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Dynamics of the nuclear envelope at mitosis and during apoptosis

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Abstract. The nuclear envelope is a highly dynamic structure that reversibly disassembles and reforms at mitosis. The nuclear envelope also breaks down – irreversibly – during apoptosis, a process essential for development and tissue homeostasis. Analyses of fixed cells, time-lapse, imaging studies of live cells and the development of powerful cell-free extracts derived from gametes or mammalian somatic cells have provided insights on the fate of nuclear envelope proteins during mitosis and apoptosis, and on the mechanisms behind nuclear envelope modifications in these processes. In this review, we discuss evidence leading to our understanding of the dynamics of the nuclear envelope alterations at mitosis and during apoptosis. We also present novel imaging and genetic approaches to the study of nuclear envelope dynamics and function.

Key words. Nuclear envelope; lamin; chromatin; mitosis; apoptosis.

Introduction

The nuclear envelope (NE) is a specialized domain of the endoplasmic reticulum (ER) that surrounds the eucaryotic cell nucleus. It contains membranes and associated lamina-pore complex structures. The nuclear membranes are divided into three distinct but interconnected domains. The outer nuclear membrane is in direct continuity with the ER membrane and shares properties with the ER. The sharply bent pore membrane domain is associated with the nuclear pore complexes (NPCs) through specific integral proteins. The inner nuclear membrane (INM) contains another set of integral proteins providing attachment sites for heterochromatin and the nuclear lamina.

The nuclear lamina is a fenestrated meshwork of lamin filaments, the most abundant peripheral proteins of the NE. Lamins are type-V intermediate filaments encoded by three different genes in mammals. B-type lamins are expressed in all somatic cells, whereas A-type lamins are lacking from some embryonic, undifferentiated and cancer cell types. Most likely, additional peripheral proteins associated with the INM also exist, such as otefin [1, 2] and Young Arrest (YA) [3–6]. Nucleoporins represent the other large set of peripheral proteins of the NE. Their characterization, localization within the NPC and specific roles in nucleoplasmic transport are now well documented [7].

Integral proteins of the either polytopic, i.e. they span the membrane several times (LBR, Nurim, MAN1), or monotopic, i.e. they harbor a single transmembrane domain (LAP1, LAP2β, emerin), with their amino-terminal domain facing the nucleoplasm and interacting with the lamina and/or chromatin. A common (LEM) motif [8] is shared by **L**AP2β, **e**merin and **M**AN1; however, the function of this domain remains unclear. Although these integral proteins are ubiquitously present in the NE of human cells, missense mutations in emerin and lamin A have been shown to be responsible for tissue-specific diseases [9–11]. Two additional monotopic proteins, gp210 and POM121, with C-terminal ends facing the cytoplasm, have been characterized in mammalian pore membranes [12, 13].

The NE is a highly dynamic structure which grows in interphase, breaks down in prophase of mitosis and reassembles after metaphase. Whereas the mitotic modifications are reversible, disassembly of NE occurring during apoptosis is irreversible. In this review, we discuss

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evidence leading to our current understanding of the dynamics of NE modifications during mitosis and apoptosis.

Dynamics of NE disassembly

The dynamics of the NE are perhaps best illustrated at mitosis, when nuclear membranes disassemble from chromatin and the nuclear lamina breaks down. Regardless of whether lamins, integral proteins of the INM, NPC proteins or all of these play essential roles in driving the disassembly and reassembly of the NE at mitosis, it is generally agreed that their cell cycle-dependent phosphorylation is critical in these processes (fig. 1). Mitotic solubilization of lamins is believed to be elicited by phosphorylation by the cyclin B1-p34^{cdc2} complex (CDK1) and protein kinase C (PKC) [14–17]. Intriguingly, breakdown of the NE in zebrafish meiotic extracts seems to require the sequential phosphorylation of lamin B first by PKC, then by CDK1 (fig. 1) [18]. In light of a model of nuclear lamina disassembly elicited by multiple kinases [19], we proposed that PKC-mediated phosphorylation of lamin B unmasks CDK1 phosphorylation sites in the amino-terminal region that are critical for lamin depolymerization [18].

