Chapter 8
The Strange Case of the Noncanonical Lamina: Deep Divisions in Nuclear Organisation?

Mark C. Field and Michael P. Rout

Abstract The nuclear envelope is subtended in most eukaryotes by a proteinaceous lamina, a network that has been recognised since the 1950s. Originally considered as a simple structural support, it is now clear that the lamina can be highly dynamic and participates in a multitude of functions, including transcriptional and epigenetic regulation, definition of chromatin domains, genome stability and the positioning of nuclear pore complexes. The major protein components of the lamina in metazoans are ~60 kDa lamins, which assemble to form fibres and a network and are regulated by multiple mechanisms. Despite a broad taxonomic distribution beyond Metazoa, lamins are not universal and, in at least three major lineages, are absent, specifically fungi, plants and kinetoplastid protists. The latter two possess lineage-specific lamin analogues, the CRWN and NUP-1/NUP-2 proteins, respectively. Here we discuss and compare the kinetoplastid and plant lamina, their origins, components and functions and spectacular examples of convergent evolution.

Keywords Nucleus · Trypanosomes · Plants · CRWN proteins · Lamina · Evolution · Gene expression · Nuclear pore complex · Eukaryogenesis

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Introduction

The origin of eukaryotic cells dates to over 10^9 years ago [1], and it is generally accepted that the last common eukaryotic ancestor (LECA) possessed a complex cellular architecture including the mitochondrion, an endomembrane system, a flagellum and the nucleus [2]. The latter was likely broadly similar in many respects to the nuclei of extant organisms, with DNA packaged into nucleosomes, possessed of a nucleolus, Cajal bodies (and likely additional subnuclear bodies) and nuclear pore complexes transversing the nuclear envelope to mediate nucleocytoplasmic communication, together with a lamina system [3, 4]. This is very much a contemporary view of the nucleus and only revealed following the advent of widescale genome sequencing and subsequent efforts towards phylogenetic reconstructions of the LECA.

The nuclear lamina was described in the 1960s based on electron micrographs as a "complex supporting layer of fine filaments… observed on the inner aspect of the nuclear envelope" (Fawcett 1966), but identification of the lamin proteins, the stoichiometry of isoforms and finally their sequences were not ascertained until the 1970s and 1980s [5, 6]. Due to their position at the periphery of the nucleus, lamins were originally proposed to function chiefly to support the nuclear envelope, but their dynamic nature (especially in metazoan organisms with an open mitosis) and multiple interactions with other intranuclear assemblies suggested a more sophisticated integration with nuclear events [7].

We are now aware that lamins are related to type V intermediate filaments on account of possession of heptad repeats [8, 9] and that they have a plethora of protein-protein interactions and post-translational modifications, including phosphorylation, prenylation and proteolytic processing [10]. Multiple isoforms are recognised in vertebrates, which assemble into coiled-coil fibres, essentially as dimers that are further assembled into head-to-tail tetrameric units [11, 12]. Type A lamins are expressed from the *LMNA* gene, and alternate splicing generates lamins A and C, while type B lamins are expressed from two separate genes, *LMNB1* and *LMNB2* [9]. Significantly, the complete tertiary structure of a lamin protein remains to be reported.

While originally considered metazoan specific, in part due to the absence of lamin genes from fungal model systems, this is not the case, and lamin genes are present across a broad range of eukaryotes, including social amoeba (antisocial ones having no truck with them), the SAR supergroup and Metazoa [4, 13]. The distribution of lamins is sufficiently deep and broad across the eukaryotes to robustly support the conclusion that a primordial lamin B was present in the LECA [4].

However, it has emerged that there are more complex levels of gene retention and loss. Specifically, there are numerous examples of closely related taxa where one retains a lamin gene and the other does not [4]. This retention behaviour also seems to scale, so that we have the fungal phylum lacking lamins while their sister lineage, the Metazoa, retains them, and within the Amoebozoa only the slime-mould amoebae have lamins while remaining lineages do not (Figs. 8.1 and 8.4). In the case of

