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The nuclear envelope: form and reformation

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Abstract

The membrane system that encloses genomic DNA is referred to as the nuclear envelope. However, with emerging roles in signaling and gene expression, these membranes clearly serve as more than just a physical barrier separating the nucleus and cytoplasm. Recent progress in our understanding of nuclear envelope architecture and composition has also revealed an intriguing connection between constituents of the nuclear envelope and human disease, providing further impetus to decipher this cellular structure and the dramatic remodeling process it undergoes with each cell division.

Introduction

The nuclear envelope (NE) consists of two concentric membrane bilayers, the inner nuclear membrane (INM) and outer nuclear membrane (ONM), which encircle chromosomes and other nuclear components (Figure 1). This double membrane is punctuated by nuclear pore complexes (NPCs), at which the INM and ONM are fused to form what is sometimes referred to as the pore membrane (POM). The lumen between the bilayers, known as the perinuclear space, spans ~50 nm in metazoans and is contiguous with the lumen of the endoplasmic reticulum (ER).

Here, we will discuss the molecular constituents of the NE and the roles that this structure plays. We will then delve into recent results that lend new insight into how formation and disassembly of the NE is orchestrated during cell division.

The nuclear envelope: form and function

The INM, ONM and pore membrane are each intimately associated with distinct proteinaceous structures; these connections ensure the integrity of the nuclear environment and aid in coordinating cellular events. Specifically, the INM contacts the underlying nuclear lamina and regions of chromatin, whereas the ONM is associated with both the cytoskeletal actin network and the centrosome. Large macromolecular pore complexes are tethered to the NE at the pore membrane.

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Systematic proteomics approaches [1,2••] have predicted 67 new integral membrane proteins to be enriched at the nuclear membrane, adding significantly to the repertoire of 13 proteins (plus splice variants) already characterized. Intriguingly, genes encoding 23 of the newly identified proteins map to chromosomal regions linked to human dystrophies [2••]. Along with previous examples that implicate INM-associated proteins in human disease [3,4], this underscores the critical need to determine whether these new candidates are disease-linked genes and, more generally, to better understand the functions of proteins resident in the NE.

The INM contains a unique subset of integral membrane proteins. Some INM proteins are involved in organization of genomic DNA. For instance, lamin B receptor has been proposed to localize to unique microdomains within the NE, to which it targets heterochromatin [5]. Other INM proteins play important roles in nuclear structure. Integrity of the NE requires contact between INM proteins and the nuclear lamina. The LEM-domain-containing proteins, including LAP2, emerin and MAN1, are examples of INM-associated proteins that interact with lamins [3]. Mutations in either emerin or lamin result in aberrant NE morphology [4]. The importance of emerin to nuclear structure may also relate to its ability to increase polymerization of actin [6•]. Nuclear actin is a topic of some debate and is considered at more length elsewhere [3,7]. It will be important to decipher if emerin influences roles proposed for actin in nuclear structure, transcription regulation or mitotic chromosome organization [7,8•,9,10].

The perinuclear compartment has not been well characterized, but is likely to share many components with the ER lumen. Recent studies focused on the AAA^+ ATPase torsinA, however, illustrate the potential for such luminal proteins to have NE-specific functions. Whereas torsinA is found predominantly in the ER, ATPase-defective torsinA mutants, which are predicted to be tightly engaged with targets, strikingly localize to the NE [11,12]. Expression of these mutants causes NE membrane herniations and alterations in spacing between the INM and ONM [12], suggesting that this ATPase plays an important role at the NE [13•,14].

Integral membrane proteins of the ONM are also in large part shared by the ER. This has been widely accepted as dogma and is consistent with the continuity between ER and ONM, which allows lateral diffusion to take place in the membrane bilayer. Interesting exceptions to this rule — ONM-enriched proteins — have come to light more recently. The first in this class to be reported was the *C. elegans* protein ANC-1, which also has an actin-binding domain and was found to play a role in positioning the nucleus within the cytoplasm [15]. ANC-1 targets to the NE in a manner dependent on the INM protein UNC-84, consistent with the notion that enrichment at the ONM can be promoted by interactions in the perinuclear space with luminal domains of INM-anchored proteins. These interactions, and indeed the role in nuclear positioning [16•], are conserved in the vertebrate counterparts of ANC-1 and UNC-84, referred to respectively as nesprins and SUN proteins [17•,18]. This protein interaction network is proposed to bridge the inside of the nucleus to actin filaments in the cytoplasm. Teasing apart the roles of these connections is a work in progress and is complicated by the fact that the nesprin proteins are extremely large and have multiple isoforms with significant distinctions in localization [18,19]. Expanding on the roles for

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ONM proteins, a recent result in *C. elegans* points toward interactions between a SUN protein and ZYG-12, which is also predicted to localize to the ONM and is required for attaching the centrosome to the NE [20].

The pore membrane houses the integral membrane proteins of the NPC. The differing properties of the two known vertebrate integral membrane nucleoporins, gp210 and POM121, suggest that these proteins play very different roles. Although gp210 is not detected in certain cell types [21•], its depletion from Hela cells results in abnormalities of the NE [22]. Consensus is lacking on the function of gp210, but an important clue may lie in the surprising observation that this transmembrane protein dynamically associates with the pore [23••]. POM121, by contrast, is a stable component of the pore [23••,24]. Thus,POM121 may provide the pore-specific component of a tether between NPC and membrane, although yet-to-be-identified vertebrate pore membrane proteins may also be involved. In addition, pore proteins that are not integral membrane proteins have been found to help in stably positioning each NPC in the NE [25,26]. Recent structural and bioinformatic characterization has determined that members of the Nup107–Nup160 complex (referred to hereafter as the Nup107 complex) contain or are predicted to contain β-propeller motifs and α-helical solenoid domains in comparable distribution to complexes, such as clathrin, that form coat-like structures on membranes [27••,28••]. This raises the intriguing possibility that nucleoporins cooperatively coat the pore membrane, potentially stabilizing its curvature. The provocative notion of a 'proto-coatomer' also provides new perspective on the evolution of the NE [27••,29].

Although the NE is often depicted as the surface of a sphere, its topology is more complex. Tubes, formed by the NE inverting into the nucleus, increase the nuclear area contacted by the INM [30,31]. These invaginations, collectively called the nucleoplasmic reticulum (NR), are found in multiple cell types and growth states, with an increased incidence reported in dedifferentiated or cancerous cells [30,32]. Recent experiments suggest that the NR contains sites of discrete calcium flux [33]. Thus, the NE appears to actively participate in signaling; calcium fluxes in the NR may drive local changes in calcium-sensitive processes such as modulation of NPC conformation [34,35] and gene expression [36].

