Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Cell Biology

Closing a gap in the nuclear envelope

Marina Vietri[1,2], Harald Stenmark[1,2,3] and Coen Campsteijn[1,2]

The nuclear envelope (NE) ensures nucleo-cytoplasmic compartmentalization, with trafficking of macromolecules across this double membrane controlled by embedded nuclear pore complexes (NPCs). The NE and associated proteins are dismantled during open mitosis and reestablishment of this barrier during mitotic exit requires dynamic remodeling of endoplasmic reticulum (ER) membranes and coordination with NPC reformation, with NPC deposition continuing during subsequent interphase. In this review, we discuss recent progress in our understanding of NE reformation and nuclear pore complex generation, with special focus on work implicating the endosomal sorting complex required for transport (ESCRT) membrane remodeling machinery in these events.

Addresses
[1] Centre for Cancer Biomedicine, Faculty of Medicine, University of Oslo, Montebello, N-0379 Oslo, Norway
[2] Department of Molecular Cell Biology, Institute for Cancer Research, Oslo University Hospital, Montebello, N-0379 Oslo, Norway
[3] Centre of Molecular Inflammation Research, Norwegian University of Science and Technology, Faculty of Medicine, N-7491 Trondheim, Norway

Corresponding authors: Stenmark, Harald (stenmark@ulrik.uio.no) and Campsteijn, Coen (coen.campsteijn@rr-research.no)

Current Opinion in Cell Biology 2016, **40**:90–97

This review comes from a themed issue on **Cell nucleus**

Edited by **Ulrike Kutay** and **Orna Cohen-Fix**

For a complete overview see the Issue and the Editorial

Available online 24th March 2016

http://dx.doi.org/10.1016/j.ceb.2016.03.001

Introduction

The cell nucleus is enclosed in a specialized double membrane called the nuclear envelope (NE). The outer nuclear membrane (ONM) displays continuity with the endoplasmic reticulum (ER) and provides connections to the cell cytoskeleton [1]. The inner nuclear membrane (INM) is a more isolated membrane environment, harboring a set of unique proteins and a meshwork of intermediate filaments called lamins that line the nuclear face of the INM and together form the nuclear lamina [2,3]. Both membranes are joined at annular junctions that are occupied by nuclear pore complexes (NPCs), large multisubunit channels that span the NE to control exchange of nuclear and cytosolic molecules [4,5]. Besides establishing nucleo-cytoplasmic compartmentalization, it has become clear that the NE and associated proteins as well as NPCs play important functions in a variety of chromatin-associated processes, such as establishment of chromosome territories, transcriptional regulation, DNA replication and DNA damage response [4,6,7]. It is therefore not surprising that compromised NE functions are linked to ageing and disease, including hereditary pathologies and cancer [8–11].

In metazoan cells, the NE breaks down during every cell division at onset of mitosis, enabling mitotic spindle microtubules to connect to kinetochores. After chromosomes have segregated, the NE and NPCs are reassembled during mitotic exit to generate functional sealed nuclei in the nascent daughter cells [12,13]. In this review we will highlight recent progress in studies of NE reformation with a special focus on the emerging role of the endosomal sorting complex required for transport (ESCRT) machinery.

Nuclear envelope dynamics during cell division

Nuclear envelope breakdown (NEBD) is initiated by membrane pulling forces exerted by microtubules (MTs) [14], but is spearheaded by mitotic kinases such as CDK1-Cyclin B1, Aurora B and PLK1 [13,15–17] that trigger extensive phosphorylation of numerous NE proteins, NPC components, lamins and INM proteins. This culminates in disruption of protein interactions, nuclear lamina integrity and connection of INM proteins to the condensing chromosomes [3,13,17,18]. Consequently, nuclear membranes together with their associated transmembrane proteins reorganize into the mitotic ER, which is excluded from the area occupied by chromosomes and the mitotic spindle by controlling contacts between ER membranes and MTs [19–24]. The structural organization of the mitotic ER is matter of debate with diverging results possibly due to the use of different cell lines and sample preparation methods. Both sheet-to-tubule reorganization [24–26] and a mitotic ER consisting mainly of sheets with a fraction of tubules have been observed [27–29].

Following anaphase onset, cells need to incorporate all chromosomes within a single nucleus and re-establish the interphase identity of their nuclear compartment [13,30,31•,32]. This involves reversal of hyper-phosphorylation of mitotic kinase substrates in a process involving inactivation (CDK1) or translocation (PLK1, Aurora B) of

mitotic kinases and the recruitment of the counteracting protein phosphatases PP1 and PP2A [33–37].

Reassembly of the nuclear envelope — membranes and nuclear pore complexes

NE reformation and reassembly of NPCs are key to re-establish nucleo-cytoplasmic compartmentalization. Recruitment of membranes derived from the mitotic ER is thought to be initiated at the rims of the chromatin discs, and several models have been put forward regarding the conformation of the ER structures that form the NE. According to the structure of the mitotic ER that was observed, ER tubules are proposed to organize in a network surrounding the chromatin discs and to flatten into sheets and expand [18,22,25]. Other studies have instead observed ER sheets that contact chromatin and expand on the surface of chromatin discs [18,29]. In cell-free systems, formation of a closed NE requires membrane fusion activity of factors such as Atlastin GTPases and SNAREs [38–40], although studies in living cells are needed to determine their direct contribution. NE reassembly and expansion of nuclear membranes on the surface of chromatin discs additionally involves ER shaping by proteins such as Reticulons and DP1/REEP protein family members [25,41–43], as well as attraction to the chromatin surface via INM proteins, such as Lamin B receptor (LBR) and Lamina-associated polypeptide 2 (Lap2β), but also transmembrane nucleoporins POM121 and NDC1 [44]. Transmembrane NE proteins can dock directly to the DNA through basic extraluminal domains, or are recruited via chromatin factors such as heterochromatin protein 1 (HP1) or barrier-to-autointegration factor (BAF), that bind LBR and LAP2-emerin-MAN1 (LEM)-domain containing proteins, respectively [45–47].

