Transcriptional repression, apoptosis, human disease and the functional evolution of the nuclear lamina

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The number and complexity of genes encoding nuclear lamina proteins has increased during metazoan evolution. Emerging evidence reveals that transcriptional regulators such as CED-4, have functional and dynamic interactions with the lamina. The discovery that mutations in nuclear lamina proteins cause heritable tissue-specific diseases, including Emery–Dreifuss muscular dystrophy, is prompting a fresh look at the nuclear lamina to devise models that can account for its diverse functions and dynamics, and to understand its enigmatic structure.

The main feature of eukaryotic cells is the nucleus, which enwraps the chromosomes and is the site of DNA replication, RNA transcription and processing, and ribosome assembly. The nuclear envelope (NE) is the boundary between the nucleus and cytoplasm. The NE is composed of the inner and outer nuclear membranes (INM and ONM, respectively), which are separated by a luminal space continuous with the ER lumen. Communication between the nucleoplasm and cytoplasm takes place through pores in the nuclear envelope, where the inner and outer membranes join. Within these pores are nuclear pore complexes (NPCs), which mediate and regulate nuclear transport. Underneath the INM is a meshwork of nuclear-specific intermediate filaments, termed the nuclear lamina, which includes lamin proteins plus a growing number of lamin-associated proteins. Near the INM is the peripheral chromatin, a large proportion of which is heterochromatin (Fig. 1).

Lamins are type V intermediate filament proteins. They range in size from 60 to 70 kDa and have a characteristic structure: a small N-terminal ‘head’, a 52-nm coiled-coil ‘rod’ and a globular C- terminal ‘tail’. Lamins form α-helical coiled-coil dimers, which are the building blocks for further assembly. In vitro, lamin dimers associate to form head-to-tail polymers. The assembly pathway and final structure(s) of lamin filaments are poorly understood. Indeed, lamin filaments are probably unlike the 10-nm diameter cytoplasmic intermediate filaments, because no 10-nm filaments have been detected in electron micrograph cross-sections, and the lamina isolated from Xenopus oocyte nuclei forms an orthogonal network rather than linear filaments. There is strong evidence that lamins are not restricted to the nuclear periphery but exist throughout the nuclear interior. It is not known if the peripheral and interior lamins form similar or different structures. However, given the growing number and types of lamin-binding proteins, some of these partners might influence the assembly or structural properties of lamins. The nuclear lamina provides structural support for chromosomes, and is required to maintain nuclear shape, space NPCs, replicate DNA and efficiently segregate chromosomes. New functions for the lamina are discussed below.

Metazoan evolution: a gradual increase in the complexity of nuclear lamina proteins

Investigators are identifying a growing number of integral and peripheral membrane proteins that associate with lamins (Fig. 2). Hence, the term ‘nuclear lamina’ is general, and includes both lamins and lamin-binding proteins. Vertebrates have three lamin genes. The LMNA gene encodes four alternatively spliced A-type lamins named A, C, Aα10 and Cγ. Two genes encode B-type lamins, LMNB1 (encoding lamin B1) and LMNB2 (encoding lamins B2 and B3). Several of these proteins are differentially expressed during development and differentiation, suggesting tissue-specific functions. In addition, vertebrates express many integral membrane proteins in the INM, including three isoforms of lamina-associated protein 1 (LAP1), at least five isoforms of LAP2, as well as emerin (mutations in which cause Emery–Dreifuss muscular dystrophy; EDMD), MAN1, lamin B receptor (LBR), nurim and probably UNC-84. A sixth isoform of LAP2, named LAP2xα, lacks a transmembrane domain and is found throughout the nuclear interior during interphase. The LAP2 isoforms plus emerin and MAN1 are members of a family defined by a 43-residue ‘LEM’ domain near their N terminus.