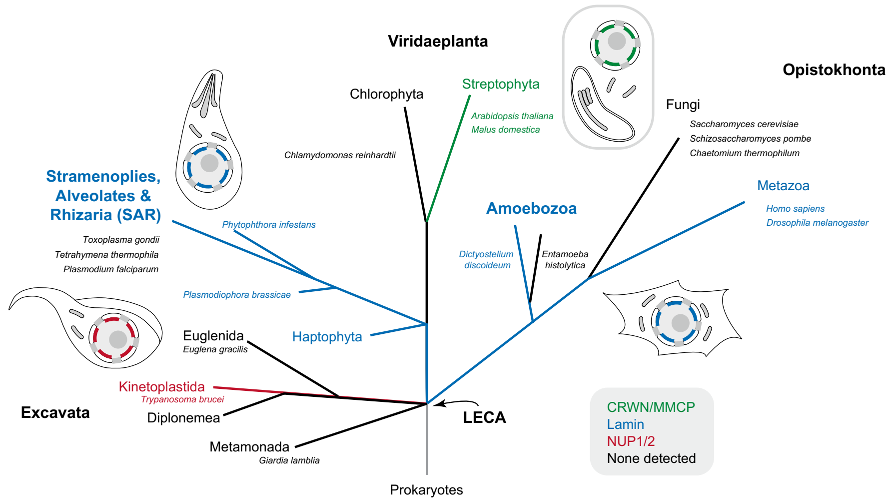


Fig. 8.1 Distribution of lamina systems across eukaryotes. A simplified eukaryote phylogeny is shown, with lineages and branches colourised according to possession of one of three presently known lamina systems. Apparent loss of the lamin system post the LECA is clear for the fungi, most Excavata and branches of the Viridiplantae and Amoebozoa. Note that for the SAR supergroup lamins are widespread, but major disease or model organisms are examples of taxa lacking the lamin system

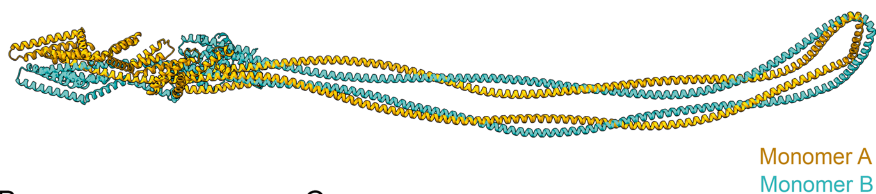
plants, none of the lineages possess lamins, although the CRWN (crowded nuclei) family of coiled-coil proteins provide a lamin analogue in the Streptophyta. Finally, the diverse Excavata supergroup also lack lamin genes, and though most lineages have no obvious analogue, the exception is the kinetoplastida, members of which possess the NUP-1/2 system.

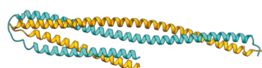
The pattern of lamin distribution across the eukarya seems to suggest an almost tenuous grip on retention, with quite frequent secondary loss. The result is that a great many lineages lack identifiable lamina components, including important model organisms such as *Saccharomyces cerevisiae* and *Chlamydomonas reinhardtii*, which begs the question of what, if anything, replaces the lamins. Importantly, we know of two analogue systems, CRWN and NUP-1/2, but there are a great many lineages that lack an obvious lamin candidate. Given the obvious central roles of any of these lamina systems, and the role of lamin defects in a multitude of diseases [14–16], it is difficult to envisage that organisms where none of these systems have been identified do not also possess an analogue that remains to be identified, *albeit* that functionality may be provided in a cryptic manner. For example, Esc1 in *S. cerevisiae* lacks any obvious architectural similarity to other lamina systems but does participate, in a lamin-like manner, in defining regions of heterochromatin [17]. Here we consider the origins, similarities and differences between the trypanosomatid and plant laminas.

CRWN (or NMCP) Proteins Constitute a Plant Lamina

Crowded nuclei proteins are essential architectural components of land plant nuclei and were first identified as nuclear matrix constituent proteins (NMCPs, renamed to CRWN [18]). CRWNs play significant roles in maintaining nuclear structure, regulating chromatin organisation and influencing gene expression, functions mirroring the lamins [19]. CRWN proteins are categorised into two main groups based on phylogeny: Group 1 includes CRWN1, CRWN2 and CRWN3, while group 2 contains CRWN4 orthologs. Within each clade, monocot sequences group independently from the dicot proteins as CRWN1- and CRWN4-related paralogues and are distinguished by the absence of a C-terminal domain present in dicot orthologs. Further, some dicot species, such as *A. thaliana*, possess multiple CRWN1 paralogues, which overall indicates ongoing evolution of the CRWN lamina system [18].

All CRWN proteins possess a coiled-coil region at their N-terminal, similar to lamins, which is required to maintain nuclear morphology [20] (Fig. 8.2). CRWN1 and CRWN4 localise predominantly at the nuclear periphery. Significantly, there is redundancy such that, depending on the paralogue, single knockouts can result in a mild nuclear morphological defect and near wild-type growth in *A. thaliana*, which becomes increasingly severe with double and triple CRWN knockouts, *albeit* that CRWN2/3 double knockouts are minimally affected, suggesting redundancy with CRWN1. Knockout of all CRWN genes is lethal [18]. More severe CRWN knockout mutants exhibit small, round nuclei, contrasting with the elongated nuclei typical of wild-type plants as well as ploidy abnormalities, again with severity varying according to the specific depleted CRWN paralogues.