Several integral proteins of the INM, including LBR, LAP2β and emerin, are also phosphorylated at mitosis [20–24]. In contrast to lamins, however, there is no evidence for a role of multiple kinases in phosphorylation of INM integral proteins (fig. 1). Specific residues phosphorylated by CDK1 have been identified for LBR in the N-terminal nucleoplasmic domain [20, 23]. Nucleoporins are also phosphorylated in a cell-cycle-dependent manner [25], as are chromatin protein HP1 variants, which are ligands for LBR [26]. This suggests that detachment of the INM from peripheral structures may result from phosphorylation of a large number of integral and peripheral proteins of NE. From in vivo and in vitro studies performed in mammalian somatic cells and in *Xenopus* eggs, it appears that protein kinases promote membrane release from chromatin [22, 27, 28], whereas protein phosphatases stimulate binding [29, 30]. When disassembled, membranes containing LBR, LAP2β and gp210 are excluded from the mitotic spindle (see fig. 2 A–C), suggesting that they are contained in membrane sheets too large to enter the microtubule network [31, 32]. Therefore, except for a fraction of emerin which is apparently localized within the spindle [33, 34], chromosomes and membranes are segregated during metaphase inside and outside of the spindle, respectively.

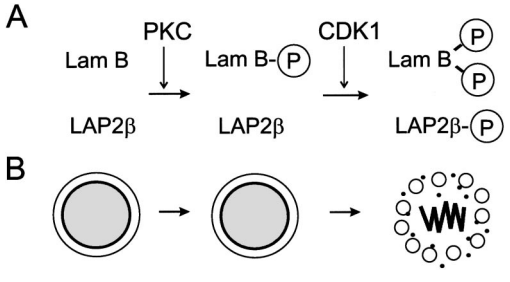

Figure 1. Model of nuclear membrane and lamina disassembly by sequential phosphorylation events elicited by PKC and CDK1. (*A*) In a zebrafish egg extract, zebrafish lamin B is phosphorylated by first PKC, and subsequently by CDK1 presumably on additional sites. LAP2β is not phosphorylated by PKC, but is phosphorylated by CDKI. (*B*) Nuclear membranes (circles) and the nuclear lamina (dots) only disassemble upon phosphorylations elicited by first PKC, then CDKI. Phosphorylation by first CDK1, then PKC, does not fully phosphorylate lamin B and does not promote NE breakdown (not shown). Adapted from [88].

Stepwise reassembly of the NE in living mammalian cells

NE reassembly at the end of mitosis has been extensively analyzed in unsynchronized mammalian cells in culture by classical immunofluorescence, and more recently by dynamic studies using recombinant proteins fused to spectral variants of the green fluorescent protein (GFP). Reconstitution of the NE is a stepwise process which starts in anaphase and is completed in early G1. The first wave of protein targeting to chromosomes occurs in anaphase A and B [32, 34] and involves INM integral proteins LBR, LAP2β and emerin, and the nucleoporin Nup153 [22, 31, 35, 36]. These markers are targeted to specific chromosome surface areas that are defined by their location with respect to the mitotic apparatus. Thus, LAP2β and LBR are associated with the lateral margins of chromosomes that emerge from the mitotic apparatus (fig. 2A, blue; fig. 2B, C, green) [31, 32, 34]. In contrast, emerin (fig. 2A, green) localizes with the central chromosome core regions located within the mitotic apparatus (fig. 2A, tubulin in red) [33, 34], whereas Nup153 decorates the entire chromosome surface [35]. At the end of telophase, when the mitotic apparatus has regressed except for pole-to-pole microtubules between the two daughter nuclei, markers of NE become uniformly distributed at the chromatin periphery [31, 32, 34, 36].