There are two lamin genes in Drosophila (lamin Dmα and lamin C), and one in Caenorhabditis elegans (Ref. 4). The number of lamins expressed in each organism fits a pattern in which more complex eukaryotes have greater lamin diversity. Eukaryotes are defined as more complex if they have more cells and more distinct cell types, tissues and organs. Thus, adult hermaphrodite C. elegans...
nematodes, which consist of 959 body cells, are
defined as less complex than adult Drosophila fruit
flies, which have ten times as many cells and can
form wings, legs, eyes, sensory bristles and other
specialized structures. The nearly complete genome
sequences for C. elegans and Drosophila
melanogaster allowed a search for homologs of
vertebrate INM proteins in ‘lower’ eukaryotes
(Fig. 2). C. elegans has three LEM-domain genes,
including homologs of vertebrate emerin and MAN1
(Ref. 7). A homolog for lamin C. elegans has not yet been
identified in vertebrates. UNC-84, an abundant
INM protein in C. elegans, is required for nuclear
migration during development8. Homologs of unc-84
are present in Drosophila and humans, but nothing
is known about their localization or function. The
Drosophila genome has at least six LEM-domain
genes, three of which are probably homologs of
MAN1, emerin and lamin. The other Drosophila
LEM-domain proteins are otefin, an otefin-like
protein and a novel protein with four putative
transmembrane domains (Fig. 2). Several INM
proteins present in vertebrates and Drosophila are
absent in C. elegans, including LBR, nurim and A-
type lamins. Not surprisingly, some lamina proteins
might be unique to vertebrates. For example,
neither Drosophila nor C. elegans encode obvious
orthologs for LAP1 or LAP2.

Metazoan nuclear lamina proteins are absent from
yeast, and probably plants
The yeast Saccharomyces cerevisiae contains no
lamin genes9, and lacks all other INM proteins
known in multicellular eukaryotes. This complete
lack of nuclear lamina proteins in S. cerevisiae
contrasts with the conservation of NPC proteins in
yeast10, and suggests that metazoan nuclear lamina
proteins provide functions unique to multicellular
animals.

What about the other multicellular eukaryotes,
plants? DNA sequence information is available in
GenBank for large fractions of the Arabidopsis
genome and many plant cDNAs. Despite previous
reports, lamina proteins appear to be absent from
plants. Conversely, plant proteins such as MAF1,
which localizes to the plant NE, lack homologs in
animals11. These results suggest that NE proteins
and functions evolved separately in plants and
animals.

Thus, several unique INM proteins first appeared
in metazoans and increased in number and
complexity during evolution. Metazoan nuclear
lamina proteins first appeared probably around the
transition between closed and open mitosis (see
below).

Increase in the efficiency and extent of nuclear envelope
disassembly during animal evolution
S. cerevisiae has a closed mitosis wherein the NE
remains intact12. During closed mitosis, tubulin
proteins are imported to allow mitotic spindles to
assemble inside the nucleus. By contrast, the
mammalian NE undergoes ‘open’ mitosis, in which
the nuclear lamina and NPCs reversibly
disassemble, the nuclear membranes merge into
the cytoplasm. During late anaphase and telophase this
process is reversed; nuclear membranes re-
associate with chromatin, NPCs assemble and
nuclear proteins including lamins are imported
back into the nucleus.

In vertebrates, the disassembly of the NE defines
the transition between prophase and
prometaphase. Based on their extent and timing
of NE breakdown, C. elegans and Drosophila
embryos are intermediate between yeast and
vertebrates (Fig. 3). For example, in C. elegans, the
nuclear membranes and lamina remain intact
except at spindle poles until after the
metaphase–anaphase transition, and completely
disassemble only during mid-late anaphase. In
Drosophila early embryos, NPCs disassemble
during prophase, similar to vertebrates.
until mid–late anaphase the nuclear membranes (and a fraction of the lamina) remain intact and are supplemented by a temporary second layer of membranes. Thus metazoan evolution might have been accompanied by an increase in the ability of different NE components to disassemble early in mitosis.

