Coaching from the sidelines: the nuclear periphery in genome regulation

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Abstract | The genome is packaged and organized nonrandomly within the 3D space of the nucleus to promote efficient gene expression and to faithfully maintain silencing of heterochromatin. The genome is enclosed within the nucleus by the nuclear envelope membrane, which contains a set of proteins that actively participate in chromatin organization and gene regulation. Technological advances are providing views of genome organization at unprecedented resolution and are beginning to reveal the ways that cells co-opt the structures of the nuclear periphery for nuclear organization and gene regulation. These genome regulatory roles of proteins of the nuclear periphery have important influences on development, disease and ageing.

HiC
A technique used to study genome organization by identifying chromosomal interactions both in *cis* and in *trans* throughout the entire genome.

Nuclear periphery
The outermost region of the nucleus, which includes the nuclear envelope and associated proteins, the nuclear lamina and the nuclear pore complexes.

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Early cytological experiments determined that the cell nucleus — the largest, most readily observed subcellular structure — contains the genetic determinants that direct the development of functional tissues[1]. We now know that the eukaryotic nucleus contains the majority of the genetic information of the cell in the form of discrete chromosomes[2], which occupy distinct, nonrandomly positioned territories within the nucleus[3]. Recent innovations in sequencing-based methods have enabled the visualization of the 3D organization of whole genomes. Chromosome conformation capture (3C) and related technologies such as HiC have made it possible to infer genome folding by detecting contact frequencies throughout the genome[4]. In general, studies using sequencing-based methods agree with those using microscopy-based approaches that intrachromosomal contacts are more frequent than interchromosomal contacts, supporting the existence of chromosome territories, and that gene-rich chromatin domains interact with each other more frequently than with gene-poor chromatin domains[5,6]. In fact, gene-rich, transcriptionally active and gene-poor, transcriptionally repressed chromatin form distinct megabase-scale compartments, designated A and B compartments, respectively[5].

Chromatin compartments adopt specific positions relative to a major structural landmark of the nucleus, the nuclear periphery (FIG. 1a). The nucleus is separated from the cytoplasm by the nuclear envelope, a double membrane bilayer that, although contiguous with the endoplasmic reticulum membrane network, is enriched for a unique set of proteins on the basis of their affinity for other structures, including chromatin and the nuclear lamina[7]. The nuclear lamina, which underlies the inner membrane of the nuclear envelope, is composed of a meshwork of filamentous proteins, the nuclear lamins, and acts as a structural scaffold for the nucleus[8]. Nuclear pore complexes (NPCs) are large multiprotein assemblies that perforate the nuclear envelope membrane and control the passage of material between the nucleus and cytoplasm (reviewed in REF.[9]). HiC analyses indicate that the B compartment preferentially occupies the nuclear periphery, whereas the A compartment is more centrally located within the nucleus[10]. As visualized by electron microscopy, heterochromatin is prominently clustered underneath the nuclear lamina in most cell types[11] and is interspersed with regions of less tightly packed euchromatin underneath the NPCs (FIG. 1b). Accordingly, the nuclear periphery is generally a repressive environment where gene activity is limited, with the prominent exception of genomic regions that are closely associated with NPCs[12].

Ongoing work is illuminating how the organization of the genome within the nucleus guides efficient gene activation and gene repression. In recent years, roles have emerged for the proteins and structures of the nuclear periphery in establishing nonrandom genome organization, maintaining nuclear organization over time and regulating gene expression. Here, from the perspective of the nuclear periphery, we follow the life cycle of the nucleus from assembly, to the supporting roles that the nuclear periphery plays in adapting the genome to cell type-specific functions, to the decline in nuclear organization during ageing. Although we focus on recent advances in mammalian systems, where relevant, we also highlight select findings from other model eukaryotes.

Building a nucleus

Cell type specificity of nuclear organization

In multicellular organisms, cells within different tissues position their chromosomes in characteristic ways. In a specific cell type, these positions are maintained in relation to other chromosomes as well as to