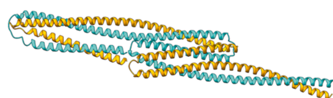


Fig. 8.2 AlphaFold 3 predictions for lamina protein tertiary structures. Panel A: *A. thaliana* CRWN1, to demonstrate the predominant α-helical topology. Panel B: A single repeat of *T. brucei* NUP-1. Panel C: Two repeats of *T. brucei* NUP-1. Each sequence was modelled as a homodimer (gold and blue for each monomer) using AlphaFold 3 and visualised using PhyMol. Models have been scaled to approximate volumetric parity. The full foldbacks of the helices are likely artifactual but do indicate reliable regions breaking the coiled-coil structure

CRWN Proteins Constitute a Flexible Network

The apparent genetic redundancy is supported by near identical expression and localisation patterns [21], with CRWN1, CRWN2 and CRWN3 all localising to the nuclear periphery. The cohort of CRWN proteins also appear to form filaments. Consistent with this, CRWN1, CRWN2 and CRWN3 interact with each other, while CRWN1 also interacts with CRWN4. This latter interaction appears rather specific as CRWN4 does not interact with CRWN2 or CRWN3, while homomeric interactions can be detected for all four CRWN proteins ([21], Fig. 8.2). The tighter associations between CRWN1, CRWN2 and CRWN3 may reflect closer evolutionary relationships and, together with homomeric association, suggest a rather more flexible and diverse system than that constructed by lamins. However, in common with both the lamin and NUP-1/2 systems, the configuration of CRWN proteins when assembled into fibres remains to be reported.

CRWN proteins are clearly involved in transcriptional regulation. CRWN1/2 and CRWN1/4 double knockouts trigger ectopic defence responses against pathogens, suggesting activity as negative regulators of immune responses. These include upregulation of salicylic acid biosynthesis genes and lamina disassembly under stress conditions [22, 23]. CRWN1 and CRWN2 play crucial roles in maintaining the repressive histone mark $H_3K_{27}me^3$ at many gene loci [14]. In CRWN1/2 double knockouts, there is reduction in $H_3K_{27}me^3$ levels at the PR1 (pathogenesis-related 1) locus, leading to elevated expression [24]. CRWN proteins also interact with the polycomb repressive complex 2 (PRC2), which is responsible for establishing the $H_3K_{27}me^3$ mark. Transcriptomes are extensively altered in CRWN mutants, with more than 2000 differentially expressed genes in the CRWN1/4 double knockout, including significant alterations in expression of copper-associated genes present as a cluster in chromosome V and critical for copper tolerance [21]. It is also likely that CRWN proteins are part of the mechanism that directs formation of lamina-associated domains (LADs), which are regions characterised by low transcriptional activity and high levels of repressive histone marks such as $H_3K_{27}me^3$ [21, 25].

CRWN1 can bind the plant-specific nucleoporins AtNup82 and AtNup136, which are components of the NPC basket. These nucleoporins possess conserved motifs shared with the plant lamina-associated protein KAKU4 that also facilitates their interaction with CRWN proteins [26]. This interaction suggests that CRWNs are both structural components of the nuclear lamina and connectors of the nucleoskeleton to the NPC. Significantly, KAKU4, AtNup82 and AtNup136 are related at the sequence level, and all three are specific to the Amborellaceae and angiosperms, which is an identical distribution to the expanded CRWN protein cohort [27]. Finally, a phase-separated large complex associated with the plant NPC basket that interacts with PWWP-domain interactor of polycombs 1 (PWO1), a histone reader that recruits PRC2 and interacts with CRWN1, indicates a bridge between the structural functions for CRWN proteins, tethering to the nuclear envelope and the heterochromatin machinery.

Overall, we can reconstruct a hypothetical pathway for the origins of the land plant lamina. The last common land plant ancestor possessed a single CRWN protein and an ancestral version of KAKU4/AtNup82/AtNup136. The ancestral system then diversified into two CRWN clades, with CRWN1–3 as one and CRWN4 as the other, while KAKU4, AtNup82 and AtNup136 also differentiated into three distinct genes. Critically, this allows continued flexibility between CRWN and the NPC together with combinatorial interactions between the CRWN proteins themselves.

The Trypanosome Lamina

T. brucei is a member of the kinetoplastids, a lineage that diverged early from the main eukaryotic lineage post the LECA as members of the Excavata supergroup. The structural and functional divergence of central nuclear activities between trypanosomatids, animals, fungi and plants has been appreciated for decades, including the presence of highly divergent histones, polycistronic transcription coupled to *trans*-splicing, transcription of some protein-coding sequences by RNA polymerase I and the presence of variant surface protein genes important to immune evasion and infectivity at telomere proximal loci [28–30].