During early steps of anaphase prior to membrane recruitment, post-mitotic nuclear pore re-assembly is initiated by chromatin binding of the nucleoporin ELYS/MEL28 [48,49]. ELYS constitutes the anchoring and recruitment factor for the NUP107-160 complex, also called Y-complex, the constituent of the nuclear pore cytoplasmic and nucleoplasmic ring [49,50,51•,52]. Together, they function as seeds for the recruitment of the transmembrane nucleoporins POM121 and NDC1, followed by the rest of the approximately thirty different nucleoporins that make up a mature NPC consisting of hundreds of proteins [50]. Underlining their importance in defining sites of NPC formation, depletion of ELYS or the Y-complex results in the formation of a closed pore-free NE [53,54]. In contrast to the post-mitotic pathway of NPC assembly, *de novo* insertion of nuclear pores during interphase proceeds much slower [55] and does not rely on ELYS [56]. Instead, it has been proposed that several nucleoporins including NUP153 recognize or induce INM curvature and recruit the Y-complex and transmembrane nucleoporins to scaffold NPC assembly [50,56,57•,58•].

ESCRTs control nuclear envelope sealing during mitotic exit

The different models for NE reformation predict the requirement for annular membrane fission to seal the NE into a continuous double membrane. Recent work has shed light on this long-standing question by uncovering a central role for the ESCRT-III membrane remodeling machinery (Box 1) in this process. Canonically, cytosolic ESCRT-III monomers polymerize into filaments lining the inside of membrane annuli and critically rely on activity of the AAA+ ATPase VPS4 to orchestrate topologically similar membrane fission events in an ever expanding range of cellular processes [59,60]. Considering the topology, ESCRT-III and VPS4 provided excellent candidates for regulation of NE sealing.

Indeed, ESCRT-III subunits have been shown to display a punctate localization pattern at chromatin discs in a brief interval (4 min) during the anaphase-to-telophase transition in human cells [61••]. Initially, ESCRT-III foci can be detected at the rims of chromatin discs, but are observed progressively towards the core region of the mitotic spindle attachments as nuclear membranes expands across the chromatin disc during mitotic exit [61••,62••]. Electron tomography has shown that the main ESCRT-III filament constituent CHMP4B localizes to the inside of nucleo-cytoplasmic channels in the reforming NE [62••]. Together with the recruitment of other ESCRT-III subunits and the requirement for VPS4, this points towards a central role for ESCRT-III in constriction and sealing of NE annuli (Box 1; Figure 1). Accordingly, perturbation of ESCRT-III function at the NE increases the number of unsealed membrane foci and results in compromised nucleo-cytoplasmic compartmentalization [61••,62••]. Even though these experiments make a strong case for the essential role of ESCRTs in NE sealing, they do not prove sufficiency, and it remains possible that downstream factors are required to complete annular fission. Arguing against this, elegant work using giant unilamellar vesicles (GUVs) and recombinant ESCRT subunits has shown that the ESCRT machinery is sufficient to induce membrane fission and formation of intraluminal vesicles *in vitro* [63]. Disruption of ESCRT-III dynamics by depletion of CHMP2A or VPS4 induced the formation of DNA damage foci at the NE [61••]. Further experiments will be needed to determine whether these foci stem from unsealed NE and reactive oxygen stress (ROS) damage or DNA herniation [9,64], or whether they are a consequence of torsional stress on the NE and underlying chromatin [65] upon unchecked CHMP4B accumulation.

ESCRT-III is prominently enriched at sites of intersection between nuclear membranes and MT bundles attached to the chromatin disc, with its recruitment coinciding with lateral MT severing [61••]. But is ESCRT-III acting exclusively at membrane annuli that

Box 1 ESCRT-III in membrane fission

The endosomal sorting complex required for transport (ESCRT)-III proteins, or CHMPs (for charged multivesicular body proteins, or, chromatin modifying proteins) constitute an evolutionarily conserved protein family encompassing 12 members in humans (CHMP1A-7 and the related protein IST1 [60,89]). CHMPs function by orchestrating topologically similar membrane fission events, classically in the formation of intraluminal vesicles in multivesicular endosomes, virus budding from the plasma membrane and cytokinetic abscission. Recent years have seen an astonishing explosion in ESCRT-III functions to include plasma membrane repair, neuron pruning, and nuclear envelope sealing [59].

ESCRT-III assembly

ESCRT-III proteins exist as small soluble monomers locked in an auto-inhibitory state with the C-terminal regulatory region folded back on the N-terminal helical core domain (α1-α4; exemplified by CHMP4B in Figure to Box 1). Activation of ESCRT-III requires membrane association and binding to the ESCRT-II–CHMP6 nucleation complex, causing conformational changes that liberate the core domain for polymerization and formation of ESCRT-III filaments that line the inside of membrane necks [89]. Bro1 domain proteins such as ALIX have been proposed to function as alternative nucleators and although they indeed recruit CHMP4 paralogs *in vivo* and *in vitro* [90,91], it remains to be elucidated whether they directly nucleate or rather stabilize ESCRT-III filaments [92].

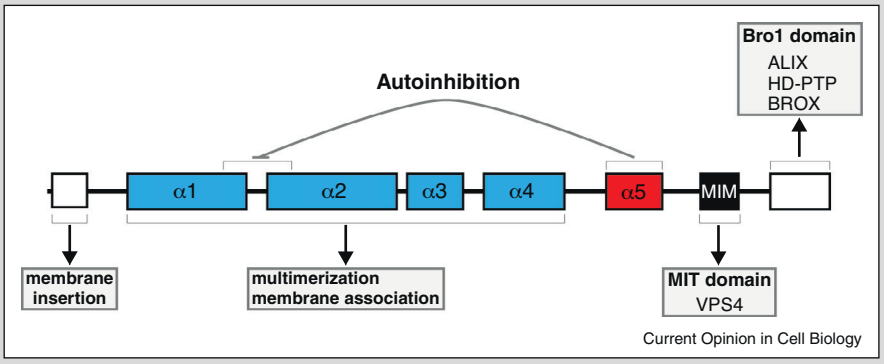
CHMP4B is the main constituent of ESCRT-III filaments, and it recruits CHMP2/3 that can convert flat CHMP4B spirals to 3-dimensional tubular filaments, with CHMP1 and IST1 the most downstream subunits recruited [61••,93]. Different ESCRT-III subunits have been shown to polymerize in a variety of structures, including linear filaments, spirals, tubes or domes *in vitro* [93–98]. However, what morphology they adopt in physiological settings and whether individual filaments are composed of different CHMP family members to form heteropolymeric filaments, or whether they constitute an assembly of individual homopolymeric filaments remains unclear [60,93,96,99•].