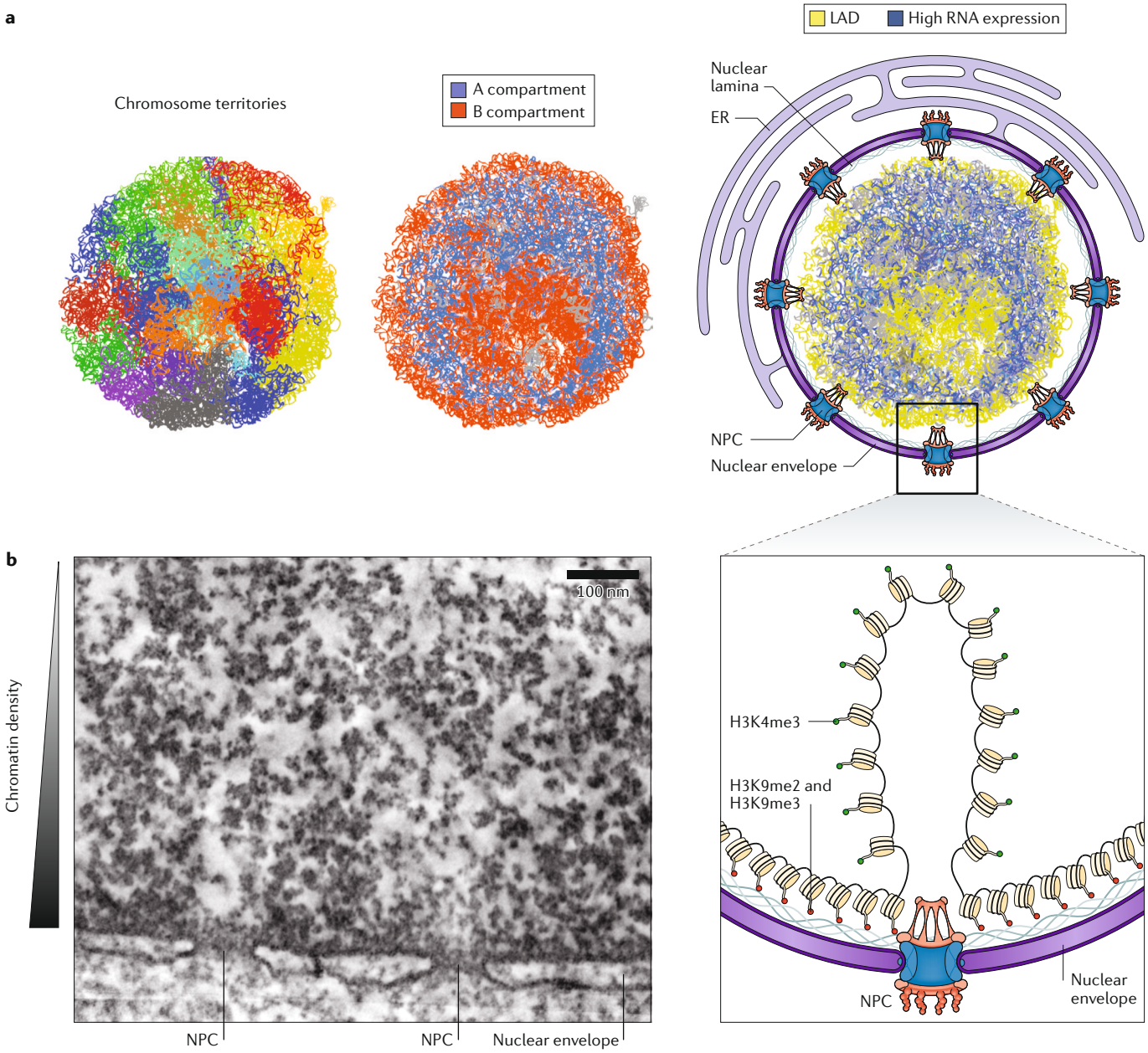

Fig. 1 | **Chromatin organization within the nucleus. a** | Cross-sections through 3D models of genome structures generated from single cell Hi-C, coloured according to individual chromosomes (left panel), whether the sequence is in the A or B compartment (middle) and whether the sequence is part of a lamina-associated domain (LAD) (yellow) or contains highly expressed genes (blue) (right). **b** | ChromEMT (chromatin electron microscopy tomography) depicts the organization of chromatin within the nucleus. Chromatin density is greatest at the nuclear periphery and decreases within the nuclear interior, with the prominent exception of decondensed euchromatin underneath nuclear pore complexes (NPCs). Gene-rich euchromatin that is transcriptionally active and enriched in histone 3 lysine 4 trimethylation (H3K4me3) marks (green) is located within the nucleoplasm, and gene-poor heterochromatin that is transcriptionally repressed and enriched for H3K9 dimethylation (H3K9me2) and H3K9me3 marks (red) is located at the nuclear periphery. ER, endoplasmic reticulum. Part **a** adapted from REF.[10], Springer Nature Limited. EM image in part **b** adapted with permission from REF.[11], Ou, H. D. et al. ChromEMT: visualizing 3D chromatin structure and compaction in interphase and mitotic cells. *Science* **357**, eaag0025 (2017) https://doi.org/10.1126/science.aag0025. Reprinted with permission from AAAS.