The correlation between NE complexity and the enhanced extent and efficiency of NE disassembly at mitosis is intriguing. Open mitosis creates new problems because mechanisms are needed to (i) ensure that all chromosomes end up in a single nucleus; (ii) reassemble the NE and interior architecture; and (iii) re-establish the interphase organization of chromatin and subnuclear organelles. Furthermore, lamin filaments, no matter how useful during interphase, might interfere with chromosome segregation during mitosis. Thus, open mitosis might have obligatorily co-evolved with nuclear lamina proteins. Having more lamina proteins probably conferred a selective advantage to metazoan creatures, perhaps related to improvements in chromatin organization, or improved nuclear signaling or gene expression. In addition, open mitosis exposes the chromatin to cytosolic proteins, which might provide new means to regulate the cell cycle through access to cytosolic replication licensing factors. Furthermore, the process of nuclear assembly itself might provide new mechanisms for regulating chromatin structure during development and differentiation.

**Roles for nuclear lamina in apoptosis**

Apoptosis, or programmed cell death, can regulate cell number, sculpt tissues during development and eliminate damaged cells. During apoptosis, nuclei undergo specific morphological changes, including proteolytic cleavage of the nuclear lamina, clustering of NPCs, detachment of chromatin from the NE and DNA cleavage. In C. elegans, the caspase CED-3 executes apoptosis by cleaving target proteins. CED-3 is activated by the APAF1 homolog, CED-4. However, CED-4 is normally prevented from activating CED-3 by the BCL2 homolog, CED-9. Cell death is triggered when the BH3-domain protein, EGL-1, binds and inhibits CED-9 (Ref. 17). In normal C. elegans cells, antibodies against CED-9 and CED-4 revealed that both proteins are localized to the mitochondria. However, when apoptosis is triggered, either by EGL-1 activation or CED-9 destruction, CED-4 rapidly translocates to the envelope (Fig. 4). This translocation does not depend on CED-3, suggesting that CED-4 translocates to the NE before caspase activation. Although the exact location of CED-4 in the NE is unknown, it appears to overlap the lamina. This result indicates that the lamina provides an attachment site for apoptotic signaling machinery.

Both A- and B-type lamins, LAP2α and LAP2β, are early targets for caspase degradation, before detectable DNA cleavage or chromatin condensation occurs. Other INM proteins, including LBR, are targeted later. Lamin α is cleaved in the α-helical rod domain, probably by caspase 6 (Ref. 22). LAP2β is cleaved by caspase 3 (Ref. 21). Cleaved fragments of lamins and LAP2β remain associated with the NE but are not known to play further roles.

Apoptotic nuclei resemble nuclei in lamin-deficient cells, which have clustered NPCs, detached chromatin and odd shapes. Thus, lamin destruction probably causes these same changes during apoptosis. Direct evidence for the importance of lamins in apoptosis was found by expressing an uncleavable mutant form of lamin in cultured cells; although caspases were activated, chromatin failed to condense and DNA cleavage was delayed. This result suggests that lamin degradation facilitates nuclear activation during apoptosis. It would be interesting to follow apoptosis in C. elegans cells that express uncleavable lamin, and to determine whether CED-4 can translocate to a lamin-depleted NE.

**Heterochromatin tends to associate with the INM**

Constitutive heterochromatin is transcriptionally repressed; it is also highly condensed, late replicating, rich in repetitive sequences, does not recombine during meiosis and has regular nucleosome spacing. Repressive chromatin structure can spread to genes near heterochromatin. A comparison between four model organisms – S. cerevisiae, C. elegans, D. melanogaster and mouse – shows that the amount of constitutive heterochromatin increases from less to more complex...
organisms. Heterochromatin in S. cerevisiae is limited to telomeres and silent mating type loci26. C. elegans has condensed chromatin at its periphery (e.g. see electron micrographs in Ref. 27) and tandem repeats, a hallmark of constitutive heterochromatin, accounting for ~2.7% of the genome28. The Drosophila genome contains an estimated 30% heterochromatin, including most of the Y chromosome. About 40% of human chromosome 22 is repetitive noncoding DNA (Ref. 29).