Components of the NPC also reconstitute sequentially onto chromosomes with an apparent polarity, with the nucleoplasmic Nup153 assembled first, followed by the p62 complex of the central core of the NPC and finally by the CAN/Nup214 complex of the cytoplasmic edge of the NPC [35]. Although the targeting topology of certain nucleoporins may be similar to that of INM markers (see mAb414 antigens in fig. 2E), it remains unknown whether NPC precursors are targeted directly to chromatin or to membranes previously bound to chromosomes. To illustrate this view, the NPC integral mem-

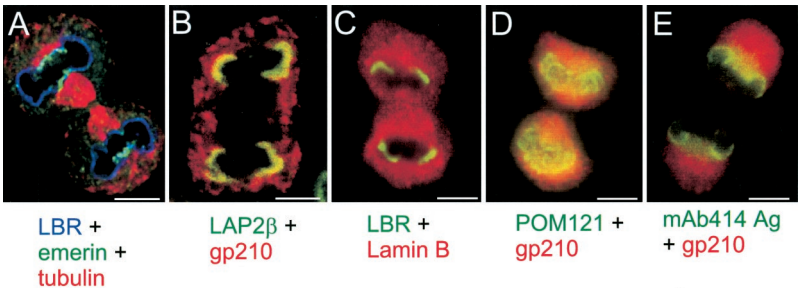

Figure 2. Differential targeting of nuclear membrane, lamina and pore components to the reforming nucleus during mitosis. (*A*) Triple labeling of LBR, emerin and tubulin in HeLa cells during late telophase. LBR is visualized by LBR-GFP fluorescence (blue), emerin is labeled with anti-emerin antibodies (green) and microtubules are stained with anti-α-tubulin antibodies (red). Taken from [34] with permission. (*B*) Immunofluorescence labeling of LAP2β (green) and gp210 (red) in HeLa cells at telophase. LAP2β is targeted to lateral chromosomes edges whereas gp210 is still cytoplasmic. Taken from [3l] with permission. (*C*) Immunofluorescence labeling of LBR (green) and lamin B (red) in HeLa cells at late telophase. (*D*) Immunofluorescence staining of the nuclear pore membrane proteins POM121 (green) and gp210 (red) during cytokinesis. Photograph kindly provided by Dr B. Burke. (*E*) Immunofluorescence labeling of gp210 (red) and mAb414 antigens (green) in HeLa cells at telophase [N. Chaudhary and J.-C. Courvalin, unpublished data]. Bars, 5 µm.

brane protein POM121 is targeted to chromosomes early in anaphase and thus may provide early membrane attachment of NPC precursors [35]. The integral nucleoporin gp210, which assembles in late telophase (fig. 2B, D, E, red) [31, 32, 35, 36], may anchor the NPC within the NE. Interestingly, gp210 also exhibits features of a fusigenic protein, which raises the attractive hypothesis that gp210 may contribute to sealing the outer and inner nuclear membranes [13]. A likely consequence of NE sealing in late telophase is the onset of NPC function [35] resulting in active nucleocytoplasmic transport [34].

Nuclear import correlates with a rapid chromatin decondensation and nucleolar assembly. Both A- and B-type lamins, which remain cytoplasmic during mitosis (fig. 2C), are imported into the daughter nuclei during cytokinesis and early G1 [32, 37], possibly through distinct pathways [38, R. L. Steen and P. Collas, unpublished observations]. At least in *Drosophila,* some lamin remains at the nuclear periphery in the "spindle envelope" [39, 40]. Assembly of lamins into the nuclear lamina has been shown to be essential for nuclear growth and chromatin decondensation, as mitotic cells microinjected with anti-lamin antibodies form daughter nuclei that remain arrested in a telophase-like configuration [41].