X chromosome inactivation
Transcriptional silencing of one X chromosome at random in XX female cells for dosage compensation between XX females and XY males.

landmarks such as the nuclear periphery[13]. This non-random positioning has important effects on genome activity and function. For example, in female mammals, X chromosome inactivation is accompanied by positioning of the inactive X close to the nuclear lamina[14], and repression of genes encoded by the inactive X requires the expression of the lamina-associated protein lamin B receptor (LBR), which interacts with the *Xist* RNA[15]. Cell type-specific positioning of specific gene loci has also been observed. For example, inactive immunoglobulin loci are positioned near the nuclear periphery in haematopoietic progenitor cells but within the nuclear

Box 1 | **Nuclear organization in pluripotency and reprogramming**

When compared with differentiated cells, the genome of pluripotent cells exists in a comparatively malleable state with low levels of heterochromatin[152] and frequent long-range interactions[153]. Chromatin is relaxed and deformable, with genomic loci capable of exploring large volumes within the nucleus[154].

By contrast, differentiated cell types follow lineage-restricted gene expression programmes that are promoted by cell type-specific chromatin modifications and more stable chromatin organization. This organization must be deconstructed in order to effectively reprogramme a differentiated cell to pluripotency. Reprogramming is extremely inefficient, suggesting that formidable barriers exist[155]. Such chromatin barriers seem to include DNA methylation[156], histone modifications[157], and positioning and composition of nucleosomes[158].

The composition of major nuclear structures including the nuclear pore complex (NPC)[45], the nuclear lamina[82] and the nuclear envelope[7] is also distinct in pluripotent cells, and the need to remodel nuclear structures may also limit reprogramming efficiency. For example, lamin A expression is abnormally high in non-viable embryos generated from nuclear reprogramming[159]. Reprogramming involves cell division and allows a differentiated cell to be transformed into almost any other cell type by passage through a pluripotent state. By contrast, transdifferentiation involves the direct transformation of one cell type into another; for example, fibroblasts can be converted into neuronal[141,160] and muscular[161] cell types. Because this process does not involve cell division, it is likely that nuclear reorganization is more limited. Further investigation into the effects of reprogramming and transdifferentiation on nuclear organization is likely to yield further insight into the mechanisms that control genome activity, as well as the means to more effectively manipulate cell identity.

interior in differentiated B cell progeny. This movement into the nuclear interior is accompanied by gene activation[16]. These examples highlight the relationship between genome organization and cellular function and indicate that cellular differentiation is often accompanied by genome reorganization[17]. Intriguingly, to revert differentiated cells to a pluripotent state, genome reorganization must also occur (BOX 1).

Nuclear organization after mitosis

In order for cells to maintain nonrandom nuclear organization, mechanisms must exist to re-establish organization after cell division. The nuclear periphery disassembles in mitotic prophase and reassembles during anaphase, telophase and early G1 phase (reviewed in detail in REF.[18]). Meanwhile, chromatin compacts into discrete mitotic chromosomes to enable the faithful separation of a complete genome into each daughter nucleus. Nevertheless, cell type-specific features of gene positioning and gene expression are maintained through cell division[19], implying that mechanisms for 'remembering' chromatin organization through cell division exist.

In recent years, some of the supporting roles that proteins of the nuclear periphery play in re-establishing this organization have been illuminated. For example, in anaphase, the DNA crosslinking protein barrier-to-autointegration factor (BAF) coats and binds chromosomes into a single chromatin mass that serves as a surface for nuclear envelope assembly[20]. Affinity for BAF recruits nuclear envelope proteins[21] and proteins of the nuclear lamina to the chromatin surface (reviewed in REF.[18]). In parallel, several components of the NPC, the nucleoporin proteins (NUPs), bind to histones and act as seeding sites for future NPC assembly[22,23]. The transcriptional repressor heterochromatin protein 1 (HP1) and associated proteins, such as proline-rich protein 14 (PRR14)[24] and LBR[25], bridge chromatin interactions with the nascent nuclear lamina.

It remains unclear whether structures of the nuclear periphery exhibit any selectivity in binding to specific genomic regions during nuclear assembly. However, the same genomic regions tend to re-establish contact with the nuclear periphery after mitosis[26]. Furthermore, single-cell HiC analyses indicate that chromatin decondenses anisotropically as cells exit mitosis. The euchromatic A compartment decondenses to a greater extent than the gene-poor, heterochromatic B compartment, and the A compartment adopts a central position whereas the B compartment moves towards the nuclear periphery and perinucleolar regions[27]. Chromatin mobility progressively decreases as cells proceed towards interphase[28]. Repressive chromatin marks, in particular histone 3 lysine 9 dimethylation and trimethylation (H3K9me2 and H3K9me3, respectively), are specifically enriched beneath the nuclear lamina by the localized actions of histone deacetylases and methylases, which are in turn recruited to the nuclear lamina through protein–protein interactions[29,30]. These types of protein–chromatin and chromatin–chromatin interactions may help to maintain the legacy of cell type-specific gene expression through cell divisions or over long periods of time in non-dividing cells.