The nuclear envelope: formation

Recent progress has given new insight into how the NE is constructed and rapidly disassembled at precise times in mitosis, but has also exposed previously unappreciated complexity in these processes. Key aspects of NE formation include targeting of membranes to the chromatin surface, membrane fusion, and incorporation of NPCs (Figure 2). Several clues about the process of NE assembly have been obtained from *in vitro* systems, in many cases using *Xenopus* egg extract, in which NEs are formed *de novo* around DNA templates by reconstituting the steps noted above.

In a paradigm established originally by its role in transport, the small GTPase Ran plays a critical role in recruiting proteins to the chromatin surface. More specifically, Ran modulates the binding activity of the nucleocytoplasmic transport receptor importin β (also known as karyopherin β1). Since RCC1/RanGEF, the guanine exchange factor for Ran, resides on

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chromatin, levels of RanGTP are high in proximity to chromosomes even at mitosis [37]. Thus, in mitosis, independently of nucleocytoplasmic transport, Ran GTP promotes the release of importin β from proteins in a spatially restricted manner and facilitates deposition of proteins on chromosomes in preparation for NE assembly [37,38••,39••]. Certain targets of this regulation are nucleoporins [39••].

Ran also serves to control membrane fusion, again by modulating interactions with importin β [38••]. In this case, the pertinent targets of importin β are not yet identified. The requirement for GTP in nuclear membrane fusion, however, can be over-ridden by the addition of phosphoinositide-specific phospholipase C (PtdIns- PLC) or of phorbol 12-myristate 13-acetate, an analogue of a product produced by this enzyme [40]. This raises speculation that an interaction reported between PtdIns- PLC and importin β [41] may be relevant to a Ran-modulated step in nuclear formation.

Recent results pointing to a Ran-regulated role for the export receptor Crm1/Exportin1 at the kinetochore [42] illustrate the potential for additional members of the importin β family to take on non-canonical roles during mitosis, although whether this is true during nuclear assembly is still an open question. Importin α, a member of a different transport receptor family and the adaptor protein that bridges importin β to NLS-bearing proteins, has been implicated in regulating NE formation [43•]. Importin α appears to have two roles: one dependent on its ability to bind NLS-containing proteins and one related to the novel observation that it associates with membrane vesicles involved in NE assembly.

Machinery involved more broadly at the membrane of different organelles also plays a role in NE formation. p97, a hexameric AAA^+ ATPase, was first implicated in ER and Golgi vesicle fusion [44]. In conjunction with Ufd1 and Npl4, p97 promotes formation of a closed NE *in vitro* [45]. Ufd1 and Npl4 can bind ubiquitin [46], and the p97/Ufd1/Npl4 complex has been found to play roles in retrotranslocation from the ER and in proteosome-mediated processing and degradation [44]. Identification of targets recognized by this protein complex during nuclear formation will yield important information about its role in this context. It will also be of interest to probe further into the canonical machinery of membrane fusion, such as SNAREs involved in vesicle targeting and fusion, as the picture of NE formation currently lacks components well characterized in other organelles [47].

The nucleoporin POM121 is key to integrating assembly of the NE with assembly of pore complexes. In the *Xenopus* system, multiple vesicle populations contribute to NE formation. Antonin *et al*. elegantly demonstrated that POM121 is not required for targeting of vesicles to the chromatin but is required for membrane vesicles to fuse during formation of the NE [48••]. Interestingly, when the Nup107 complex is absent, POM121-bearing vesicles are not incorporated, resulting in an NE devoid of pores [48••,49]. Consistent with this, formation of such a ‘pore-less’ NE occurs when both the Nup107 complex and POM121 are absent [48••]. This latter result suggests that absence of the Nup107 complex alleviates the requirement for POM121 in membrane fusion at the newly forming NE. Thus, a regulatory interplay between the Nup107 complex and POM121 coordinates membrane recruitment, membrane fusion and NPC formation.

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The nuclear envelope: growth

After the formation of a closed membrane around the chromatin, the NE must expand by addition of membrane and new NPCs (Figure 2). Although the net result of growth is simply more of the same membrane system, the mechanisms involved are quite distinct from its initial formation. For instance, rather than side-to-side fusion of vesicles, followed by flattening to create a double-layered stretch of envelope, membrane must presumably be added at the ONM during growth, followed by redistribution to maintain the regular spacing of ONM and INM. Again, important clues about this second phase in NE formation have been obtained from *in vitro* analysis. For instance, p97 has also been implicated in nuclear growth; however, consistent with the notion that the mechanics have changed, a different partner protein, p47, is required for this step [45].

A further distinction between NE formation and expansion, in some cases, is whether the membranes are in storage pools or whether new lipids must be synthesized, a process that occurs primarily in the ER. Rather than cycling through NE disassembly and reassembly, *S. cerevisiae* undergoes a 'closed mitosis' in which the nucleus is divided, but this mode of nuclear division also necessitates NE growth. Santos-Rosa *et al*. recently found that the yeast homologue of Lipin, Smp2p, is a target of the ER/nuclear membrane-associated phosphatase complex Nem1p–Spo7p [50•]. Dephosphorylated Smp2p is thought to repress transcription of lipid biosynthetic enzymes. Consistent with this, in yeast lacking Smp2p the NE expands to create extraneous membrane structures, whereas in wild-type yeast deactivation of Smp2p is temporally controlled by a mitotic kinase and NE expansion ensues before nuclear division. Notably, a mammalian protein related to the phosphatase Nem1p is a putative INM protein [2••]. Thus, there may be a conserved signaling pathway at the NE involved in modulating levels of lipids that will incorporate into the NE.

NE expansion also takes place when the complexity of the NR increases. A link between the number of NR tubules and CTP:phosphocholine cytidylyltransferase-α, an enzyme involved in phosphatidylcholine synthesis, has been observed [51•]. This enzyme could influence nuclear membranes in two ways, as both its physical association and its biosynthetic product alter bilayer topology [51•]. The connection between alterations in membrane bilayers and promotion of NR formation, however, has not been elucidated. The observation that glyceraldehyde 3-phosphate dehydrogenase, and in particular its putative phosphatidylserine recognition motif, may play a role in vesicle fusion during nuclear assembly points to an early role for lipid recognition during NE formation [52]. Integrating information about lipid biosynthesis with our understanding of nuclear formation and growth is an important focus for the future. Feedback mechanisms, although poorly understood, clearly exist. For instance, overproduction of INM-associated proteins has been observed to cause proliferation of NE-like structures (for example [53,54]).

Nuclear growth involves targeting of integral membrane proteins as well as expansion of membranes. Integral membrane proteins have been proposed to diffuse laterally from ONM to INM via the pore membrane, fitting through gaps in the pore structure [55]. Antibodies to the POM protein gp210 interfere with targeting of reporters to the INM, suggesting that this nucleoporin in particular may participate in the movement from ONM to INM [56••].