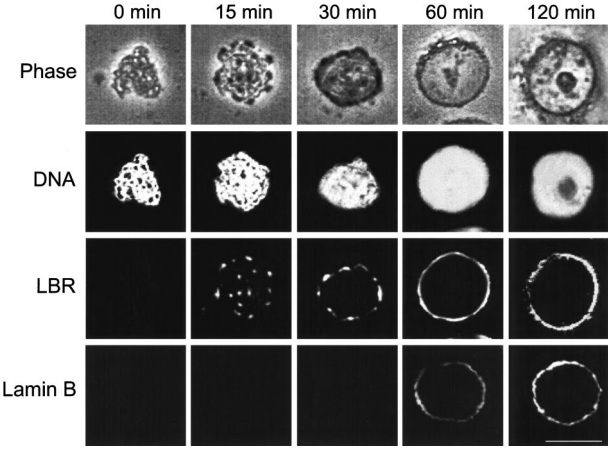

Figure 3. Sequential targeting of LBR and lamin B to chromosomes during somatic nuclear assembly in vitro. HeLa cell chromosomes condensed in a mitotic cell extract were incubated in an extract derived from interphase HeLa cells. The extract contained an ATP-generating system, GTP and membrane vesicles purified from mitotic HeLa cells. Nuclear reconstitution occurred over a 2-h period at 30 °C. Nuclear reassembly was examined over time by phase contrast microscopy, DNA labeling and immunofluorescence analysis of LBR and B-type lamins. Bar, 10 µm. Rcproduced from [42].

In vitro NE reconstitution

The sequential assembly of NE components to chromatin has also been illustrated in a recently developed in vitro somatic nuclear reconstitution system (fig. 3) [42]. A condensed chromatin substrate can be prepared from isolated interphase nuclei exposed to a mitotic extract (fig. 3, 0 min). Purification of such chromatin masses and further incubation in an interphase extract together with membrane vesicles derived from mitotic cells promotes targeting of the vesicles around the chromatin. In this system, the first vesicles detected on the chromatin surface by phase contrast microscopy harbor LBR (fig. 3). Lamin B, in contrast, is incorporated into the NE by nuclear import only after nuclear membranes have formed [42], strongly supporting earlier findings in sea urchin egg extracts [43].

INM proteins have specific functions in NE disassembly and reformation at mitosis. Lamins bind chromatin and DNA and, based on lamin depletion experiments using extracts from somatic cells, *Xenopus* eggs or *Drosophila* embryos, they have been proposed to play a key role in promoting mitotic NE disassembly and reassembly [41, 44, 45]. This conclusion, however, is still debated. Im-

munodepletion of lamins in some experiments conducted in *Xenopus* extracts [46, 47] and in experiments using sea urchin egg extracts [43] did not prevent reformation of a nuclear membrane containing nuclear pores, although the assembled nuclei were unable to undergo nuclear swelling and DNA replication [46–48]. These results suggest that lamins are dispensable for NE reassembly, though they undoubtedly play a critical role in various other nuclear functions. Consistent with this idea, the presence of LBR in NEs has been correlated with the occurrence of chromatin-membrane contacts but not with lamins in the NE of *Xenopus* oocytes and blastomeres [49].

Vesiculation or a continuum of nuclear membranes at mitosis?

Early ultrastructural analysis of mitotic cells demonstrated a progressive fragmentation of nuclear membranes, suggesting a disassembly by vesiculation [50]. In the context of available models of membrane traffic by vesiculation, it was suggested that the structure of the NE might represent a balance between membrane scission and fusion activities, with prevailing scission producing vesicles at mitosis [51]. Subsequently, the use of antibodies elicited against a battery of NE markers showed a sequential assembly of NE components after metaphase, and the enrichment of LBR and LAP2β in biochemically separable mitotic membrane fractions [31, 32]. These observations suggested a domain-specific vesiculation of nuclear membranes. Other arguments in favor of the vesiculation of nuclear membranes during mitosis have emerged from electron microscopic observations and isolation by ultracentrifugation of membrane fractions with distinct abilities to bind chromatin and fuse with one another [52, 53]. Furthermore, INM markers including LBR and LAP2β have been characterized or cloned in sea urchin and *Xenopus* [49, 52, 54–56]. This has enabled the recovery of egg membrane fractions enriched in proteins of the nuclear outer, inner or pore membrane. Using these markers, sequential targeting of proteins of the inner nuclear membrane and pore membrane to sperm chromatin was demonstrated, suggesting the existence of several populations of nuclear membrane vesicles in egg cytoplasm [43, 49, 54]. These data indicate that either nuclear vesicles exist in eggs or are generated during extract preparation from portions of a membrane continuum enriched in INM proteins.