NUP-1, the first identified component of the trypanosome nuclear lamina, was described in 2001 [31]. Using a monoclonal antibody (Mab) with nuclear rim staining, but unknown protein antigen [32, 33], the NUP-1 protein was revealed as a band by SDS-PAGE migrating at about 450 kDa and strongly enriched in nuclear fractions [31]. Further, the anti-NUP-1 MAb localises NUP-1 to the inner face of the nuclear envelope under immunoEM, *albeit* being excluded from the region directly beneath the nuclear pore complex. Furthermore, peptide sequencing identified a partial open reading frame containing near-perfect 144 amino acid repeats, with a predicted coiled-coil architecture and short spacers of lower coiled-coil propensity.

NUP-1 Is Dynamic, with Ordered Assembly

T. brucei NUP-1 is a protein of 3647 amino acids, with a central region composed of near prefect repeats. There are orthologs across kinetoplastids, all of which are smaller than *T. brucei* and with varying repeat patterns, but all retain a prediction of essentially a coiled-coil core with periodic breaks, together with distinct α-helical N- and C-terminal domains [34]. If fully extended, this would indicate a protein spanning some ~400 nm; given that the diameter of the trypanosome nucleus is under 2 μm, this suggests that an individual TbNUP-1 protein can access much of the nuclear volume, in stark contrasts to CRWN or lamin proteins [35].

When visualised via epitope tagging of the N- or C-terminus, TbNUP-1 appears as nuclear rim puncta, suggesting a concentration of termini at the nuclear periphery

[34]. This localisation is retained throughout the cell cycle, consistent with closed mitosis in trypanosomes with no evidence for nuclear envelope breakdown. However, persistence of the lamina does not preclude dynamic behaviour, and indeed there is both an apparent less compact spacing during mitosis compared with interphase and a polarised location during anaphase where NUP-1 termini partially accumulate at the distal poles of the separating nuclei [34].

Dual staining using both a C-terminal GFP-fusion protein and an antibody against the repeats indicates considerable spatial differentiation between the termini and the core repeat region, suggesting that at least some TbNUP-1 proteins have an extended configuration [34]. More extensive analysis using cells harbouring differentially tagged TbNUP-1 at both termini and stained using an antibody against the amino acid repeats revealed complex dynamics during the cell cycle ([29], Fig. 8.3). During interphase, all three regions of the protein are predominantly at the nuclear periphery, but during S-phase and through to chromosomal segregation in anaphase, the repeat region migrates deeper within the nucleus, suggesting that TbNUP-1 forms part of the chromosomal segregation machinery. At nuclear division, the protein migrates to the distal poles, with this being more pronounced for the N-terminus. This complexity indicates a central role for TbNUP-1 in nuclear and chromosomal organisation.

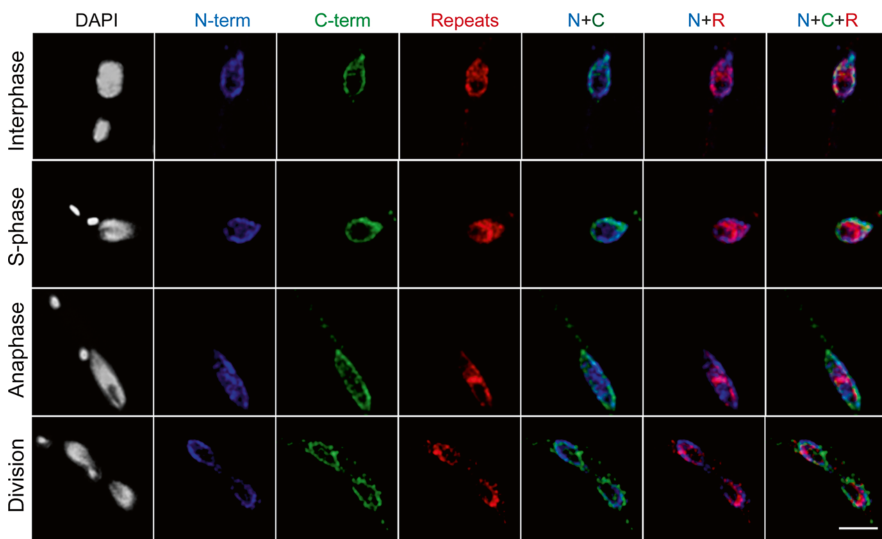


Fig. 8.3 NUP-1 domains relocate during the cell cycle. Cells expressing a double-tagged version of NUP-1 were imaged by confocal microscopy. DAPI was used to visualise DNA (pseudo-colour white). The N-terminal (N-term) and the C-terminal (C-term) domains were tagged with HA (pseudo-colour blue) and GFP (pseudo-colour green), respectively. To visualise the repeat region of NUP-1 (repeats), affinity-purified rabbit antibodies raised against the repeat were used (pseudo-colour red). The typical distribution of NUP-1 at the nuclear periphery is clear throughout the cell cycle stages as indicated (see text for details). Scale bar: 2 µm. (Figure is reproduced and adapted from Ref. [29]. Copyright retained by the authors as article open access)

An AlphaFold 3 [36] prediction of TbNUP-1 structure offers a possible mechanism for this dynamic behaviour (Fig. 8.2). The prediction highlights the coiled-coil repeats but also the links between these repeats which in the AlphaFold 3 representation are collapsed upon each other, *albeit* the collapse likely an artefact. However, this alternating rod and linker suggests that the repeats can contract and extend, in a manner similar to a Hoberman sphere [37]. This does also suggest the possible formation of TbNUP-1 fibres that are not in register, allowing for more complex branching and network formation than for the lamins.