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According to the diffusion-retention model, widely accepted for many years to explain how a specific subset of integral membrane proteins locates exclusively to the INM, certain proteins that diffuse to the INM become tethered here due to interactions with the underlying lamina and chromatin.

Recent work challenges whether this mechanism is universal by demonstrating an energy requirement, which would not be expected for simple diffusion and trapping [56••].

Other observations are consistent with the idea that early steps in protein biogenesis, not simply a tether that is encountered following diffusion into the INM, could influence targeting. Specifically, a sorting motif dictating INM localization was found to make distinct contacts with machinery that cotranslationally integrates proteins into membrane [57•]. Learning more about the energy requirement, the role of the nuclear pore, and the role of cofactors that participate from an early stage in routing proteins to the INM will lend new insight into mechanisms involved in creating the specialized INM environment.

Nuclear envelope disassembly: prelude to reformation

In higher eukaryotic cells, the conundrum of how to allow spindle microtubules access to chromosomes at mitosis is resolved by dismantling the NE with each cell cycle (referred to as 'open mitosis'). This rapid remodeling is physically coordinated with the rearrangement of many other cellular constituents and temporally coordinated with other cell cycle events. Several mechanisms appear to contribute to NE disassembly. Given that NE breakdown has been characterized in different experimental systems (Figure 3), one current challenge is to determine which mechanisms are conserved between species and how the pathways differ between different cell types and growth states.

In starfish oocytes, NE permeability increases before loss of envelope integrity. This correlates with early remodeling events at the NPC [58]. An early alteration in NE permeability also occurs in the unicellular organism *Ustilago maydis*, a basidiomycete fungi that undergoes open mitosis [59••]. Notably, various degrees of mitotic pore remodeling have been observed even in organisms that undergo closed mitosis [60,61]. The contribution of altered barrier/transport function of the NPC at mitosis is still poorly understood. An increase in permeability may simply facilitate nuclear access for mitotic signaling components. However, when nuclei were artificially permeabolized, wheat germ agglutinin, a pore-binding lectin, still blocked nuclear disassembly, raising the possibility that NPCs (and remodeled intermediates) provide important scaffolds for the coordinate breakdown of nuclear components [62].

Different characteristics of membrane remodeling have been observed across species during NE breakdown. In starfish oocytes, remodeling is proposed to emanate from the disassembling NPCs [58]. Microtubule-dependent events also drive alterations at the NE. In *U. maydis*, the spindle pole body, which is attached to the ONM, appears to drag the NE in a dynein-dependent manner toward the daughter cell. Disruption of nuclear integrity occurs after the nucleus elongates and has one end positioned in the bud. Dynein-mediated movement likewise facilitates NE breakdown in higher eukaryotic somatic cells, although rather than analogously pulling the NE via centrosomes, this microtubule-dependent event

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gathers the NE in at the centrosomes [63,64], eventually causing a rupture at distal regions of the NE.

As mitosis progresses, markers of the NE intermix with markers of the ER in somatic cells [65,66]. This loss of distinct membrane domains could result simply from lateral diffusion. NPC disassembly, as well as concurrent events such as lamina breakdown, may remove tethers and barriers that otherwise prevent free flow between the INM and the ER.

Distinct membrane vesicle populations, derived from the NE, are present in extracts made from *Xenopus* eggs [67]. The presence of multiple vesicle populations suggests that NE breakdown involves vesicle formation and, although this is a difficult intermediate to document, some direct evidence for vesicle formation exists [68]. More recently, the coatomer complex COPI was found to participate in NE breakdown in the *Xenopus* egg extract system [69••]. The role of COPI in coating membranes and, in doing so, promoting the formation of vesicles has been characterized in the context of secretory trafficking [47]. COPI may similarly remodel the nuclear membrane into vesicles during mitosis.

Nuclear pore proteins appear to be involved in attracting COPI to the vicinity of the NE. Specifically, COPI was found to associate with Nup153 [69••] and with Nup358, which has recently been found to have a non-redundant role in nuclear disassembly [70]. Interestingly, these two nucleoporins are located on the nuclear and cytoplasmic faces of the nuclear pore, respectively, suggesting that efficient nuclear breakdown requires COPI recruitment near both the INM and the ONM. Although the observed increase in NPC permeability early in mitosis discussed above [58] may facilitate COPI access to the nuclear face of the pore, many questions remain about how recruitment of COPI is regulated.

Vesicle formation and NE/ER mixing are not mutually exclusive. In some situations, vesicles may persist as a storage form of NE membranes; in other cell types, vesicles could go on to fuse with the ER, providing a more active means of mixing the ER and NE membranes at mitosis. Alternatively, COPI components could participate in NE remodeling in a non-conventional manner, such as in the surprising involvement of clathrin at the mitotic spindle [71]. The recent hypothesis that the Nup107 complex is ‘coat-like’ in structure [27••] raises the additional possibility that, during nuclear disassembly, COPI collaborates with these nucleoporins already juxtaposed on the pore membrane. More regulatory and mechanistic detail is needed to create an integrated picture of the multi-layered NE disassembly process.

Conclusions

The double membrane of the NE and the presence of the NR create unique functional environments. The contribution of the NE to gene expression, signal transduction and nuclear positioning hinges on its specialized architecture and unique protein composition. These same features present challenges to efficient disassembly and reassembly of the NE. Many players and paradigms that contribute to cell-cycle-driven remodeling of the NE have been identified, but significant questions remain about how the NE is rapidly dispersed and accurately reformed. More experimental scrutiny will contribute to a better understanding of

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normal nuclear function and also lend molecular insight to pathogenic states that arise from alterations at the nuclear envelope.

Update

Notably, there is paper in press [72] that further probes the connection between the ONM localized protein, nesprin 2 (nesp2G), and Sun proteins in mammalian cells. Specifically, both Sun1 and Sun2 were found to contribute to nesp2G localization. This network of interactions, which bridges INM and ONM proteins, is given the term LINC for 'complex that links the nucleoskeleton and cytoskeleton'.