In contrast to the above studies, several observations argue against vesiculation and suggest instead that the disappearance of nuclear membrane domains at mitosis occurs by diffusion of their integral proteins throughout a continuous ER membrane. First, immunofluorescence and confocal imaging studies have shown that INM proteins colocalize with ER markers at mitosis [36]. Second, using fluorescence recovery after photobleaching and fluorescence loss in photobleaching, Ellenberg et al. [57] have demonstrated that the amino-terminal domain of LBR fused to GFP diffuses rapidly and freely within ER membranes of metaphase cells, then immobilizes again when ER domains contact chromatin in late anaphase and early telophase. This study performed in living cells favors the hypothesis that integral proteins of nuclear membranes diffuse throughout the mitotic ER, which remains as an intact tubular network in many cell types. As cell homogenization unavoidably provokes membrane vesiculation and fragmentation, isolation of membrane fractions enriched in one marker may also reflect its concentration in microdomains of a continuous membrane system. The use of sophisticated imaging techniques in living cells, such as fluorescence resonance energy transfer (FRET), may help resolve whether proteins derived from a particular membrane domain fully disperse throughout the ER or remain associated in microdomains of the ER membrane. Indeed, FRET has proven successful in detecting molecular interactions between proteins tagged with spectral variants of GFP [58] and in detecting microdomains in the plasma membrane [59]. Similarly, to what extent nuclear membrane disassembly and reassembly processes in eggs and embryos are similar to, or different from, those in somatic cells may conceivably be unraveled by live dynamics studies associating FRET with multiphoton excitation microscopy.

Dynamics of the NE during apoptosis

Apoptosis is crucial for development and tissue homeostasis in metazoans. Apoptosis has primarily been studied in *Caenorhabditis elegans*, cultured cells and cell-free systems. A unified mechanism of apoptosis has emerged in which diverse death signals activate different signaling pathways that converge toward a conserved execution machinery composed of specific apoptotic proteases (caspases) [60]. Hierarchical activation of caspases provokes the cleavage of key proteins, leading to lesions characteristic of apoptotic cells.

Apoptosis induced in cultured cells or in cell-free extracts generates stereotyped NE alterations. These include budding, detachment of membranes from chromatin and clustering of NPCs [61–63]. Figure 4 illustrates such modifications using markers of the INM and NPCs. Despite these NE alterations, the NE persists and in vivo and in vitro until the late stages of apoptosis, and the ultrastructure of chromatin-detached nuclear membranes and NPCs is conserved [63, 64].

Cleavage of lamins 1–3 h after apoptosis induction is the earliest biochemical modification occurring at the NE [62, 63, 65–67]. The caspase 6 cleavage site of A- and B-

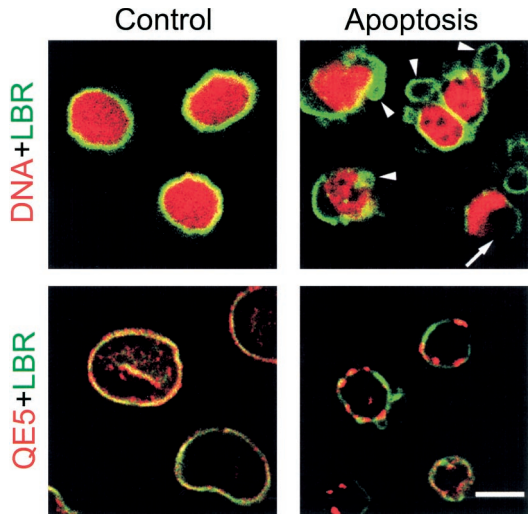

Figure 4. Nuclear envelope breakdown and nuclear pore clustering during apoptosis in human cells. (*Upper panels*) Immunofluorescence staining of LBR (green) in control and actinomycin D-treated (Apoptosis) KE37 cells. Note the detachment and budding of the nuclear membranes (green) from chromatin in apoptotic cells (arrow heads), preceding disappearance of the labeling at ultimate stages of apoptosis (arrow). DNA is labeled red. (*Lower panels*) Immunofluorescence labeling of LBR (green) and Nup153/Nup214/p62 (mAbQE5, red) in control and apoptotic KE37 cells. Note the clustering of nuclear pore proteins (red). Bar, 5 µm. Taken from [63].

type lamins has been mapped to a conserved aspartic residue located in a hinge region upstream of coil 2 [62, 66, 68, 69]. This cleavage generates a carboxy-terminal proteolytic fragment of 46 kDa containing coil 2, which is necessary for the formation of stable B-type lamin homodimers and for their lateral interactions in filaments. The features of this proteolytic fragment may explain the persistent association of lamina remnants with nuclear membranes until late stages of apoptosis [67, 70].

