interact with nuclear factors including the genome and the nuclear lamina[6]. The continuity of the NE-ER system and the inability to distinguish between the INM and ONM by conventional fluorescence microscopy has made defining the INM proteome a persistent challenge in the field. Proteomic studies support that there are dozens of NE integral (NETs) proteins in mammalian cells[51], some of which are differentially expressed in tissues[91] but not all are known to localize exclusively to the INM; yeast have far fewer with only a handful clearly identified (Fig. I). In Metazoans, many integral INM proteins bind to the nuclear lamina, which is a network of intermediate-filament-like proteins made up of A and B-type lamins (Fig. I). The lamins provide mechanical stability to the nucleus, contribute to the NE-tethering of chromatin that is largely transcriptionally silent and influence signaling pathways[92]. While not present in all eukaryotes, the prevalence of human diseases associated with mutations in the lamin-encoding genes (*LMNA* and *LMNB*) have made understanding lamin function a priority[6,83].

The most conserved integral INM proteins from yeast to humans are members of the LAP2-emerin-MAN1(LEM)[93] and the Sad1-Unc84 (SUN) families[94], categorized by the presence of the 'LEM' and 'SUN' domains (Fig. I). The LEM domain is a 40–50 amino acid helix extension helix motif that interacts with (at least in Metazoans) the chromatin-binding factor Barrier-to-Autointegration factor (BAF), but likely also with DNA and other proteins[93,95]. The SUN domain proteins are found within the perinuclear space/NE lumen and exist as trimers that bind three KASH-domain proteins at the ONM (Fig. I). The binding of the KASH proteins with cytoplasmic cytoskeletal elements mechanically couples the cytoskeleton with chromatin, raising the possibility of the direct mechanical modulation of genomic processes[96].

Box 2**Nuclear pore complexes**

NPCs are built from ~30 proteins, termed nucleoporins or nups, in (likely) all eukaryotes. These 30 nups are found in distinct subcomplexes, which assemble in multiples of 8 to generate the iconic 8-fold radial symmetry of the full assembly. Indeed, by using an amazing variety of structural, imaging and modeling approaches, we are approaching an atomic-level understanding of the structure of the scaffold of the NPC[97], which once formed is incredibly stable[66]. A major challenge remains in understanding the organization of a group of disordered nups rich in phenylalanine and glycine amino acid residues found within repetitive motifs (FG-nups). The FG-nups likely line the central channel of the NPC and play two major roles: (1) they establish a diffusion barrier to soluble proteins over ~40 kD and (2) they facilitate the active transport of macromolecules (and some membrane proteins) bound to a family of soluble nuclear transport receptors, through a mechanism that is still debated in the field[4,5].

Another major challenge is to understand how the NPC is assembled. The general thinking is that NPC assembly occurs through a post-mitotic and interphase mechanism[3], although recent work supports that there are likely more similarities between these two scenarios than initially thought (see [2] for discussion). Both mechanisms ultimately rely on the step-wise assembly of individual nups (likely within their cognate complexes), which is coupled to events at the membrane to form and stabilize a nuclear pore. Several membrane-bending nups[98–100] and ER-shaping proteins[101] likely play key roles in forming a pore but no fusogen has been identified that is capable of fusing the INM and ONM. Understanding the fusion mechanism and how it is coupled to the ultimate insertion of hundreds of individual proteins represents a significant hurdle to elucidating the mechanism of assembly. Moreover, since the misassembly of even one NPC could contribute to a loss of the integrity of the diffusion barrier across the NE, it will be necessary to understand the processes that ensure assembly proceeds correctly[60].

Box 3**The ESCRT machinery**

The ESCRT machinery consists of several modules, ESCRT-0, I, II, III and the AAA ATPase, Vps4 (and associated factors)[57], all of which are well conserved from yeast to man[102]. It has been widely studied as a key player in multivesicular body biogenesis where it plays a role in the sorting of ubiquitylated transmembrane receptors into intraluminal vesicles formed from the invagination and scission of endosomal membranes. While ESCRT's 0, I and II are thought to play roles in the sorting process, ESCRT-III and Vps4 are required for the ultimate membrane scission event that forms the intraluminal vesicle[57]. While the precise scission mechanism remains debated, there are several models that have been proposed that incorporate ESCRT-III's remarkable capacity to oligomerize into a filament that forms a helical or conical spiral that is capable of invaginating membranes away from the cytosol and lining the neck of an invagination[57,102,103]. Vps4 is thought to play a role in disassembling the ESCRT-III filament in a terminal step that remodels the complex in a way that might promote scission[104]. Interestingly, recent studies in yeast and in mammalian cells support that it is only ESCRT-III and the AAA-ATPase Vps4 that function at the NE[55,56,60]. Since ESCRT-II is thought to play a role in recruiting ESCRT-III to endosomes, analogous recruitment factors likely contribute to ESCRT-III's NE binding and might include UFD1[55], CHMP7[56] or LEM domain integral membrane proteins[60].