Assembly of TbNUP-1 has been examined by expression of fragments *in cellulo*, specifically the N- and C-terminal domains alone together with a construct where the repeats have been deleted [29]. All three produce regular spherical assemblies, indicating that both the N- and C-termini are able to oligomerise. These analyses suggest that the N-terminus is more dominant in terms of assembly and anchoring to the nuclear envelope. The domain constructs also recruit endogenous TbNUP-1, indicating that these domains mediate bona fide protein-protein interactions, while migration of the construct polymers to distal positions within dividing nuclei indicates retention of endogenous protein domain targeting. We propose that TbNUP-1 likely assembles via a hub-and-spoke arrangement and with clear homotypic (i.e. N- to N-terminal, C- to C-terminal) and possibly heterotypic (N- to C-terminal) interactions, facilitating great flexibility within the lamina structure, once more an echo of the flexible CRWN system.

TbNUP-1 is a phosphoprotein, with multiple phosphorylation sites within the N- and C-termini and likely a trypanosome-specific CDK substrate (Koreny et al., in preparation). Specifically, mutation of all phosphorylation sites (ser/thr to ala) leads to the appearance of an umbilicus between separating nuclei (Fig. 8.4), which is phenocopied by knockdown of the CDK, CRK3. CRK3 impacts the cell cycle and is highly expressed during cell division [38]. We suggest that phosphorylation (and possibly other post-translational modifications) is responsible for regulating packing and unpacking of TbNUP-1 and specifically during nuclear division and hence an altered conformation and localisation across the cell cycle.

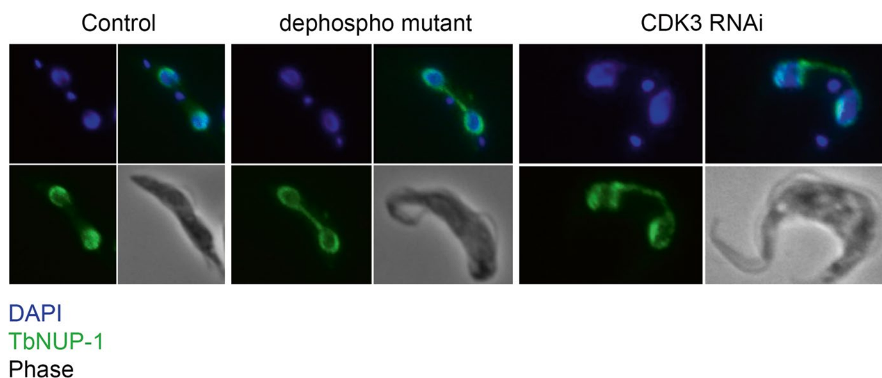


Fig. 8.4 Phosphorylation mediates TbNUP-1 relocation during nuclear division. Examples of cells possessing wild-type TbNUP-1 (control), a TbNUP-1 mutant with all identified phosphorylation sites mutated to alanine (dephospho-mutant) and cells with CDK3 silenced (CDK3 RNAi). All examples are TbNUP-1::GFP (green) and insect stage parasites, with DAPI stain in blue

NUP-1 Functions as a Lamin Analogue

Gene silencing of TbNUP-1 reveals functions which align with those reported for lamins in metazoan cells. These impacts include loss of normal nuclear morphology and the emergence of nuclear blebs. The distribution of NPCs is also impacted with the regular spacing of these structures being lost and resulting in pseudocrystalline arrays [34]. NUP-1 also functions in chromatin organisation. The *T. brucei* nuclear genome is organised as 22 megabase chromosomes, several intermediate-sized chromosomes of two to eight hundred kilo- base pairs and ~100 minichromosomes. Originally considered to segregate with distinct timing [33], all classes of chromosome segregate together using the same kinetochore and other machinery [39]. Telomeres during interphase are arranged at the nuclear periphery but become polarised during chromosomal segregation and closely follow TbNUP-1, but this peripheral organisation is, as expected, lost in TbNUP-1 silenced cells [34].