In addition to lamins, other proteins of the INM and NPCs are also selectively cleaved during apoptosis, although with a short delay (~ 1 h) compared with lamin B. Figure 5, from Buendia and co-workers [63], shows that B-type lamins, LAP2β and Nup153 are proteolyzed early in apoptosis (fig. 5, right panel), whereas LBR, gp210 and nucleoporin p62 are not cleaved (fig. 5, left panel). This does not preclude partial proteolysis of the latter markers at a later stage of apoptosis [70]. Protein sequence analysis [71] and the use of synthetic inhibitor peptides have identified caspase 3 as the protease responsible for apoptotic cleavage of LAP2β and Nup153 [63].

Interestingly, LBR and gp210, which remain uncleaved during apoptosis, do not harbor any consensus sites for caspases 3 and 6 in their nucleoplasmic domains. In contrast, p62 displays a consensus site for cleavage by caspase 3, but also remains intact during apoptosis possibly as a result of its protection from protease activity by its location within the compact core structure of the NPC.

Proteolysis of lamins, LAP2β, and filamentous nucleoplasmic Nup153, i.e. proteins connecting INM and NPC to chromatin, may explain the detachment of the NE from condensed apoptotic chromatin. Since NPCs are embedded into the lamina meshwork and connected to the cytoplasm by Nup153 filaments, proteolysis of these NE components may also explain the diffusion of NPCs in the plane of the membrane leading to their clustering.

In addition to proteolysis, key proteins may undergo changes in phosphorylation during apoptosis. Unscheduled activation of CDK1 has been reported during apoptosis [72–75]; however, the requirement for apoptotic CDK1 activation remains controversial [70, 76]. Other kinases may also phosphorylate NE proteins during apoptosis. For example, PKC-δ has recently been proposed as an apoptotic lamin kinase cooperating with caspase 6 to mediate efficient lamina disassembly [77].

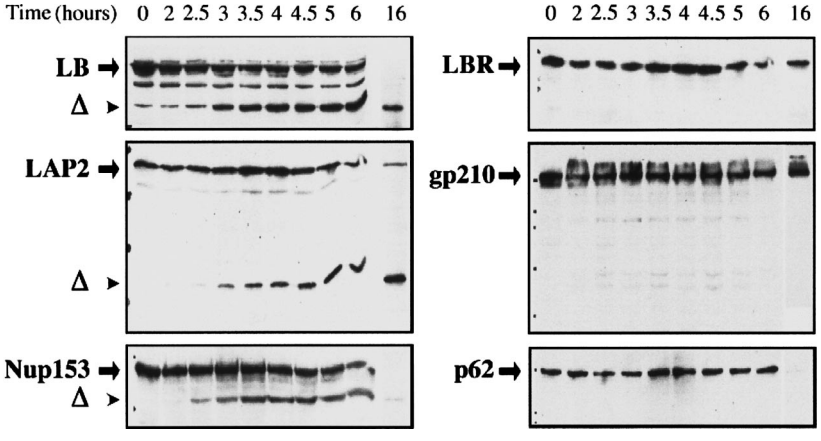

Figure 5. Selective proteolysis of lamin B (LB), LAP2β and Nup153 in apoptotic human cells. KE37 cells were exposed to actinomycin D (Act. D) for 0–16 h and analyzed at regular time intervals by SDS-polyacrylamide gel electrophoresis and immunoblotting. Not proteolysis of lamin B (LB, Δ), LAP2β (LAP2, Δ) and Nup153 (Nup153, Δ) (*left panels*), whereas LBR, gp210 and p62 remain intact (*right panels*). Taken from [63].