Upon activation, exposure of C-terminal elements regulates recruitment of a number of effector proteins through MIT-interacting motifs (MIMs) [94]. This short motif bestows individual CHMPs with remarkable selectivity for microtubule interaction and trafficking (MIT) domain-containing effectors, including kinases (ULK3 [84]) and AAA+ ATPases involved in microtubule severing (Spastin [60]) or ESCRT-III filament remodeling and membrane fission (VPS4 [89]).

ESCRT-III membrane association and constriction

ESCRT-III functions at regions of complex membrane architecture, involving both negative and positive curvature inside membrane necks. Based on structural evidence, it has been proposed that the upstream ESCRT-I complex bridges the positive curvature found at the rim of the membrane neck [100]. This targets the convex membrane interaction surface of the Y-shaped ESCRT-II–CHMP6 nucleator complex to negative curvature [101] with possible additional targeting by N-myristoylation of CHMP6 [102].

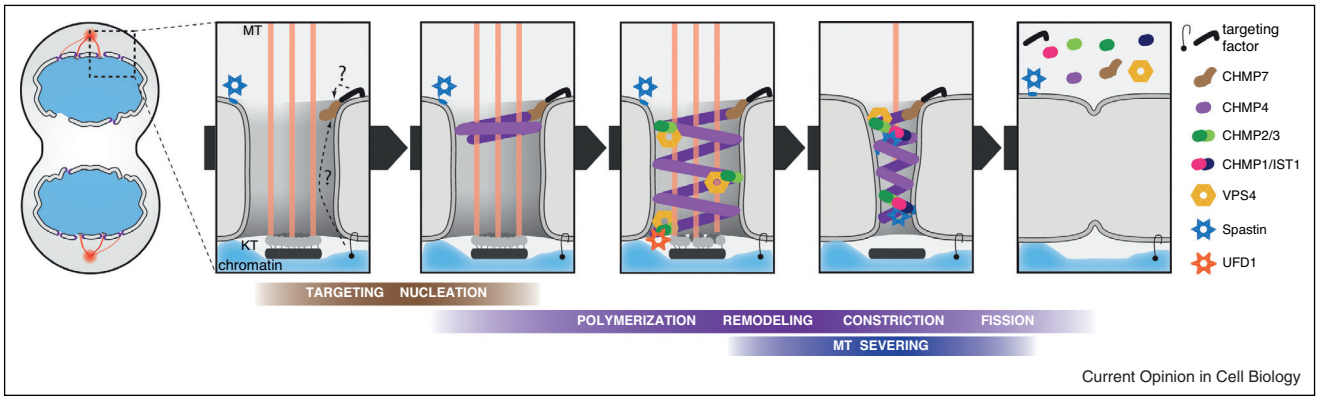
Upon nucleation, polymerizing ESCRT-III subunits establish extensive contacts with membranes through basic surfaces on their core domain [103•,104], and CHMP4B possesses an additional N-terminal amphipathic helix that inserts in regions of positive membrane curvature [103•] (Figure to Box 1). Subsequent recruitment of VPS4 by several ESCRT-III subunits plays a central role in the remodeling of ESCRT-III filaments [60,89,105,106] (Figure 1). However, how this translates to membrane constriction and fission remains to be conclusively established, with *in vitro* filament architectures and microscopy analyses leading to models involving ESCRT-III spirals, whorls, domes, or sliding elements [99•,107]. To add to the complexity, this dynamic process is subject to extensive regulation by signaling pathways, as exemplified by cytokinetic abscission checkpoint signaling where Aurora B and ULK3 dependent regulation of CHMP4C and IST1 controls ESCRT-III function in the presence of chromatin bridges, likely via restraining the activity of VPS4 [69,70,83,84].



are intersected by MTs? Interestingly, mitotic slippage experiments showed that the number of observable ESCRT-III foci was dramatically reduced during NE reformation in the presence of the MT depolymerizing drug nocodazole. This could be taken to indicate a role for ESCRTs specifically at NE-MT intersections, although it could also reflect a difference in ESCRT-III recruitment kinetics or quantity at sites lacking MT impediments.

With a striking analogy to cytokinetic abscission, the ESCRT-III-like subunit IST1 was found to recruit cytosolic and ER-associated isoforms of the MT severing AAA+ ATPase Spastin to chromatin discs (Figure 1). Perturbation of ESCRT-III function delays MT severing and vice versa, perturbation of Spastin function delays completion of ESCRT-III dependent NE sealing. This suggests that ESCRT-III could coordinate membrane

Figure 1



Model for ESCRT-III function during nuclear envelope reformation. During late anaphase, CHMP7 is targeted through unknown mechanisms to sites of microtubule (MT)-nuclear envelope (NE) intersections, and possibly also at MT-free annuli. CHMP7 activation nucleates CHMP4 polymerization, followed by recruitment of CHMP2/3 and VPS4 and finally IST1-dependent recruitment of Spastin. CHMP2 recruitment depends on UFD1 that could additionally contribute by extraction of Aurora B through the p97-UFD1-NPL4 complex. VPS4-dependent ESCRT-III filament remodeling in concert with Spastin-mediated MT severing drives membranes constriction and fission. It should be stressed that it remains unresolved whether ESCRT-III nucleation is initiated from the outer or inner nuclear membrane. Abbreviations: KT, kinetochore.

sealing with removal of MT filaments that impede annular membrane fission. Importantly though, disruption of ESCRT-III or Spastin only mildly affects MT severing, highlighting the existence of independent functionalities that control spindle disassembly during mitotic exit such as kinetochore-based detachment [66,67], other MT severing enzymes [68] or mechanical MT buckling [67].

With none of the canonical upstream regulators of ESCRT-III found to be required, such as upstream ESCRTs and Bro1 domain proteins [61••], how are ESCRT-III proteins recruited to and activated at the NE? Interestingly, a direct interaction between the key downstream ESCRT-III subunit CHMP2A and the UFD1 subunit of the p97 complex was identified and proposed to mediate recruitment of CHMP2A to the reforming NE [62••]. Since the p97-UFD1-NPL4 complex has previously been implicated in NE reformation as well as extraction of chromatin-bound Aurora B during mitotic exit, it is conceivable that UFD1 integrates these established functions with direct regulation of ESCRT-III function during NE sealing (Figure 1 [69–73]). However, since this study did not relate UFD1 function to upstream recruitment of the CHMP4B subunit, it remains unclear whether UFD1 function extends beyond CHMP2A. A tight reciprocal regulation of UFD1 and ESCRT-III functions would be expected in order to preclude uncontrolled CHMP4B polymerization, as observed in the absence of CHMP2A and its effector VPS4 [61••,62••].