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References and recommended reading

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

• of special interest

•• of outstanding interest

1. Dreger M, Bengtsson L, Schoneberg T, Otto H, Hucho F. Nuclear envelope proteomics: novel integral membrane proteins of the inner nuclear membrane. Proc Natl Acad Sci USA. 2001; 98:11943–11948. [PubMed: 11593002]
2. Schirmer EC, Florens L, Guan T, Yates JR 3rd, Gerace L. Nuclear membrane proteins with potential disease links found by subtractive proteomics. Science. 2003; 301:1380–1382. [PubMed: 12958361] A subtractive proteomic approach is used to identify 67 previously uncharacterized nuclear envelope proteins. This adds to an earlier proteomic approach where 19 uncharacterized nuclear envelope proteins were identified [1].
3. Gruenbaum Y, Margalit A, Goldman RD, Shumaker DK, Wilson KL. The nuclear lamina comes of age. Nat Rev Mol Cell Biol. 2005; 6:21–31. [PubMed: 15688064]
4. Burke B, Stewart CL. Life at the edge: the nuclear envelope and human disease. Nat Rev Mol Cell Biol. 2002; 3:575–585. [PubMed: 12154369]
5. Makatsori D, Kourmouli N, Polioudaki H, Shultz LD, McLean K, Theodoropoulos PA, Singh PB, Georgatos SD. The inner nuclear membrane protein lamin B receptor forms distinct microdomains and links epigenetically marked chromatin to the nuclear envelope. J Biol Chem. 2004; 279:25567–25573. [PubMed: 15056654]
6. Holaska JM, Kowalski AK, Wilson KL. Emerin caps the pointed end of actin filaments: evidence for an actin cortical network at the nuclear inner membrane. PLoS Biol. 2004; 2:E231. [PubMed: 15328537] The INM protein emerin was found to have the unexpected property of increasing actin polymerization *in vitro*. The authors suggest that emerin may help form an actin cortical network at the INM.
7. Pederson T, Aebi U. Nuclear actin extends, with no contraction in sight. Mol Biol Cell. 2005; 16:5055–5060. [PubMed: 16148048]
8. Kiseleva E, Drummond SP, Goldberg MW, Rutherford SA, Allen TD, Wilson KL. Actin- and protein-4.1-containing filaments link nuclear pore complexes to subnuclear organelles in *Xenopus* oocyte nuclei. J Cell Sci. 2004; 117:2481–2490. [PubMed: 15128868] Using field emission scanning electron microscopy of *Xenopus* oocytes nuclei, the authors observe filaments, attached to

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the NPCs, that collapse when treated with the actin depolymerizing agent, latrunculin A. These potential actin filaments may relate to recently observed functions for actin, including roles in chromatin congression during mitosis [9], nuclear assembly [10] and transcription (reviewed in [7]).

9. Lenart P, Bacher CP, Daigle N, Hand AR, Eils R, Terasaki M, Ellenberg J. A contractile nuclear actin network drives chromosomecongression in oocytes. Nature. 2005; 436:812–818. [PubMed: 16015286]
10. Krauss SW, Chen C, Penman S, Heald R. Nuclear actin and protein 4.1: essential interactions during nuclear assembly in vitro. Proc Natl Acad Sci U S A. 2003; 100:10752–10757. [PubMed: 12960380]
11. Goodchild RE, Dauer WT. Mislocalization to the nuclear envelope: an effect of the dystonia-causing torsinA mutation. Proc Natl Acad Sci U S A. 2004; 101:847–852. [PubMed: 14711988]
12. Naismith TV, Heuser JE, Breakefield XO, Hanson PI. TorsinA in the nuclear envelope. Proc Natl Acad Sci USA. 2004; 101:7612–7617. [PubMed: 15136718]
13. Goodchild RE, Dauer WT. The AAA+ protein torsinA interacts with a conserved domain present in LAP1 and a novel ER protein. J Cell Biol. 2005; 168:855–862. [PubMed: 15767459] Along with the two previous references [11,12], this study focuses in on the role of torsin A at the NE. Here, candidate NE proteins are over-expressed to identify torsin A substrates that can trap torsin A at the NE. LAP1 is identified as a potential NE target and LULL1 (luminal domain like LAP1) is implicated as a torsinA target on the basis of its homology to the LAP1 luminal domain. The identification of substrates of torsin A is an important step in understanding its role at the NE and in the disease mechanism of torsion dystonia.
14. Gerace L. TorsinA and torsion dystonia: unraveling the architecture of the nuclear envelope. Proc Natl Acad Sci USA. 2004; 101:8839–8840. [PubMed: 15187229]
15. Starr DA, Han M. Role of ANC-1 in tethering nuclei to the actin cytoskeleton. Science. 2002; 298:406–409. [PubMed: 12169658]
16. Grady RM, Starr DA, Ackerman GL, Sanes JR, Han M. Syne proteins anchor muscle nuclei at the neuromuscular junction. Proc Natl Acad Sci USA. 2005; 102:4359–4364. [PubMed: 15749817] Mammalian Syne-1 (a nesprin family member), like its *C. elegans* homolog ANC-1 [12], is required for nuclear positioning *in vivo*.
17. Padmakumar VC, Libotte T, Lu W, Zaim H, Abraham S, Noegel AA, Gotzmann J, Foisner R, Karakesisoglou I. The inner nuclear membrane protein Sun1 mediates the anchorage of Nesprin-2 to the nuclear envelope. J Cell Sci. 2005; 118:3419–3430. [PubMed: 16079285] The authors demonstrate that mammalian Sun-1 localizes nesprin to the NE and acts as an important ‘structural bridge’ connecting the lamina to the actin cytoskeleton in a manner similar to its C. elegans homolog UNC-84.
18. Warren DT, Zhang Q, Weissberg PL, Shanahan CM. Nesprins: intracellular scaffolds that maintain cell architecture and coordinate cell function? Expert Rev Mol Med. 2005; 7:1–15. [PubMed: 15953398]
19. Libotte T, Zaim H, Abraham S, Padmakumar VC, Schneider M, Lu W, Munck M, Hutchison C, Wehnert M, Fahrenkrog B, et al. Lamin A/C-dependent localization of Nesprin-2, a giant scaffolder at the nuclear envelope. Mol Biol Cell. 2005; 16:3411–3424. [PubMed: 15843432]
20. Malone CJ,Misner L, Le Bot N, Tsai MC, Campbell JM, Ahringer J, White JG. The C. elegans hook protein, ZYG-12, mediates the essential attachment between the centrosome and nucleus. Cell. 2003; 115:825–836. [PubMed: 14697201]
21. Olsson M, Scheele S, Ekblom P. Limited expression of nuclear pore membrane glycoprotein 210 in cell lines and tissues suggests cell-type specific nuclear pores in metazoans. Exp Cell Res. 2004; 292:359–370. [PubMed: 14697343] Expression of the nuclear pore protein gp210 is found to vary by cell type. This is the first report suggesting cell-type differences in nuclear pore composition.
22. Cohen M, Feinstein N, Wilson KL, Gruenbaum Y. Nuclear pore protein gp210 is essential for viability in HeLa cells and Caenorhabditis elegans. Mol Biol Cell. 2003; 14:4230–4237. [PubMed: 14517331]
23. Rabut G, Doye V, Ellenberg J. Mapping the dynamic organization of the nuclear pore complex inside single living cells. Nat Cell Biol. 2004; 6:1114–1121. [PubMed: 15502822] Nucleoporins expressed as GFP fusion proteins are systematically evaluated by iFRAP to determine their residence times at the NPC. Surprisingly, given that the NPC as a whole is a stable structure

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composed of relatively few different proteins, several nuclear pore proteins are very dynamic, including the integral membrane protein gp210.