Chromatin condensation and DNA cleavage are major nuclear changes occurring simultaneously with NE alterations during apoptosis. Distinct pathways downstream of the effector caspase 3 are responsible for oligonucleosomal DNA cleavage [78] and chromatin condensation [79], although some factors may also induce peripheral chromatin condensation in a caspase-independent manner [80]. Chromosome condensation is independently facilitated by the loss of integrity of the nuclear matrix secondary to the cleavage of some of its components such as NuMA, topoisomerase II, and snRNP and hnRNP proteins. Cleavage of the lamina network and nucleoporin filaments, which are integral parts of the nuclear skeleton, cooperatively enhances the process of chromatin condensation.

It appears, therefore, that biochemical modifications during apoptosis are conserved, in particular the caspase-dependent proteolysis of lamins and of a subset of nuclear membrane and NPC proteins. As cleavage of proteins of the nuclear matrix and chromatin effectors is also conserved, it is becoming possible to identify nuclear components that cooperate to trigger the dramatic nuclear modifications induced by apoptosis.

Perspectives

The dawn of the 21st century has been marked by the emergence of powerful approaches for understanding NE dynamics during the cell cycle, development and in disease. The development of imaging software is expected to render dynamic analyses of nuclear reconstruction after mitosis in live cells more amenable. As an example, the group of R. Eils at the European Molecular Biology Laboratory (Heidelberg, Germany) has recently developed fully automated image analysis tools for visualization and quantitative evaluation of dynamic three-dimensional data using multicolor GFP confocal time-lapse microscopy. Such analyses have allowed the visualization and quantification of LBR-YFP targeting to chromosomes (labeled using histone H2B-CFP) during mitosis [R. Eils, personal communication].

The study of new organisms is also likely to provide exciting novel insights on NE dynamics during the somatic cell cycle and during development. Recent studies of the genetically tractable nematode *C. elegans* have identified orthologues of mammalian integral proteins of the INM [81]. These studies also characterized a novel timing of disassembly of NE components during mitosis in *C. elegans* relative to that of the open mitosis of higher eucaryotes, the closed mitosis of *S. cerevisiae* and the morphologically semiopen mitosis of *Drosophila.* Labeling of the *C. elegans* NE markers Ce-emerin, Ce-MAN1 and Ce-lamin revealed that nuclear membranes and the lamina remain essentially intact during metaphase and break down only in mid-late anaphase [81]. Interestingly, the timing of NPC disassembly at mitosis is dependent on C. *elegans* embryo age, raising the possibility that NE dynamics may also be developmentally regulated. Further studies are awaited to elucidate the genetic basis for developmental regulation of NE dynamics in *C. elegans* and other species.

Genetic studies of NE proteins are also anticipated to shed light on the function of NE proteins in interphase during development and in disease. An interaction has recently been identified between *Drosophila* lamin Dm0 and BICD, a protein involved in oocyte development [82]. A mutation causing the dominant bicaudal Bic-D phenotype alters binding of BICD to lamin Dm0, suggesting that interaction with the lamina plays a role in Bic-D function [82]. Additionally, the maternally-derived lamin-interacting protein YA has been shown to be essential for the transition from meiosis to mitosis in *Drosophila* eggs and is required for the first mitotic division [3, 5, 6]. Thus, females lacking YA function are healthy but sterile. It has been proposed that YA function is required either for completion of meiosis or for the behavior of the female meiotic products and male pronucleus after fertilization [83]. Furthermore, lamin B3 has been suggested to be involved in nuclear shaping during mouse spermiogenesis [84]. Lastly, mutations in the emerin gene have been shown to be responsible for a rare X-linked recessive form of Emery-Dreifuss muscular dystrophy (EDMD) [85]. An autosomal dominant form of EDNM is also caused by mutations in the *LMNA* (lamin A/C) gene [9]. Moreover, specific mutations in lamin A/C can elicit two other disorders, Dunningan-type familial partial lipodystrophy [10] and dilated cardiomyopathy [86]. Further investigations using cell lines derived from patients, or a recently developed lamin A knockout mouse model for EDMD [87] are expected to provide information on how mutations in emerin and lamin A/C cause disease.

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