Interestingly, the poorly characterized ESCRT-III like-protein CHMP7 localizes to NE holes (Vietri, Campsteijn, Stenmark, unpublished data) and was found to be essential for CHMP4B recruitment [61••], indicating that it presents a key regulator of ESCRT-III filaments at the reforming NE (Figure 1). Although CHMP7 function remains to be explored, its domain composition resembles that of established ESCRT-III nucleating complexes (Box 1), consisting of an ESCRT-II-like winged helix and a CHMP6-like ESCRT-III core domain [74]. Considering its ability to directly bind to CHMP4B [75], it is tempting to speculate that CHMP7 could function as an ESCRT-III filament nucleator. Whether CHMP7 architecture is sufficient to target ESCRT-III to membrane annuli or whether this entails additional factors for recruitment and activity, such as LEM domain proteins, curvature sensing proteins or regulators, remains to be elucidated [35,36,41,76••].

ESCRTs and nuclear pore complexes

Recent work has raised the question whether ESCRT-III activity at the NE extends beyond telophase NE sealing to NPC assembly and quality control. In budding yeast, VPS4 and core ESCRT-III components were shown to genetically interact with various NPC components, as well as the INM LEM domain protein HEH2 and the NPC assembly and NE liquidity regulator APQ12 [76••,77]. Furthermore, Snf7p (the yeast homolog of CHMP4B) was found to associate with Heh2p and colocalize at the NE. In yeast mutant strains with perturbed NPC assembly (*pom152Δ*) or in *snf7Δ* or *vps4Δ* strains, a fraction of NPCs clustered in a single focus at the NE, coined 'SINC' (for storage of improperly assembled NPCs). These data highlight a functional interaction between ESCRT-III and NPCs, although the nature of this intersection remains unclear. The authors propose a model where ESCRT-III clears misassembled NPCs from the NE, possibly by intraluminal vesicle budding

[76••], analogous to a previous report on ESCRT-dependent nuclear egress of Epstein-Barr virus (EBV) [78]. Whereas this scenario is conceivable for early NPC intermediates, it is more difficult to envision for clearance of defective NPCs that span the NE. In the absence of mechanistic data, other functional models such as ESCRT-III dependent membrane resealing at sites of misassembled NPCs are also plausible [79]. It would be interesting to explore the involvement of p97-UFD1-NPL4 as well as CHMP7 in this ESCRT-III functionality, with CHMP7 shown to genetically interact with APQ12 [74].

Whereas this work links ESCRTs to NPC biogenesis during interphase, ESCRT-III does not colocalize with NPC markers or affect overall NPC dynamics during mitotic exit in human cells [61••,62••]. This could be taken to indicate that such function is species-specific, is restricted to interphase or is detectable only upon severe perturbation of NPC assembly. In this light it is interesting to note that depletion of NPC subunits can result in a dramatic delay in completion of cytokinesis [80,81], with partial knockdown of NUP153 shown to induce Aurora B-dependent abscission checkpoint signaling [82]. The abscission checkpoint is thought to signal via Aurora B-dependent phosphorylation of CHMP4C to restrict VPS4 activity, and as such directly impinges on ESCRT-III function in the intercellular bridge [69,70,83,84]. Considering the mechanistic and temporal overlaps, it will be of interest to explore whether this signaling cascade affects ESCRT-III functions at the NE during mitotic exit, and as such connects ESCRT-dependent roles in NE closure with NPC integrity.

Conclusions

The reformation of the NE during mitotic exit requires interplay between a variety of proteins regulating membrane dynamics and fusion events, and is tightly coordinated with NPC assembly as well as chromosome movement and decondensation [13,18,85]. Despite impressive progress over the last years, many questions remain regarding membrane dynamics during NE reformation. The discovery of ESCRT-III proteins as regulators of post-mitotic NE sealing adds a key new membrane modulator to the playing field. Further work is needed to unravel ESCRT-III functions during mitotic exit, its regulation by the established mitotic machineries and its intersection with cytokinetic abscission. The intriguing links between ESCRT-III and NPCs during mitosis [82] and interphase [79] also warrant further mechanistic exploration. Indeed, additional interphase ESCRT-III functions at the NE should now be considered, extending to resealing of NE ruptures and budding events as well as micronuclear integrity [9,86,87••]. Considering the defects observed at the NE during ageing, cancer and in laminopathies [8–11,88], dissecting ESCRT-dependent membrane dynamics in these phenomena could prove central to understanding their etiology.