24. Daigle N, Beaudouin J, Hartnell L, Imreh G, Hallberg E, Lippincott-Schwartz J, Ellenberg J. Nuclear pore complexes form immobile networks and have a very low turnover in live mammalian cells. J Cell Biol. 2001; 154:71–84. [PubMed: 11448991]
25. Hawryluk-Gara LA, Shibuya EK, Wozniak RW. Vertebrate Nup53 interacts with the nuclear lamina and is required for the assembly of a Nup93-containing complex. Mol Biol Cell. 2005; 16:2382–2394. [PubMed: 15703211]
26. Walther TC, Fornerod M, Pickersgill H, Goldberg M, Allen TD, Mattaj IW. The nucleoporin Nup153 is required for nuclear pore basket formation, nuclear pore complex anchoring and import of a subset of nuclear proteins. Embo J. 2001; 20:5703–5714. [PubMed: 11598013]
27. Devos D, Dokudovskaya S, Alber F, Williams R, Chait BT, Sali A, Rout MP. Components of coated vesicles and nuclear pore complexes share a common molecular architecture. PLoS Biol. 2004; 2:e380. [PubMed: 15523559] Computational modeling and biochemical characterization leads the authors to predict that all members of the Nup107 nuclear pore subcomplex contain β-sheets and/or α-solenoid motifs similar to those found in proteins known to coat membranes during vesicular trafficking. The role for a coat-like structure at the pore and in the evolution of the NE is discussed.
28. Berke IC, Boehmer T, Blobel G, Schwartz TU. Structural and functional analysis of Nup133 domains reveals modular building blocks of the nuclear pore complex. J Cell Biol. 2004; 167:591–597. [PubMed: 15557116] This study is one of only a few to study nucleoporin domains at the level of crystal structure. Here, the N terminus of Nup133 is determined to contain a seven-bladed β-propeller. Similar β-propeller motifs are predicted in a third of all nucleoporins, suggesting NPCs may be comprised of limited, reiterative modules.
29. Fuerst JA. Intracellular compartmentation in Planctomycetes. Annu Rev Microbiol. 2005; 59:299–328. [PubMed: 15910279]
30. Fricker M, Hollinshead M, White N, Vaux D. Interphase nuclei of many mammalian cell types contain deep, dynamic, tubular membrane-bound invaginations of the nuclear envelope. J Cell Biol. 1997; 136:531–544. [PubMed: 9024685]
31. Lui PP, Kong SK, Kwok TT, Lee CY. The nucleus of HeLa cell contains tubular structures for Ca^{2+} signalling. Biochem Biophys Res Commun. 1998; 247:88–93. [PubMed: 9636660]
32. Johnson N, Krebs M, Boudreau R, Giorgi G, LeGros M, Larabell C. Actin-filled nuclear invaginations indicate degree of cell dedifferentiation. Differentiation. 2003; 71:414–424. [PubMed: 12969334]
33. Echevarria W, Leite MF, Guerra MT, Zipfel WR, Nathanson MH. Regulation of calcium signals in the nucleus by a nucleoplasmic reticulum. Nat Cell Biol. 2003; 5:440–446. [PubMed: 12717445]
34. Stoffler D, Fahrenkrog B, Aebi U. The nuclear pore complex: from molecular architecture to functional dynamics. Curr Opin Cell Biol. 1999; 11:391–401. [PubMed: 10395558]
35. Moore-Nichols D, Arnott A, Dunn RC. Regulation of nuclear pore complex conformation by IP(3) receptor activation. Biophys J. 2002; 83:1421–1428. [PubMed: 12202368]
36. Chawla S, Hardingham GE, Quinn DR, Bading H. CBP: a signal-regulated transcriptional coactivator controlled by nuclear calcium and CaM kinase IV. Science. 1998; 281:1505–1509. [PubMed: 9727976]
37. Harel A, Forbes DJ. Importin : conducting a much larger cellular symphony. Mol Cell. 2004; 16:319–330. [PubMed: 15525506]
38. Harel A, Chan RC, Lachish-Zalait A, Zimmerman E, Elbaum M, Forbes DJ. Importin β negatively regulates nuclear membrane fusion and nuclear pore complex assembly. Mol Biol Cell. 2003; 14:4387–4396. [PubMed: 14551248] This study determines that, besides its roles in transport, importin β has a critical role as a negative regulator of membrane fusion and nuclear pore complex formation during nuclear assembly.
39. Walther TC, Askjaer P, Gentzel M, Habermann A, Griffiths G, Wilm M, Mattaj IW, Hetzer M. RanGTP mediates nuclear pore complex assembly. Nature. 2003; 424:689–694. [PubMed: 12894213] A molecular mechanism underlying the role of the small GTPase Ran in nuclear

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assembly is characterized. Specifically, RanGTP is found to release certain nucleoporins from importin β in close proximity to the chromatin, providing spatial control over NE/NPC assembly.