Many megabase chromosome telomeres contain a specialised expression site (ES) for the variant surface glycoprotein, a critical component for antigenic variation [40]. The ES contains a cohort of additional protein-coding genes, collectively referred to as ES-associated genes or ESAGs. All ES genes are, unusually, transcribed by RNA polymerase I as a polycistronic unit, and only a single ES is active at any time; all others are repressed. The single active ES locates to a nuclear interior position, distinct from the inactive ES which are positioned closer to the nuclear periphery [41]. A second protein-coding locus is also transcribed by RNA PolI which encodes the insect stage surface procyclin proteins together with a small number of procyclin-associated genes (PAGs), also transcribed in a polycistronic manner, and repressed in bloodstage parasites [42]. Significantly, both silent VSG ESs and the procyclin locus are derepressed in TbNUP-1 knockdown cells, concordant with the loss of a peripheral location of telomeres [34]. Hence, TbNUP-1 has a very specific impact on maintaining the correct expression profile of a select group of RNA PolI-transcribed developmentally regulated surface protein encoding genes.

NUP-1 Interacts with the NPC and NUP-2

Protein-protein interactions are central to understanding mammalian lamin biology as they underpin the plethora of processes that lamins mediate: The same is true for TbNUP-1. Direct pullouts of TbNUP-1 identify a large number of nucleoporins, including components of the NPC core scaffold, parts of the transport machinery, TbRan, TbRanBP1 and TbTBC-RootA, a putative RanGAP [43, 44], and components of the nuclear basket [45]. It is likely that many of these represent secondary interactions, for example, the full complement of the trypanosome equivalent of the Nup85 Y-shaped complex is recovered and the entire Ran machinery, the latter a GTPase mediating directional coupling of transport to a GTP/GDP gradient. FG-repeat-containing TbNup98 interacts with multiple regions of the NPC

including other FG Nups, the nuclear basket and the scaffold rings and is likely a direct TbNUP-1 interaction partner [29, 43, 44]. TbNUP-1 immunoprecipitations also identify a second large coiled-coil protein, which we designated as TbNUP-2 [43].

An interaction between TbNUP-1 and TbNup98 was also observed by proteomic analysis of the N- and C-terminal domain and N + C-terminal domain constructs [29]. TbNup98 localises as puncta at the NE, consistent with being an NPC component [34, 46], but this is disrupted by overexpression of the N- and C-terminal domain or N + C-terminal domain constructs. Specifically, at short times of overexpression, TbNup98 colocalises with the C-terminal domain, but not N- or N + C domains, while after more protracted induction periods TbNup98 colocalises with all three NUP-1 domain constructs and implies that the TbNUP-1 C-terminus is more dominant in interacting with TbNup98.

Furthermore, silencing TbNup98 disrupts the classical distribution of TbNUP-1 at the nuclear periphery and TbNUP-1 clusters as puncta at the NE. Nuclei in TbNup98-depleted cells are deformed, with evident defects in morphology and aberrant chromatin distribution. DNA content is also disrupted with an increase in >4n cells in the population, with these silenced cells also possessing extranuclear chromatin-containing bodies. Overall, these data suggest a model for TbNUP-1 binding the NPC via TbNup98, and potentially with a preference for the C-terminus, with this interaction being critical to coordinating nuclear division. However, the ability to anchor both termini at the NPC, independent of the central repeats, is fully consistent with the nuclear peripheral localisation of TbNUP-1 N- and C-termini and the more nuclear internal repeat location during chromosomal replication and segregation.

NUP-2 Is a Second Lamina Component

TbNUP-2 is a 170 kDa protein, predominantly α-helical with several predicted coiled-coil regions, and a phosphoprotein [47]. Orthologs are present across the kinetoplastids and extend to the free-living *Bodo saltans* and maintain a similar architecture in all cases. The absence of a repeat structure differentiates TbNUP-2 from TbNUP-1. The protein localises to the nuclear periphery and, while proximal to TbNUP-1, does not fully colocalise but does concentrate at the distal poles during nuclear division, similar to TbNUP-1. Having both lamina proteins concentrate at the same position in the nucleus where the chromosomes segregate most likely provides additional mechanical support to the spindle.

Similar to TbNUP-1, silencing of TbNUP-2 leads to nuclear blebbing, defective progression through the cell cycle, accumulation of cells with abnormal DNA content and mislocalisation of NPCs. Furthermore, the positioning of telomeres is abnormal, with telomeres frequently accumulating within nuclear blebs. Similar defects are present for the minichromosomes, which overall mirror the impact of TbNUP-1 on chromosomal organisation. Likely in consequence there is a

prominent increase in ɣH2A-like marker signal, which indicates double-strand DNA breaks, probably a result of disorganisation of the nucleus. Significantly, silenced cells demonstrate a highly specific altered transcriptome, whereby both the procyclin and silent VSG-ES RNA PolI-transcribed loci are derepressed. The remarkable overall similarity between TbNUP-1 and TbNUP-2 phenotypes is almost certainly due to their codependence, such that silencing of either leads to mislocalisation of the other [43].