Acknowledgements

MV is at postdoctoral fellow of the South-Eastern Norway Regional Health Authority. CC is a postdoctoral fellow of the Norwegian Cancer Society. Research in the HS lab was also supported by the Norwegian Cancer Society (grant no. 605009). This work was partly supported by the Research Council of Norway through its Centres of Excellence funding scheme, project number 179571.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Chang W, Worman HJ, Gundersen GG: **Accessorizing and anchoring the LINC complex for multifunctionality**. *J Cell Biol* 2015, **208**:11-22.
2. Ungricht R, Kutay U: **Establishment of NE asymmetry-targeting of membrane proteins to the inner nuclear membrane**. *Curr Opin Cell Biol* 2015, **34**:135-141.
3. Hetzer MW: **The nuclear envelope**. *Cold Spring Harb Perspect Biol* 2010, **2**:a000539.
4. Ibarra A, Hetzer MW: **Nuclear pore proteins and the control of genome functions**. *Genes Dev* 2015, **29**:337-349.
5. Raices M, D'Angelo MA: **Nuclear pore complex composition: a new regulator of tissue-specific and developmental functions**. *Nat Rev Mol Cell Biol* 2012, **13**:687-699.
6. Bermejo R, Kumar A, Foiani M: **Preserving the genome by regulating chromatin association with the nuclear envelope**. *Trends Cell Biol* 2012, **22**:465-473.
7. Mekhail K, Moazed D: **The nuclear envelope in genome organization, expression and stability**. *Nat Rev Mol Cell Biol* 2010, **11**:317-328.
8. Capell BC, Collins FS: **Human laminopathies: nuclei gone genetically awry**. *Nat Rev Genet* 2006, **7**:940-952.
9. Hatch E, Hetzer M: **Breaching the nuclear envelope in development and disease**. *J Cell Biol* 2014, **205**:133-141.
10. Chow KH, Factor RE, Ullman KS: **The nuclear envelope environment and its cancer connections**. *Nat Rev Cancer* 2012, **12**:196-209.
11. Dauer WT, Worman HJ: **The nuclear envelope as a signaling node in development and disease**. *Dev Cell* 2009, **17**:626-638.
12. Drechsler H, McAinsh AD: **Exotic mitotic mechanisms**. *Open Biol* 2012, **2**:120140.
13. Guttinger S, Laurell E, Kutay U: **Orchestrating nuclear envelope disassembly and reassembly during mitosis**. *Nat Rev Mol Cell Biol* 2009, **10**:178-191.
14. Beaudouin J, Gerlich D, Daigle N, Eils R, Ellenberg J: **Nuclear envelope breakdown proceeds by microtubule-induced tearing of the lamina**. *Cell* 2002, **108**:83-96.
15. Alvarez-Fernandez M, Malumbres M: **Preparing a cell for nuclear envelope breakdown: spatio-temporal control of phosphorylation during mitotic entry**. *BioEssays* 2014, **36**:757-765.
16. Bollen M, Gerlich DW, Lesage B: **Mitotic phosphatases: from entry guards to exit guides**. *Trends Cell Biol* 2009, **19**:531-541.
17. Medema RH, Lindqvist A: **Boosting and suppressing mitotic phosphorylation**. *Trends Biochem Sci* 2011, **36**:578-584.
18. Schellhaus AK, De MP, Antonin W: **Nuclear reformation at the end of mitosis**. *J Mol Biol* 2015.

19. Schlaitz AL, Thompson J, Wong CC, Yates JR III, Heald R: **REEP3/4 ensure endoplasmic reticulum clearance from metaphase chromatin and proper nuclear envelope architecture**. *Dev Cell* 2013, **26**:315-323.

20. Smyth JT, Beg AM, Wu S, Putney JW Jr, Rusan NM: **Phosphoregulation of STIM1 leads to exclusion of the endoplasmic reticulum from the mitotic spindle**. *Curr Biol* 2012, **22**:1487-1493.

21. Vedrenne C, Klopfenstein DR, Hauri HP: **Phosphorylation controls CLIMP-63-mediated anchoring of the endoplasmic reticulum to microtubules**. *Mol Biol Cell* 2005, **16**:1928-1937.

22. Anderson DJ, Hetzer MW: **Nuclear envelope formation by chromatin-mediated reorganization of the endoplasmic reticulum**. *Nat Cell Biol* 2007, **9**:1160-1166.

23. Muhlhausser P, Kutay U: **An in vitro nuclear disassembly system reveals a role for the RanGTPase system and microtubule-dependent steps in nuclear envelope breakdown**. *J Cell Biol* 2007, **178**:595-610.

24. Puhka M, Vihinen H, Joensuu M, Jokitalo E: **Endoplasmic reticulum remains continuous and undergoes sheet-to-tubule transformation during cell division in mammalian cells**. *J Cell Biol* 2007, **179**:895-909.

25. Anderson DJ, Hetzer MW: **Reshaping of the endoplasmic reticulum limits the rate for nuclear envelope formation**. *J Cell Biol* 2008, **182**:911-924.

26. Puhka M, Joensuu M, Vihinen H, Belevich I, Jokitalo E: **Progressive sheet-to-tubule transformation is a general mechanism for endoplasmic reticulum partitioning in dividing mammalian cells**. *Mol Biol Cell* 2012, **23**:2424-2432.

27. Poteryaev D, Squirrell JM, Campbell JM, White JG, Spang A: **Involvement of the actin cytoskeleton and homotypic membrane fusion in ER dynamics in *Caenorhabditis elegans***. *Mol Biol Cell* 2005, **16**:2139-2153.

28. Lu L, Ladinsky MS, Kirchhausen T: **Cisternal organization of the endoplasmic reticulum during mitosis**. *Mol Biol Cell* 2009, **20**:3471-3480.

29. Lu L, Ladinsky MS, Kirchhausen T: **Formation of the postmitotic nuclear envelope from extended ER cisternae precedes nuclear pore assembly**. *J Cell Biol* 2011, **194**:425-440.

30. Mora-Bermudez F, Gerlich D, Ellenberg J: **Maximal chromosome compaction occurs by axial shortening in anaphase and depends on Aurora kinase**. *Nat Cell Biol* 2007, **9**:822-831.

31. • Afonso O, Matos I, Pereira AJ, Aguiar P, Lampson MA, Maiato H: **Feedback control of chromosome separation by a midzone Aurora B gradient**. *Science* 2014, **345**:332-336.
This study highlights the existence of an Aurora-B dependent mechanism that delays nuclear envelope closure in primary nucleus to allow incorporation of lagging chromosomes during mitotic exit in Drosophila S2 cells.

32. Neurohr G, Naegeli A, Titos I, Theler D, Greber B, Diez J, Gabaldon T, Mendoza M, Barral Y: **A midzone-based ruler adjusts chromosome compaction to anaphase spindle length**. *Science* 2011, **332**:465-468.

33. Trinkle-Mulcahy L, Lamond AI: **Mitotic phosphatases: no longer silent partners**. *Curr Opin Cell Biol* 2006, **18**:623-631.

34. Vagnarelli P, Ribeiro S, Sennels L, Sanchez-Pulido L, de Lima AF, Verheyen T, Kelly DA, Ponting CP, Rappsilber J, Earnshaw WC: **Repo-Man coordinates chromosomal reorganization with nuclear envelope reassembly during mitotic exit**. *Dev Cell* 2011, **21**:328-342.

35. Wurzenberger C, Gerlich DW: **Phosphatases: providing safe passage through mitotic exit**. *Nat Rev Mol Cell Biol* 2011, **12**:469-482.

36. Vagnarelli P: **Repo-man at the intersection of chromatin remodelling, DNA repair, nuclear envelope organization, and cancer progression**. *Adv Exp Med Biol* 2014, **773**:401-414.