40. Byrne RD, Barona TM, Garnier M, Koster G, Katan M, Poccia DL, Larijani B. Nuclear envelope assembly is promoted by phosphoinositide-specific phospholipase C with selective recruitment of phosphatidylinositol-enriched membranes. Biochem J. 2005; 387:393–400. [PubMed: 15554872]
41. Okada M, Ishimoto T, Naito Y, Hirata H, Yagisawa H. Phospholipase Cd1 associates with importin β1 and translocates into the nucleus in a Ca^{2+}-dependent manner. FEBS Lett. 2005; 579:4949–4954. [PubMed: 16115628]
42. Arnaoutov A, Azuma Y, Ribbeck K, Joseph J, Boyarchuk Y, Karpova T, McNally J, Dasso M. Crm1 is a mitotic effector of Ran-GTP in somatic cells. Nat Cell Biol. 2005; 7:626–632. [PubMed: 15908946]
43. Hachet V, Kocher T, Wilm M, Mattaj IW. Importin alpha associates with membranes and participates in nuclear envelope assembly *in vitro*. Embo J. 2004; 23:1526–1535. [PubMed: 15014441] This paper reports the discovery that importin α can associate with membranes and plays a dual role in NE assembly.
44. Bays NW, Hampton RY. Cdc48–Ufd1–Npl4: stuck in the middle with Ub. Curr Biol. 2002; 12:R366–R371. [PubMed: 12015140]
45. 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. [PubMed: 11781570]
46. Meyer HH, Wang Y, Warren G. Direct binding of ubiquitin conjugates by the mammalian p97 adaptor complexes, p47 and Ufd1–Npl4. Embo J. 2002; 21:5645–5652. [PubMed: 12411482]
47. Bonifacino JS, Glick BS. The mechanisms of vesicle budding and fusion. Cell. 2004; 116:153–166. [PubMed: 14744428]
48. Antonin W, Franz C, Haselmann U, Antony C, Mattaj IW. The integral membrane nucleoporin pom121 functionally links nuclear pore complex assembly and nuclear envelope formation. Mol Cell. 2005; 17:83–92. [PubMed: 15629719] Depleting POM121, an integral membrane nucleoporin, or vesicles containing POM121 from Xenopus egg extract significantly disrupts membrane fusion during NE assembly. This phenotype is dependent on the presence of the nuclear pore Nup107 subcomplex, suggesting an important connection between pore assembly and membrane fusion.
49. Harel A, Orjalo AV, Vincent T, Lachish-Zalait A, Vasu S, Shah S, Zimmerman E, Elbaum M, Forbes DJ. Removal of a single pore subcomplex results in vertebrate nuclei devoid of nuclear pores. Mol Cell. 2003; 11:853–864. [PubMed: 12718872]
50. Santos-Rosa H, Leung J, Grimsey N, Peak-Chew S, Siniossoglou S. The yeast lipin Smp2 couples phospholipid biosynthesis to nuclear membrane growth. Embo J. 2005; 24:1931–1941. [PubMed: 15889145] This paper adds important insight into the regulation of NE biogenesis. In yeast, the phosphorylation status of Smp2p appears to control its ability to bind to promoter regions and repress transcription of phospholipid enzymes.
51. Lagace TA, Ridgway ND. The rate-limiting enzyme in phosphatidylcholine synthesis regulates proliferation of the nucleoplasmic reticulum. Mol Biol Cell. 2005; 16:1120–1130. [PubMed: 15635091] Proliferation of the NR is regulated by CTP:phosphocholine cytidylyl-transferase-α, an enzyme that is implicated in lipid synthesis and in deforming membrane into tubules.
52. Nakagawa T, Hirano Y, Inomata A, Yokota S, Miyachi K, Kaneda M, Umeda M, Furukawa K, Omata S, Horigome T. Participation of a fusogenic protein, glyceraldehyde-3-phosphate dehydrogenase, in nuclear membrane assembly. J Biol Chem. 2003; 278:20395–20404. [PubMed: 12651855]
53. Ralle T, Grund C, Franke WW, Stick R. Intranuclear membrane structure formations by CaaX-containing nuclear proteins. J Cell Sci. 2004; 117:6095–6104. [PubMed: 15546917]
54. Prufert K, Alsheimer M, Benavente R, Krohne G. The myristoylation site of meiotic lamin C2 promotes local nuclear membrane growth and the formation of intranuclear membranes in somatic cultured cells. Eur J Cell Biol. 2005; 84:637–646. [PubMed: 16032931]
55. Worman HJ, Courvalin JC. The inner nuclear membrane. J Membr Biol. 2000; 177:1–11. [PubMed: 10960149]

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56. Ohba T, Schirmer EC, Nishimoto T, Gerace L. Energy- and temperature-dependent transport of integral proteins to the inner nuclear membrane via the nuclear pore. J Cell Biol. 2004; 167:1051–1062. [PubMed: 15611332] The authors use a novel assay and find that targeting of a reporter from the ER to the INM requires energy but not vesicle trafficking. They propose that the reporter moves through the pore membrane, since antibodies to the nucleoporin gp210 inhibit targeting to the inner membrane.
57. Saksena S, Shao Y, Braunagel SC, Summers MD, Johnson AE. Cotranslational integration and initial sorting at the endoplasmic reticulum translocon of proteins destined for the inner nuclear membrane. Proc Natl Acad Sci U S A. 2004; 101:12537–12542. [PubMed: 15306686] These results suggest that targeting of proteins to the INM requires more than diffusion and retention. The authors propose that as INM proteins are inserted into the ER they interact in a unique way with the translocation machinery, which could contribute to targeting to the INM.
58. Lenart P, Rabut G, Daigle N, Hand AR, Terasaki M, Ellenberg J. Nuclear envelope breakdown in starfish oocytes proceeds by partial NPC disassembly followed by a rapidly spreading fenestration of nuclear membranes. J Cell Biol. 2003; 160:1055–1068. [PubMed: 12654902]
59. Straube A, Weber I, Steinberg G. A novel mechanism of nuclear envelope break-down in a fungus: nuclear migration strips off the envelope. Embo J. 2005; 24:1674–1685. [PubMed: 15861140] In basidiomycetes, a novel mechanism of NE breakdown, involving nuclear migration toward the daughter cell followed by removal of the NE from the DNA, is observed.
60. De Souza CP, Osmani AH, Hashmi SB, Osmani SA. Partial nuclear pore complex disassembly during closed mitosis in Aspergillus nidulans. Curr Biol. 2004; 14:1973–1984. [PubMed: 15556859]
61. Makhnevych T, Lusk CP, Anderson AM, Aitchison JD, Wozniak RW. Cell cycle regulated transport controlled by alterations in the nuclear pore complex. Cell. 2003; 115:813–823. [PubMed: 14697200]
62. Collas P. Nuclear envelope disassembly in mitotic extract requires functional nuclear pores and a nuclear lamina. J Cell Sci. 1998; 111:1293–1303. [PubMed: 9547309]
63. 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. [PubMed: 11792323]
64. Salina D, Bodoor K, Eckley DM, Schroer TA, Rattner JB, Burke B. Cytoplasmic dynein as a facilitator of nuclear envelope breakdown. Cell. 2002; 108:97–107. [PubMed: 11792324]
65. Ellenberg J, Siggia ED, Moreira JE, Smith CL, Presley JF, Worman HJ, Lippincott-Schwartz J. Nuclear membrane dynamics and reassembly in living cells: targeting of an inner nuclear membrane protein in interphase and mitosis. J Cell Biol. 1997; 138:1193–1206. [PubMed: 9298976]
66. Yang L, Guan T, Gerace L. Integral membrane proteins of the nuclear envelope are dispersed throughout the endoplasmic reticulum during mitosis. J Cell Biol. 1997; 137:1199–1210. [PubMed: 9182656]
67. Collas I, Courvalin JC. Sorting nuclear membrane proteins at mitosis. Trends Cell Biol. 2000; 10:5–8. [PubMed: 10603470]
68. Cotter LA, Goldberg MW, Allen TD. Nuclear pore complex disassembly and nuclear envelope breakdown during mitosis may occur by both nuclear envelope vesicularisation and dispersion throughout the endoplasmic reticulum. Scanning. 1998; 20:250–251. [PubMed: 9604402]
69. Liu J, Prunuske AJ, Fager AM, Ullman KS. The COPI complex functions in nuclear envelope breakdown and is recruited by the nucleoporin Nup153. Dev Cell. 2003; 5:487–498. [PubMed: 12967567] This paper implicates the Golgi COPI vesiculation machinery in breakdown of the nuclear envelope. In addition, COPI recruitment to the nuclear envelope is facilitated by its interaction with the nuclear pore protein, Nup153.
70. Prunuske AJ, Liu J, Elgort S, Joseph J, Dasso M, Ullman KS. Nuclear envelope breakdown is coordinated by both Nup358/ RanBP2 and Nup153, two nucleoporins with zinc finger modules. Mol Biol Cell. 2005 in press.
71. Royle SJ, Bright NA, Lagnado L. Clathrin is required for the function of the mitotic spindle. Nature. 2005; 434:1152–1157. [PubMed: 15858577]