Immunoisolation of TbNUP-2 identified robust interaction with TbNup110, a component of the nuclear basket and a suite of seven additional proteins. Subsequent analysis reveals that several are part of an extensive trypanosome NPC nuclear basket [45], and hence while overall structural and functional similarities between TbNUP-1 and TbNUP-2 are significant, it is certainly the case that these proteins are nevertheless nonidentical in their roles.

Of the cohort of proteins identified from TbNUP-1 or TbNUP-2 immunoisolations that are neither NPC or transport factors, only two are conserved across eukaryotes. TbLAP59 is an ortholog of a sphingomyelinase implicated in mediating NPC insertion [45], and TbLAP173 is orthologous to Sac3, the sole component of the TREX-2 complex identified to date in trypanosomes. Of the remainder, TbLAP333 provides a considerable surprise: Lacking any sequence similarity to organisms beyond the kinetoplastida, this protein is predicted by AlphaFold 3 to contain 11 Ig-fold repeats as well as a cluster of transmembrane domains at the C-terminus and hence is structurally related to the NPC membrane proteins yeast Pom152 and human Nup210. The remaining four proteins are predicted to be extensively coiled coil and, based on their interactions, robustly placed as nuclear basket proteins, revealing an amalgam of conserved and lineage-specific components associated with the trypanosome NPC and lamina.

Evolution of NUP-1 Repeats

T. brucei NUP-1 contains multiple coiled-coil segments with linkers separating them. In contrast, lamin rod domains are non-repetitive and consist of unique coiled-coil segments of various lengths separated by short linker regions, which are highly conserved [4] and more similar to the CRWN proteins in this regard [20]. The TbNUP-1 rod domain is actually comprised of two repetitive elements, designated type A and B. Sixteen type A and 15 type B repeats form a coiled-coil rich span with shorter coiled coils separated by multiple non-coiled-coil linker sequences. The repeat core starts and ends with an A-type element ([48], Fig. 8.5).

Analysis of repeat variation and coiled-coil predictions across kinetoplastids identified three groups of sequences; those related to the regular A|B TbNUP-1 architecture, those closely related to *L. major* NUP-1 and a group with more divergent characteristics. *Leishmania* NUP-1 orthologs all possess head and tail domains of very similar lengths and nearly identical sequence. Their rod domains consist of just two types of repeats arranged in the $(A\text{-}B)_n$-A pattern. Unlike the trypanosome

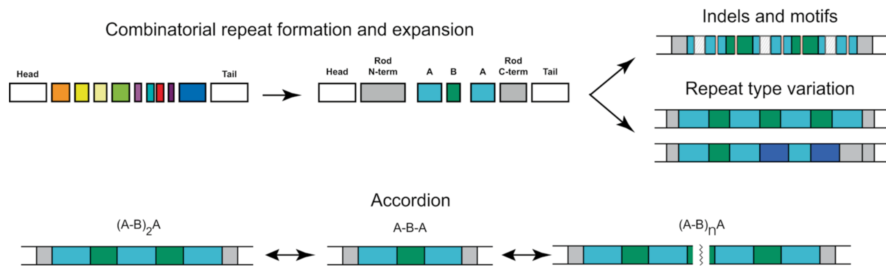


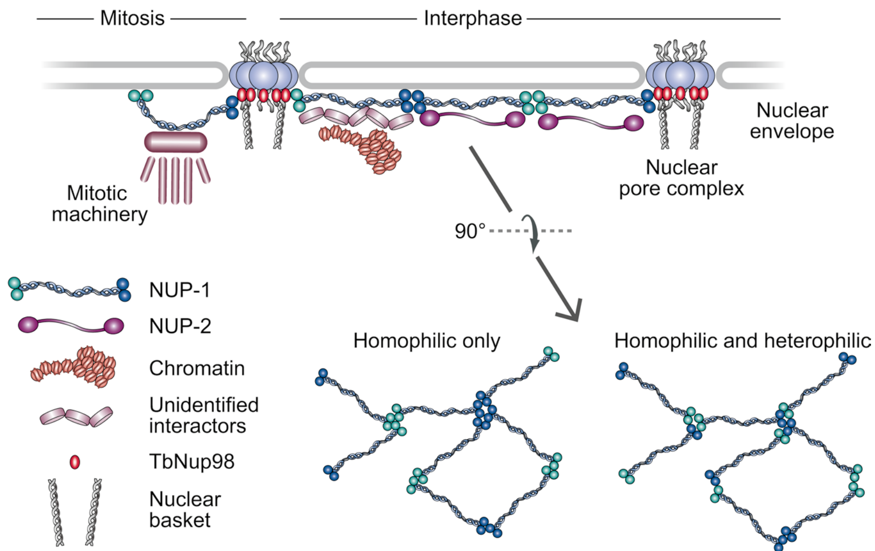
Fig. 8.5 Modes of expansion and variation of NUP-1 rod domain. We suggest that the highly regular repeats present in most kinetoplastids have evolved from a less regular progenitor, as exemplified by the NUP-1 ortholog in *C. fasciculata* (rainbow repeats top left). This resolved into a regular repeat with A (light blue) and B (green) elements, which could be diversified by indels or other types of sequence variation. Further, we suggest that repeats can evolve through variation in the number of repeats, allowing increases or decreases, similar to an accordion