37. Schmitz MH, Held M, Janssens V, Hutchins JR, Hudecz O, Ivanova E, Goris J, Trinkle-Mulcahy L, Lamond AI, Poser I, Hyman AA, Mechtler K, Peters JM, Gerlich DW: **Live-cell imaging RNAi screen identifies PP2A-B55alpha and importin-beta1 as key mitotic exit regulators in human cells**. *Nat Cell Biol* 2010, **12**:886-893.

38. Wang S, Romano FB, Field CM, Mitchison TJ, Rapoport TA: **Multiple mechanisms determine ER network morphology during the cell cycle in Xenopus egg extracts**. *J Cell Biol* 2013, **203**:801-814.

39. Baur T, Ramadan K, Schlundt A, Kartenbeck J, Meyer HH: **NSF- and SNARE-mediated membrane fusion is required for nuclear envelope formation and completion of nuclear pore complex assembly in *Xenopus laevis* egg extracts**. *J Cell Sci* 2007, **120**:2895-2903.

40. Lee M, Ko YJ, Moon Y, Han M, Kim HW, Lee SH, Kang K, Jun Y: **SNAREs support atlastin-mediated homotypic ER fusion in *Saccharomyces cerevisiae***. *J Cell Biol* 2015, **210**:451-470.

41. Lu L, Kirchhausen T: **Visualizing the high curvature regions of post-mitotic nascent nuclear envelope membrane**. *Commun Integr Biol* 2012, **5**:16-18.

42. Shemesh T, Klemm RW, Romano FB, Wang S, Vaughan J, Zhuang X, Tukachinsky H, Kozlov MM, Rapoport TA: **A model for the generation and interconversion of ER morphologies**. *Proc Natl Acad Sci U S A* 2014, **111**:E5243-E5251.

43. Voeltz GK, Prinz WA, Shibata Y, Rist JM, Rapoport TA: **A class of membrane proteins shaping the tubular endoplasmic reticulum**. *Cell* 2006, **124**:573-586.

44. Anderson DJ, Vargas JD, Hsiao JP, Hetzer MW: **Recruitment of functionally distinct membrane proteins to chromatin mediates nuclear envelope formation in vivo**. *J Cell Biol* 2009, **186**:183-191.

45. Ulbert S, Platani M, Boue S, Mattaj IW: **Direct membrane protein–DNA interactions required early in nuclear envelope assembly**. *J Cell Biol* 2006, **173**:469-476.

46. Zierhut C, Funabiki H: **Nucleosome functions in spindle assembly and nuclear envelope formation**. *BioEssays* 2015, **37**:1074-1085.

47. Wandke C, Kutay U: **Enclosing chromatin: reassembly of the nucleus after open mitosis**. *Cell* 2013, **152**:1222-1225.

48. Schooley A, Moreno-Andres D, De MP, Vollmer B, Antonin W: **The lysine demethylase LSD1 is required for nuclear envelope formation at the end of mitosis**. *J Cell Sci* 2015, **128**:3466-3477.

49. Dultz E, Zanin E, Wurzenberger C, Braun M, Rabut G, Sironi L, Ellenberg J: **Systematic kinetic analysis of mitotic dis- and reassembly of the nuclear pore in living cells**. *J Cell Biol* 2008, **180**:857-865.

50. Schwartz M, Travesa A, Martell SW, Forbes DJ: **Analysis of the initiation of nuclear pore assembly by ectopically targeting nucleoporins to chromatin**. *Nucleus* 2015, **6**:40-54.

51. • Bui KH, von AA, DiGuilio AL, Ori A, Sparks L, Mackmull MT, Bock T, Hagen W, Andres-Pons A, Glavy JS, Beck M: **Integrated structural analysis of the human nuclear pore complex scaffold**. *Cell* 2013, **155**:1233-1243.
This study combines electron microscopy and crosslinking mass spectrometry to comprehensively dissect the composition and structure of the Nup107 Y-complex.

52. Szymborska A, de MA, Daigle N, Cordes VC, Briggs JA, Ellenberg J: **Nuclear pore scaffold structure analyzed by super-resolution microscopy and particle averaging**. *Science* 2013, **341**:655-658.

53. Franz C, Walczak R, Yavuz S, Santarella R, Gentzel M, Askjaer P, Galy V, Hetzer M, Mattaj IW, Antonin W: **MEL-28/ELYS is required for the recruitment of nucleoporins to chromatin and postmitotic nuclear pore complex assembly**. *EMBO Rep* 2007, **8**:165-172.

54. Walther TC, Alves A, Pickersgill H, Loiodice I, Hetzer M, Galy V, Hulsmann BB, Kocher T, Wilm M, Allen T, Mattaj IW, Doye V: **The conserved Nup107-160 complex is critical for nuclear pore complex assembly**. *Cell* 2003, **113**:195-206.

55. D'Angelo MA, Anderson DJ, Richard E, Hetzer MW: **Nuclear pores form de novo from both sides of the nuclear envelope**. *Science* 2006, **312**:440-443.

56. Doucet CM, Talamas JA, Hetzer MW: **Cell cycle-dependent differences in nuclear pore complex assembly in metazoa**. *Cell* 2010, **141**:1030-1041.

57. • Vollmer B, Lorenz M, Moreno-Andres D, Bodenhofer M, De MP, Astrinidis SA, Schooley A, Flotenmeyer M, Leptihn S, Antonin W: **Nup153 recruits the Nup107-160 complex to the inner nuclear membrane for interphasic nuclear pore complex assembly**. *Dev Cell* 2015, **33**:717-728.
This study shows that association of the nuclear basket protein NUP153 with the inner nuclear envelope is key for nucleation of interphase nuclear pore complex assembly.

58. • Meszaros N, Cibulka J, Mendiburo MJ, Romanauska A, Schneider M, Kohler A: **Nuclear pore basket proteins are tethered to the nuclear envelope and can regulate membrane curvature**. *Dev Cell* 2015, **33**:285-298.
This study shows that the nuclear basket proteins NUP1 and NUP60 directly associate with the inner nuclear envelope in budding yeast and induce membrane curvature to shape the nuclear pore membrane.

59. Hurley JH: **ESCRTs are everywhere**. *EMBO J* 2015.

60. Henne WM, Stenmark H, Emr SD: **Molecular mechanisms of the membrane sculpting ESCRT pathway**. *Cold Spring Harb Perspect Biol* 2013:5.