72. Crisp M, Liu Q, Roux K, Rattner JB, Shanahan C, Burke B, Stahl PD, Hodzic D. Coupling of the nucleus and cytoplasm: role of the LINC complex. J Cell Biol. 2005 in press.

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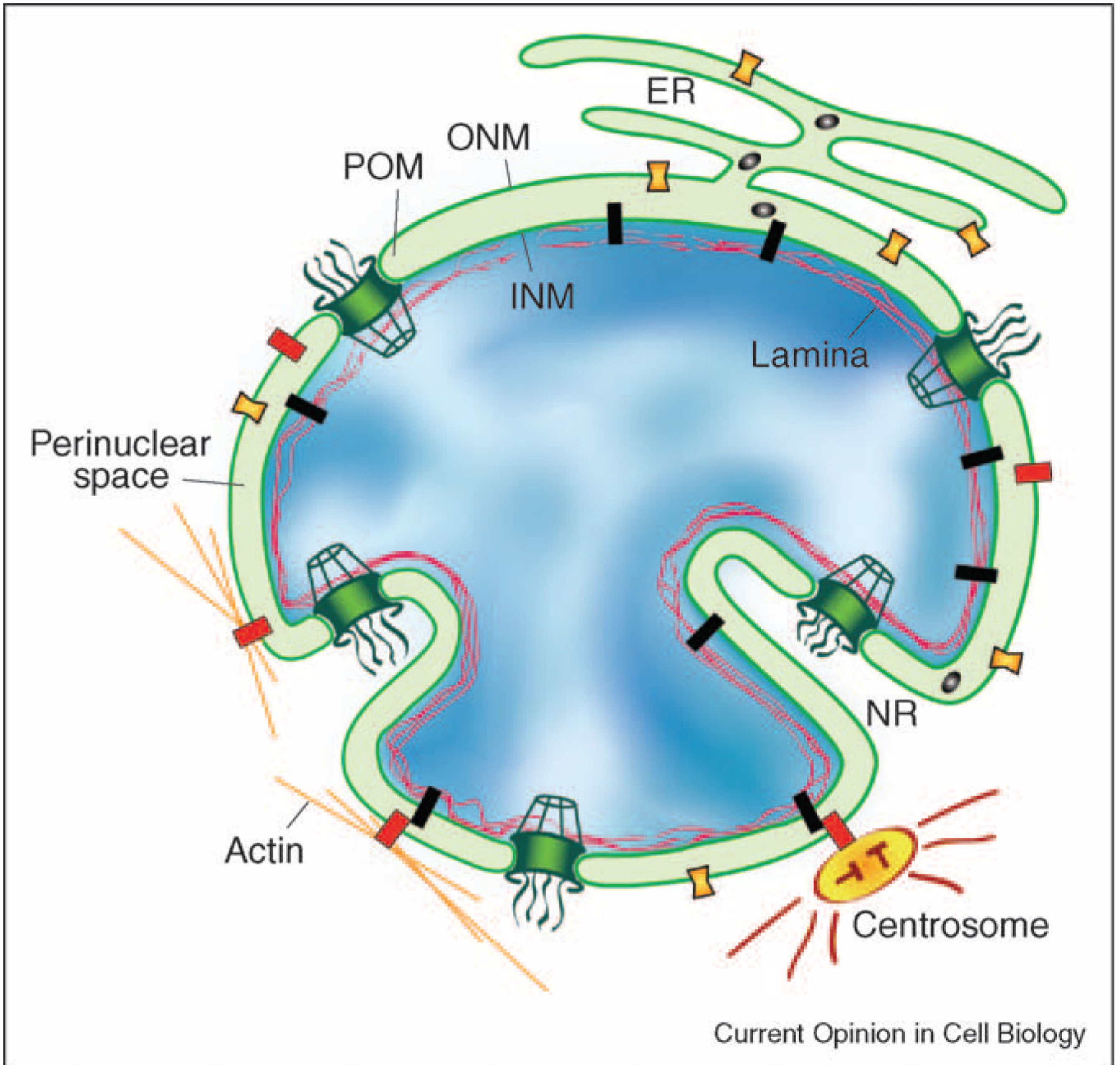

Figure 1.
Schematic diagram of the nucleus, highlighting membrane domains of the nuclear envelope (NE) and associated structures. The membrane system of the nuclear envelope consists of the outer nuclear membrane (ONM), the inner nuclear membrane (INM) and the pore membrane (POM). The ONM is contiguous with the endoplasmic reticulum (ER). Portions of the NE extend into the nucleus forming the nucleoplasmic reticulum (NR). The INM contains many distinct proteins (black) that contact the underlying lamina and chromatin. The pore membrane houses integral membrane proteins of nuclear pore complexes (green). Some ONM proteins (yellow) are also present within the ER and others (red) preferentially localize to the ONM and are proposed to bridge INM proteins to such cytoplasmic structures

as the centrosome and actin filaments. Finally, another category of protein (blue ovals) is able to diffuse within the perinuclear space and to interact with luminal domains of NE proteins.