The use of site-specific adaptors is thought to be an important mechanism to target ESCRT-III to multiple subcellular locations[57]. For example, ESCRT-III has been implicated in viral egress from the plasma membrane, membrane abscission during cytokinesis, centrosome maintenance, and plasma membrane wound repair[57,89,90]. In most of these contexts, it acts on membranes that share a common topology exemplified by a NE hole (Fig. 1C). It is likely that there are nuances to the fission mechanism that could be impacted by interactions with the adaptors themselves[105], or the use of unique combinations of ESCRT-III subunits[103,106].

Trends Box

- Protein QC is compartmentalized in eukaryotes: a dedicated arm of ERAD functions at the INM to protect its unique proteome, and perhaps its lipidome as well.
- The ESCRT machinery ensures the integrity of the NE membranes by sealing them at the end of mitosis.
- ESCRTs help ensure the proper assembly and function of NPCs during interphase.
- NPC malfunction is linked to aging: in analogy to spatial quality control mechanisms, budding yeast sequester malfunctioning NPCs into a cluster that helps prevent their inheritance to daughter cells.
- The loss of nuclear compartmentalization and the intermixing of cytosolic and nuclear contents is a feature of the cellular pathology of many human diseases, which might be mitigated by NE-specific QC mechanisms.

Outstanding Questions Box

- What are the mechanisms by which mistargeted and/or damaged integral INM proteins are recognized before clearance by ERAD? This is a critical question that goes to the heart of how cellular compartments are capable of assessing the functionality of their unique proteomes.
- How are ESCRTs recruited to holes in the NE? More specifically, providing the protein-adaptors have been identified, how are they capable of recognizing the unique topology of the NE holes, and is this putative mechanism used in other subcellular locations in which ESCRTs act, like, for example, during NPC assembly?
- Are cells capable of assessing the functionality of individual NPCs, and if so, how does this occur?
- How are damaged nups removed and replaced in old post-mitotic neurons?
- Is there a unique INM lipidome that is required to enable NE dynamics?
- Are there mechanisms analogous to piece-meal autophagy or viral nuclear egress that allow the removal of large protein aggregates from the nucleus?
- How do QC mechanisms impact the cellular and organismal pathology of the laminopathies and other diseases where NE malfunction has been observed?

Figure 1. Emerging mechanisms of QC at the NE

A. The proteome of the INM is protected through an arm of ERAD mediated by the Asi1/3 E3 ubiquitin ligases (orange), which are integral INM proteins. Other ERAD E3 ligases include Hrd1 (brown) and Doa10 (purple); Doa10 is also capable of accessing the INM, but does not accumulate there. In red are substrates of the Asi complex including Erg11 and mistargeted membrane proteins. Their removal and degradation are facilitated by the AAA-ATPase Cdc48 (and associated factors, not shown) and the proteasome. B. The biogenesis of NPCs is surveilled by the integral INM proteins, Heh1/2 (yellow) bound to nups (other shapes), which recruits ESCRT-III (blue) and the AAA-ATPase Vps4 (hexamer). ESCRT-III is thought to oligomerize into a filament, which might invaginate the membrane. The clearance of the defective NPC assembly intermediate could occur through different pathways including a vesicular intermediate in the NE lumen/PNS, or by being stripped from the membrane by Vps4. C. At the end of mitosis, membrane sheets enclose the genome and require annular fusion events to seal the NE. This is mediated by the recruitment of ESCRT-III to NE holes by the ESCRT-III like protein CHMP7 and/or UFD1 (yellow circles). The remodeling of the ESCRT-III filament by Vps4 drives membrane fusion (indicated by arrows). At sites of MT (red) attachment to chromatin, the ESCRT-III, Ist1, also recruits the AAA-ATPase Spastin (red hexamer), which is capable of severing MTs, thus coupling membrane sealing and MT-removal. D. In systems like yeast where the NE does not break down, defective NPCs are clustered into a compartment termed the SINC, which facilitates their retention in mother cells. In all panels, INM and ONM is inner and outer nuclear membrane, respectively.

Figure I. Common integral membrane proteins of the NE and their evolutionary conservation

The most conserved membrane proteins at the INM contain the LEM (blue) and SUN (green) domains. The KASH domain (red) is also well conserved although no clear KASH domain orthologue has been identified in *S. cerevisiae*. INM and ONM are inner nuclear membrane and outer nuclear membrane, respectively; PNS is perinuclear space.