Cytological studies show that a large proportion of condensed chromatin, including centromeres and telomeres, borders the INM (Ref. 30). The positioning of inactive DNA is not random, because the inactive X chromosome in female mammalian cells is located near the NE, whereas the active X chromosome extends into the nuclear interior31. Even in S. cerevisiae, INM proximity helps to silence partially silenced genes: when the DNA-binding domain of the yeast transcription factor Gal4 was fused to a transmembrane protein, Gal4 became localized to the nuclear periphery, as did target genes containing the Gal4 operator. Furthermore, these genes became completely silenced, in a Sir (silent information regulatory protein)-dependent manner32.

Many nuclear lamina proteins interact directly with chromosomal proteins and might thereby affect chromatin structure at the nuclear periphery. For example, LBR interacts with the heterochromatin-specific protein HP1 (Ref. 33), and LEM-domain protein LAP2β interacts with barrier-to-autointegration factor (BAF)34, a DNA-binding protein that inhibits autointegration of retroviral DNA (Ref. 35). Lamins themselves can bind specific histones36. Young arrest (YA) is a Drosophila protein with developmentally regulated expression that is required for the transition from meiosis to mitosis; YA binds to both lamin B and chromatin37. A key question for the future is whether interactions between NE proteins and chromatin directly modulate the higher-order structure of chromatin.

**Nuclear lamina and transcription**

A growing number of transcription factors, most of which are repressors, localize at the nuclear periphery. Oct-1, a transcription factor containing a
POU domain that represses the aging-associated collagenase gene, co-localizes with lamin B (Ref. 38). In aging cells, the departure of Oct-1 from the NE coincides with increased collagenase activity; both events are reverted in immortalized cells. This result suggests that Oct-1 is active as a repressor only when it is located at the NE. The retinoblastoma (Rb) protein binds transcription factor E2F and represses transcription by recruiting histone deacetylases (not depicted). The model is based on binding observed between pairs of proteins in vitro: lamins A/C –Rb, Rb–E2F, E2F–DP, GCL (germ cell-less), GCL–LAP2β (see text for details). It is not yet known which, if any, of these putative interactions occur in vivo in the context of gene regulation. Nuclear lamina filaments are depicted generically as an orange rod, with round knobs to represent specific binding sites for A-type lamins, and triangular knobs to represent specific binding sites on B-type lamins.

Fig. 5. A speculative model for the involvement of the nuclear lamina in transcriptional repression. Transcriptional repressor retinoblastoma (Rb) associates with the nuclear lamina when it is active as a repressor, whereas hyperphosphorylated Rb is not associated with the lamina. Rb represses genes required for entry into S-phase by binding to E2F–DP heterodimers, and recruiting histone deacetylases (not depicted). The model is based on binding observed between pairs of proteins in vitro: lamins A/C –Rb, Rb–E2F, E2F–DP–GCL (germ cell-less), GCL–LAP2β, LAP2β–BAF, and LAP2β–lamin B (see text for details). It is not yet known which, if any, of these putative interactions occur in vivo in the context of gene regulation. Nuclear lamina filaments are depicted generically as an orange rod, with round knobs to represent specific binding sites for A-type lamins, and triangular knobs to represent specific binding sites on B-type lamins.