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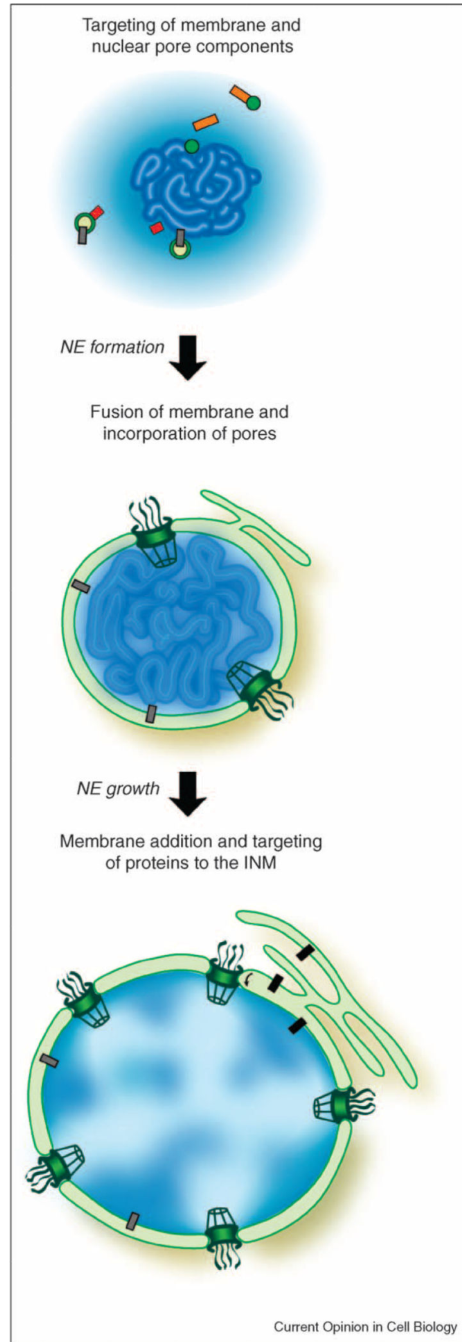

Figure 2.

Model of reformation of the nuclear envelope (NE) at the end of mitosis. Chromatin-associated RanGEF creates a gradient of RanGTP around DNA, which induces the localized release of nucleoporins (green balls) chaperoned at mitosis by importin β (orange). Importin α (red) also participates in nuclear formation and is, in part, membrane-associated. Some inner nuclear membrane (INM) proteins (grey), present on membrane, target to the chromatin during assembly. Formation of a closed NE requires incorporation of nuclear pore complexes into the fusing membrane. NE growth requires the addition of more membrane

and pores as well as import through the nuclear pore complexes. Additional INM proteins (black), synthesized in the ER, target to the INM via the pore membrane (POM).

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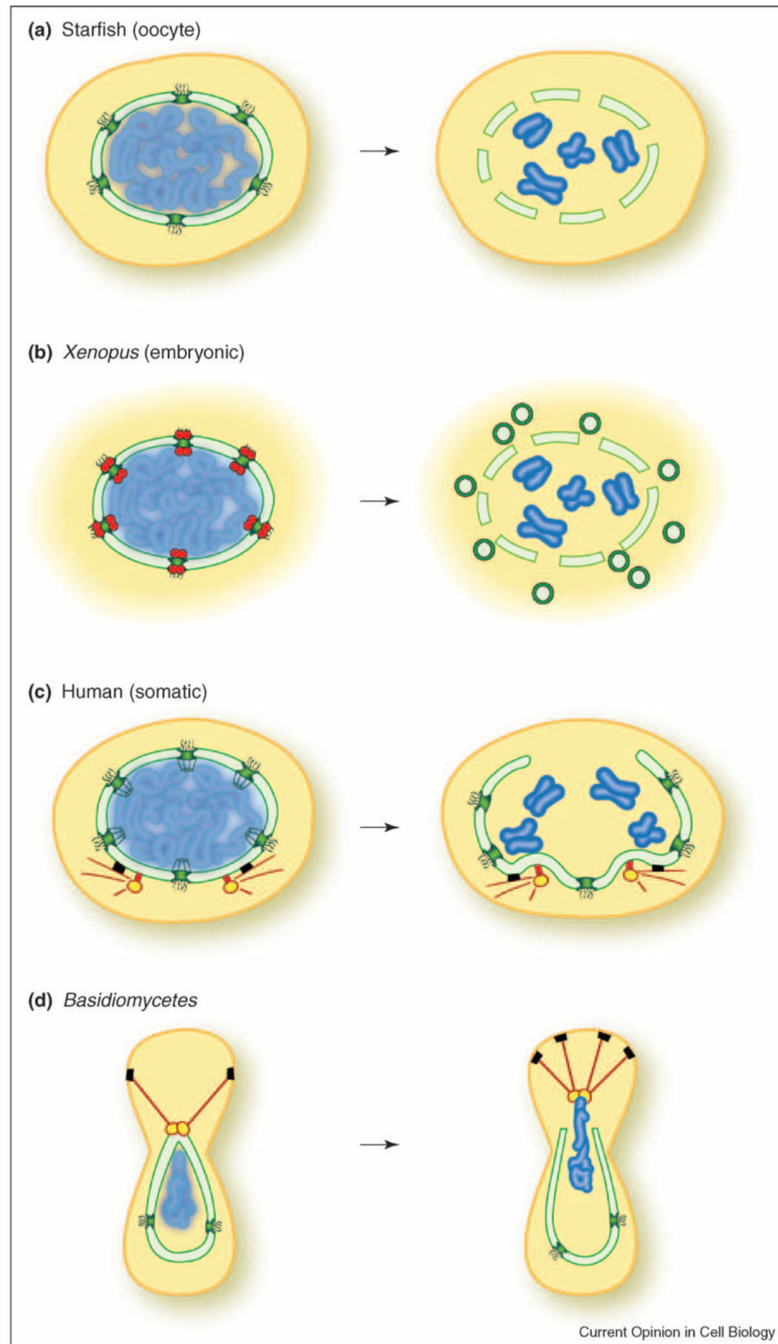

Figure 3.

Mechanistic models of nuclear envelope (NE) disassembly. Key findings in various experimental systems are schematically depicted. Cells are not drawn to scale; it is notable that the oocyte is very large compared to a somatic cell, since size may impose specific constraints on the mechanics of NE breakdown. (**a**) In starfish oocytes, early alterations in permeability at the nuclear pore (green) have been observed, and correlate with an early phase of nuclear pore complex (NPC) disassembly. During the second phase of disassembly, larger holes in the NE are proposed to emanate from the site of disassembled pores. (**b**) In

embryonic-like nuclei formed *in vitro* from *Xenopus* egg extract, nuclear pore proteins recruit the COPI complex (red) to the NE. Local concentration of this coatomer complex may then lead to vesiculation of the NE, as depicted, or to a non-conventional role for COPI. (**c**) In human tissue culture cells (somatic), microtubules originating from the centrosomes (yellow) connect to the NE via the microtubule motor dynein (black). Dynein-mediated movement is thought to then pull the NE toward the centrosomes, eventually causing a rupture at a distal region of the NE. (*d*) In *Ustilago maydis*, a basidiomycete, the NE is dragged from the mother cell to the bud by microtubules and dynein (black). There is an early increase in permeability, suggestive of pore remodeling, and then an obvious opening in the NE near the spindle pole body (yellow). Subsequently, the chromosomes enter the daughter cell where the spindle is formed, and the remnant of the NE collapses into the mother cell.

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