61. •• Vietri M, Schink KO, Campsteijn C, Wegner CS, Schultz SW, Christ L, Thoresen SB, Brech A, Raiborg C, Stenmark H: **Spastin and ESCRT-III coordinate mitotic spindle disassembly and nuclear envelope sealing**. *Nature* 2015, **522**:231-235.
This study shows that ESCRT-III subunits localize transiently to chromatin discs during mitotic exit and recruit Spastin to coordinate nuclear envelope reformation and spindle disassembly. Together with Ref. [62••], this is the first study implicating the ESCRT-III machinery in nuclear envelope reformation.

62. •• Olmos Y, Hodgson L, Mantell J, Verkade P, Carlton JG: **ESCRT-III controls nuclear envelope reformation**. *Nature* 2015, **522**:236-239.
This study shows that CHMP2A localizes to chromatin discs in a p97-dependent manner to ensure nuclear envelope sealing during mitotic exit. Together with Ref. [61••] this is the first study implicating the ESCRT-III machinery in nuclear envelope reformation.

63. Wollert T, Hurley JH: **Molecular mechanism of multivesicular body biogenesis by ESCRT complexes**. *Nature* 2010, **464**:864-869.

64. Richards SA, Muter J, Ritchie P, Lattanzi G, Hutchison CJ: **The accumulation of un-repairable DNA damage in laminopathy progeria fibroblasts is caused by ROS generation and is prevented by treatment with N-acetyl cysteine**. *Hum Mol Genet* 2011, **20**:3997-4004.

65. Kumar A, Mazzanti M, Mistrik M, Kosar M, Beznoussenko GV, Mironov AA, Garre M, Parazzoli D, Shivashankar GV, Scita G, Bartek J, Foiani M: **ATR mediates a checkpoint at the nuclear envelope in response to mechanical stress**. *Cell* 2014, **158**:633-646.

66. Gascoigne KE, Cheeseman IM: **Induced dicentric chromosome formation promotes genomic rearrangements and tumorigenesis**. *Chromosome Res* 2013, **21**:407-418.

67. Gascoigne KE, Cheeseman IM: **CDK-dependent phosphorylation and nuclear exclusion coordinately control kinetochore assembly state**. *J Cell Biol* 2013, **201**:23-32.

68. Sharp DJ, Ross JL: **Microtubule-severing enzymes at the cutting edge**. *J Cell Sci* 2012, **125**:2561-2569.

69. Carlton JG, Caballe A, Agromayor M, Kloc M, Martin-Serrano J: **ESCRT-III governs the Aurora B-mediated abscission checkpoint through CHMP4C**. *Science* 2012, **336**:220-225.

70. Thoresen SB, Campsteijn C, Vietri M, Schink KO, Liestol K, Andersen JS, Raiborg C, Stenmark H: **ANCHR mediates Aurora-B-dependent abscission checkpoint control through retention of VPS4**. *Nat Cell Biol* 2014, **16**:550-560.

71. Hetzer M, Meyer HH, Walther TC, Bilbao-Cortes D, Warren G, Mattaj IW: **Distinct AAA-ATPase p97 complexes function in discrete steps of nuclear assembly**. *Nat Cell Biol* 2001, **3**:1086-1091.

72. Cao K, Nakajima R, Meyer HH, Zheng Y: **The AAA-ATPase Cdc48/p97 regulates spindle disassembly at the end of mitosis**. *Cell* 2003, **115**:355-367.

73. Ramadan K, Bruderer R, Spiga FM, Popp O, Baur T, Gotta M, Meyer HH: **Cdc48/p97 promotes reformation of the nucleus by extracting the kinase Aurora B from chromatin**. *Nature* 2007, **450**:1258-1262.

74. Bauer I, Brune T, Preiss R, Kolling R: **Evidence for a non-endosomal function of the *Saccharomyces cerevisiae* ESCRT-III like protein Chm7**. *Genetics* 2015.

75. Horii M, Shibata H, Kobayashi R, Katoh K, Yorikawa C, Yasuda J: **CHMP7, a novel ESCRT-III-related protein, associates with CHMP4b and functions in the endosomal sorting pathway**. *Biochem J* 2006, **400**:23-32.

76. •• Webster BM, Colombi P, Jager J, Lusk CP: **Surveillance of nuclear pore complex assembly by ESCRT-III/Vps4**. *Cell* 2014, **159**:388-401.
This study shows that ESCRT-III and VPS4 genetically and physically interact with the nuclear pore complex (NPC) and associated factors, and control clearance of defective NPC intermediates in budding yeast.

77. Yewdell WT, Colombi P, Makhnevych T, Lusk CP: **Lumenal interactions in nuclear pore complex assembly and stability**. *Mol Biol Cell* 2011, **22**:1375-1388.

78. Lee CP, Liu PT, Kung HN, Su MT, Chua HH, Chang YH, Chang CW, Tsai CH, Liu FT, Chen MR: **The ESCRT machinery is recruited by the viral BFRF1 protein to the nucleus-associated membrane for the maturation of Epstein-Barr Virus**. *PLoS Pathog* 2012, **8**:e1002904.

79. Webster BM, Lusk CP: **ESCRTs breach the nuclear border**. *Nucleus* 2015, **6**:197-202.

80. Rasala BA, Orjalo AV, Shen Z, Briggs S, Forbes DJ: **ELYS is a dual nucleoporin/kinetochore protein required for nuclear pore assembly and proper cell division**. *Proc Natl Acad Sci U S A* 2006, **103**:17801-17806.

81. Platani M, Santarella-Mellwig R, Posch M, Walczak R, Swedlow JR, Mattaj IW: **The Nup107-160 nucleoporin complex promotes mitotic events via control of the localization state of the chromosome passenger complex**. *Mol Biol Cell* 2009, **20**:5260-5275.

82. Mackay DR, Ullman KS: **Coordinating postmitotic nuclear pore complex assembly with abscission timing**. *Nucleus* 2011, **2**:283-288.

83. Steigemann P, Wurzenberger C, Schmitz MH, Held M, Guizetti J, Maar S, Gerlich DW: **Aurora B-mediated abscission checkpoint protects against tetraploidization**. *Cell* 2009, **136**:473-484.