Nuclear lamina and genetic diseases

Interactions between the nuclear lamina and transcription factors might explain how mutations in nuclear lamina proteins cause inherited diseases. For example, the X-linked form of Emery–Dreifuss muscular dystrophy (EDMD) is caused by mutations in the emerin gene, whereas autosomal–dominant EDMD is caused by mutations in LMNA (Ref. 47; Fig. 6). Other mutations in LMNA cause cardiomyopathy and lipodystrophy. These diseases are proposed to result from defects in gene expression, due to loss of specific attachment sites on the nuclear lamina needed to establish or maintain particular patterns of gene expression. In this model, a missense mutation that disrupts the binding of just one factor to lamin filaments would cause a limited phenotype, whereas complete loss of LMNA would disrupt all proteins that depend on A-type lamin filaments. This prediction is supported by the severe combined phenotype seen in LMNA-knockout mice, which have defects in muscle, fat, and possibly bone (B. Burke, pers. commun.), consistent with defects in tissue mesenchymal stem cells.
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Fig. 6. A schematic map of mutations in human lamin A that cause three distinct inherited diseases. Shown is the lamin A protein (Ref. 50). The N-terminal head, coiled-coil rod and C-terminal tail domains are shown in different shades of blue. Mutations clinically associated with inherited dilated cardiomyopathy type 1A are shown in red. The mutations associated with autosomal–recessive and autosomal–dominant Emery–Dreifuss muscular dystrophy (EDMD) are shown in green and blue, respectively. Mutations associated with Dunnington-type partial lipodystrophy are shown in brown (mutation data taken from Ref. 47). More than half of the mutated residues that are associated with human diseases are identical in the lamin A gene in Xenopus and lamin A genes in human. Numbers indicate exons, which are drawn approximately to scale according to the number of amino acid residues encoded. Mutations associated with sporadic (non-inherited) disease are omitted. The clustering of mutations for dilated cardiomyopathy type 1A and Dunnington-type lipodystrophy to distinct regions of the lamin protein indicates that these regions are used as attachment sites for specific binding partners relevant to each disease.

Conclusions

The nuclear lamina cannot be viewed merely as the ‘nuts and bolts’ of nuclear structure. Although the lamina is certainly an essential structural element in the nucleus, the increasing evidence for human ‘laminopathy’ diseases suggests that some lamin-associated proteins have highly specialized, and in some cases possibly tissue-specific, functions. Recent studies suggest that activities such as transcription repression, growth control and apoptotic signaling each depend on the dynamic assembly of lamin-based structures. These findings join previous work showing that the lamina is essential for DNA replication. The evolutionary theory suggests that metazoan evolution has been accompanied by an expansion in the number and ‘flavors’ of nuclear lamins and lamin-binding proteins. Thus, more lamin-associated proteins will probably emerge, including new and known proteins whose functions are finally linked to a 3D context within the nucleus.


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Unwinding the ‘Gordian knot’ of helicase action

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Helicases are enzymes involved in every aspect of nucleic acid metabolism. Recent structural and biochemical evidence is beginning to provide details of their molecular mechanism of action. Crystal structures of helicases have revealed an underlying common structural fold. However, although there are many similarities between the mechanisms of different classes of helicase, not all aspects of the helicase activity are the same in all members of this enzyme family.

The sequence of the bases in the DNA of each organism contains the genetic information that determines its characteristics. However, this genetic information is locked within a double helix formed by two interacting and antiparallel DNA strands. Specific hydrogen-bonded pairs are formed between the bases of the interacting strands. Gaining access to the genetic information is of crucial importance for the replication and repair of this information as well as to translate it into proteins. For RNA, there is a need to remove unwanted secondary structures and to dissociate RNA–DNA heteroduplexes. Therefore, a diverse class of enzymes has evolved whose functions are to allow access to the genetic information. These are known as ‘helicases’.

Since the discovery of the first helicase from Escherichia coli nearly a quarter of a century ago, a large number of these enzymes have been identified and characterized in many organisms. Primary-structure comparisons have identified several conserved regions of amino acid sequence homology thought to be characteristic of helicases (so-called ‘helicase signature motifs’) and have resulted in the classification of these proteins into distinct superfamilies based upon the extent of homology of their primary sequences. Superfamilies I and II represent the largest and most closely related groups of helicases. They

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