group, the *Leishmania* A- and B-type repeats are conserved both within and between species, and the major difference between orthologs is the number of repeats comprising the rod. Considering that the number of repeats in *Leishmania* varies in a similar way in trypanosomes but shares relatively little sequence similarity, this suggests an "accordion" effect where, in species with two types of repeats, repeats likely multiplied different numbers of times via domain expansion while maintaining an $(A\text{-}B)_n$-A pattern arrangement (Fig. 8.5).

In the monoxenous parasite, *Crithidia fasciculata*, which is considered a basal kinetoplastid form, the regular repeat structure is not present. The syntenic CfNUP-1 gene retains the overall head-rod-tail architecture with the rod consisting of multiple short elements that have a complex repeat organisation. However, despite this, the rod remains predicted as predominantly coiled coil. Hence, we suggest that the highly regular repeats in the trypanosomatids/*Leishmania* are the evolutionary result of a simplification of repeat elements, followed by independent expansion and variation within each lineage [48]. This simplification may allow the interaction between repeats to be flexible and hence allow modification of the network.

Conclusions

The nucleus originated prior to the LECA, with all reconstructions predicting that the nucleus in the LECA possessed a lamina (most probably related to lamin B), NPCs and other systems that are represented in the vast majority of described extant species. That said, it is also apparent that the period subsequent to the LECA provided ample opportunity for evolution to diversify these systems and that the lamina provides an excellent example. There are at least three systems presently described, lamins, CRWN and NUP-1/2, which have striking similarities despite no evidence for vertical descent and together are a potent example of convergent evolution (Fig. 8.6). We speculate that additional systems may well remain to be uncovered.



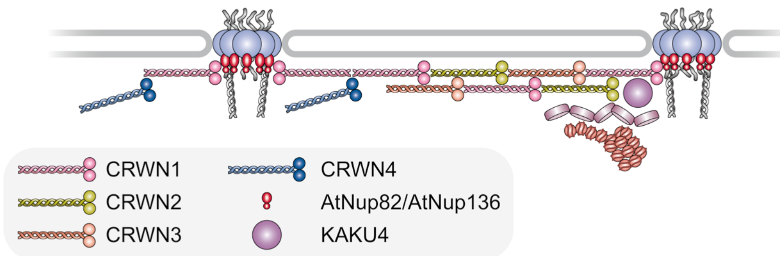


Fig. 8.6 Organisation of the lamina systems in trypanosomes and plants. Schematics of the lamina systems discussed in the text. Top panel the NUP-1/2 system in kinetoplastida. In this representation, it is assumed that NUP-1 forms a homodimer but that interactions between dinners are primarily mediated by the terminal domains. Lower panel represents the CRWN proteins in vascular plants. Note that the stoichiometry is unknown, but all CRWN proteins can self-interact as well as form promiscuous heterophilic connections. Note that a number of factors that connect the lamina and chromatin remain unknown in both systems, although for plants some components of the polycomb repressive complex 2 (PRC2), responsible for establishing the $H_3K_{27}me^3$ mark, are associated with CRWN isoforms. Top panel is redrawn and expanded from Ref. [29]. Note that protein/complex sizes are not drawn to scale

Each lamina possesses a predominant coiled-coil element, which leads to a common architecture and which is likely modulated by post-translational mechanisms. While NUP-1/2 are only present in organisms with a closed mitosis, lamins are present in organisms with both open and closed mechanisms, while the vast majority of land plants utilise open mitosis. We suggest that the large trypanosome NUP proteins

are unsuitable for open mitosis where the lamina has to disassemble, *albeit* that there remains flexibility within the NUP-1/2 network facilitating dynamic reorganisation during the cell cycle. This does lead to diversification in the manner of oligomerisation, with NUP-1 primarily assembling via the non-repetitive N- and C-terminal domains, whereas lamins form fibres that are in register as head-to-tail oligomers. Furthermore, all lamina systems interact with the NPC and mediate spacing of the pores and control gene expression, *albeit* by divergent mechanisms. It remains to be determined how any lamina system is fully built and for the structure of a full monomer to be reported.

The evolutionary distribution of classical lamins is clearly the broadest, and the patchy retention across most eukaryotic lineages contrasts with the CRWN and NUP-1/2 systems, which are clear examples of lineage-specific evolution. The multiple losses of lamins suggest that circumstances can arise comparatively frequently where lamin function is non-essential, and at least in the case of the euglenids, it is clear that lamins were lost prior to their replacement by NUP-1/2.

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