84. Caballe A, Wenzel DM, Agromayor M, Alam SL, Skalicky JJ, Kloc M, Carlton JG, Labrador L, Sundquist WI, Martin-Serrano J: **ULK3 regulates cytokinetic abscission by phosphorylating ESCRT-III proteins**. *Elife* 2015, **4**:e06547.

85. Maiato H, Afonso O, Matos I: **A chromosome separation checkpoint: a midzone Aurora B gradient mediates a chromosome separation checkpoint that regulates the anaphase–telophase transition**. *BioEssays* 2015, **37**:257-266.

86. Hatch EM, Fischer AH, Deerinck TJ, Hetzer MW: **Catastrophic nuclear envelope collapse in cancer cell micronuclei**. *Cell* 2013, **154**:47-60.

87. •• Zhang CZ, Spektor A, Cornils H, Francis JM, Jackson EK, Liu S, Meyerson M, Pellman D: **Chromothripsis from DNA damage in micronuclei**. *Nature* 2015, **522**:179-184.
This study describes loss of compartmentalization in micronuclei (MN), MN nuclear envelope (NE) collapse, and accumulation of massive DNA damage. This provides an important link between MN NE defects and genome integrity.

88. Worman HJ, Ostlund C, Wang Y: **Diseases of the nuclear envelope**. *Cold Spring Harb Perspect Biol* 2010, **2**:a000760.

89. McCullough J, Colf LA, Sundquist WI: **Membrane fission reactions of the mammalian ESCRT pathway**. *Annu Rev Biochem* 2013, **82**:663-692.

90. Carlson LA, Hurley JH: **In vitro reconstitution of the ordered assembly of the endosomal sorting complex required for transport at membrane-bound HIV-1 Gag clusters**. *Proc Natl Acad Sci U S A* 2012, **109**:16928-16933.

91. Christ L, Wenzel EM, Liestol K, Raiborg C, Campsteijn C, Stenmark H: **ALIX and ESCRT-I/II function as parallel ESCRT-III recruiters in cytokinetic abscission**. *J Cell Biol* 2016, **212**:499-513.

92. Wemmer M, Azmi I, West M, Davies B, Katzmann D, Odorizzi G: **Bro1 binding to Snf7 regulates ESCRT-III membrane scission activity in yeast**. *J Cell Biol* 2011, **192**:295-306.

93. Henne WM, Buchkovich NJ, Zhao Y, Emr SD: **The endosomal sorting complex ESCRT-II mediates the assembly and architecture of ESCRT-III helices**. *Cell* 2012, **151**:356-371.

94. Hurley JH, Hanson PI: **Membrane budding and scission by the ESCRT machinery: it's all in the neck**. *Nat Rev Mol Cell Biol* 2010, **11**:556-566.

95. Lata S, Schoehn G, Jain A, Pires R, Piehler J, Gottlinger HG, Weissenhorn W: **Helical structures of ESCRT-III are disassembled by VPS4**. *Science* 2008, **321**:1354-1357.

96. Ghazi-Tabatabai S, Saksena S, Short JM, Pobbati AV, Veprintsev DB, Crowther RA, Emr SD, Egelman EH, Williams RL: **Structure and disassembly of filaments formed by the ESCRT-III subunit Vps24**. *Structure* 2008, **16**:1345-1356.

97. Cashikar AG, Shim S, Roth R, Maldazys MR, Heuser JE, Hanson PI: **Structure of cellular ESCRT-III spirals and their relationship to HIV budding**. *Elife* 2014:3.

98. Schuh AL, Audhya A: **The ESCRT machinery: from the plasma membrane to endosomes and back again**. *Crit Rev Biochem Mol Biol* 2014, **49**:242-261.

99. • McCullough J, Clippinger AK, Talledge N, Skowyra ML, Saunders MG, Naismith TV, Colf LA, Afonine P, Arthur C, Sundquist WI, Hanson PI, Frost A: **Structure and membrane remodeling activity of ESCRT-III helical polymers**. *Science* 2015.

This study uses cryo-EM to determine the structure of a double-stranded copolymer of CHMP1B and IST1 and provides a novel model for filament composition and inter-subunit interactions.

100. Boura E, Rozycki B, Chung HS, Herrick DZ, Canagarajah B, Cafiso DS, Eaton WA, Hummer G, Hurley JH: **Solution structure of the ESCRT-I and -II supercomplex: implications for membrane budding and scission**. *Structure* 2012, **20**:874-886.

101. Im YJ, Wollert T, Boura E, Hurley JH: **Structure and function of the ESCRT-II-III interface in multivesicular body biogenesis**. *Dev Cell* 2009, **17**:234-243.

102. Yorikawa C, Shibata H, Waguri S, Hatta K, Horii M, Katoh K, Kobayashi T, Uchiyama Y, Maki M: **Human CHMP6, a myristoylated ESCRT-III protein, interacts directly with an ESCRT-II component EAP20 and regulates endosomal cargo sorting**. *Biochem J* 2005, **387**:17-26.

103. • Buchkovich NJ, Henne WM, Tang S, Emr SD: **Essential N-terminal insertion motif anchors the ESCRT-III filament during MVB vesicle formation**. *Dev Cell* 2013, **27**:201-214.

This study characterizes the membrane binding surfaces of ESCRT-III proteins and identifies an N-terminal membrane insertion motif in the CHMP4 homolog Snf7p.

104. Muziol T, Pineda-Molina E, Ravelli RB, Zamborlini A, Usami Y, Gottlinger H, Weissenhorn W: **Structural basis for budding by the ESCRT-III factor CHMP3**. *Dev Cell* 2006, **10**:821-830.

105. Adell MA, Vogel GF, Pakdel M, Muller M, Lindner H, Hess MW, Teis D: **Coordinated binding of Vps4 to ESCRT-III drives membrane neck constriction during MVB vesicle formation**. *J Cell Biol* 2014, **205**:33-49.

106. Yang B, Stjepanovic G, Shen Q, Martin A, Hurley JH: **Vps4 disassembles an ESCRT-III filament by global unfolding and processive translocation**. *Nat Struct Mol Biol* 2015, **22**:492-498.

107. Hanson PI, Roth R, Lin Y, Heuser JE: **Plasma membrane deformation by circular arrays of ESCRT-III protein filaments**. *J Cell Biol* 2008, **180**:389-402.