Temporal and physical constraints

Reorganizing the genome is usually not a rapid process. Specific DNA sequences have been identified that possess an intrinsic ability to localize to particular nuclear regions and can direct the positioning of other genomic loci when introduced in *trans*. However, this re-positioning requires passage through mitosis[26,31]. Similarly, it may be that haematopoietic progenitor cells can undergo rapid and dramatic changes to genome organization as they differentiate into B cells because of their rapid proliferation rate[16]. However, there are examples of chromatin reorganization in the absence of cell division. The terminal differentiation of rod photoreceptor cells in the mouse retina involves complete inversion of the relative positions of euchromatic and heterochromatic domains over the course of several weeks as peripheral tethers for heterochromatin are lost[32]. On a faster timescale and smaller physical scale, damaged telomeres can explore large volumes of the nucleus over minutes to hours[33], although whether DNA lesions elsewhere in the genome also exhibit increased mobility remains controversial[34,35]. Movement of a chromatin locus is profoundly affected by the level of compaction of its immediate chromatin environment[36], and loci embedded within heterochromatin are generally less mobile than loci positioned within euchromatin[37]. In fact, drug-induced or transcription-induced decompaction of chromatin can promote the movement of genomic loci within the nucleus[38]. Decatenation of chromatin by topoisomerases may help to resolve tangled chromatin and promote movement or reorganization of chromatin domains[39]. As we discuss in subsequent sections, association of chromatin with the nuclear periphery generally serves as a stable anchor for genome organization rather than as a platform for the dynamic movement of loci.

Telomeres
Repetitive sequences found at the ends of chromosomes for maintenance of genomic integrity.

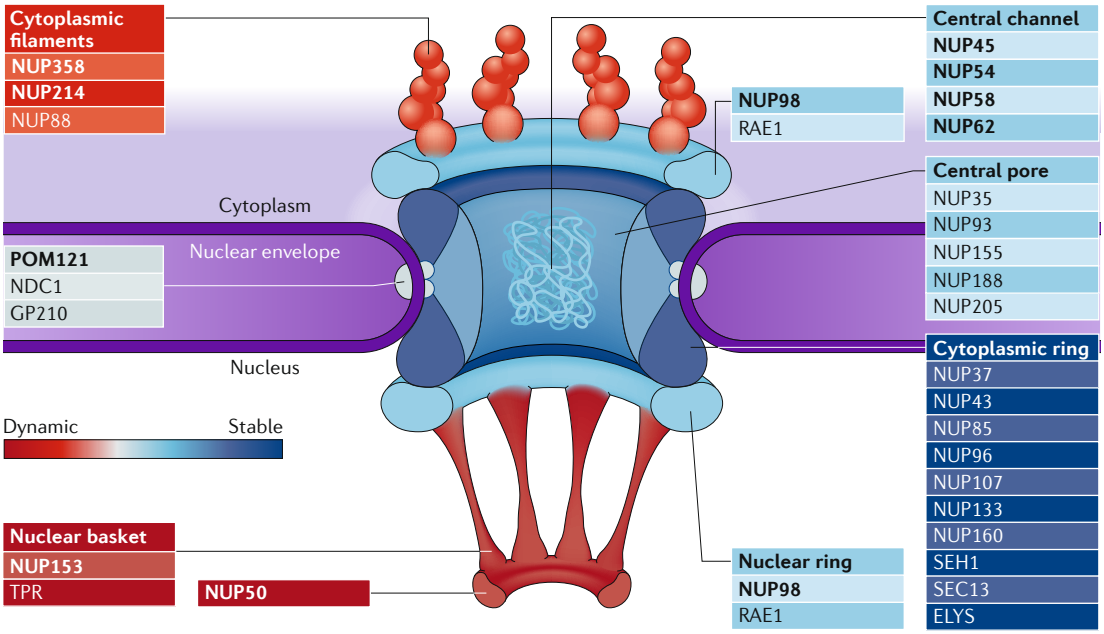

Fig. 2 | **NPC structure and dynamics.** The nuclear pore complex (NPC) is the major gateway for transport between the nucleus and the cytoplasm. Transport through NPCs involves the nucleoporin proteins (NUPs), the GTPase Ran, importin and exportin transport receptors and specialized factors that promote transport of large protein and RNA complexes (not shown). Whereas core structural elements of the NPC are very stable, other NUPs shuttle dynamically on and off the NPC[40]. Average NUP dynamics are represented for each subcomplex on the basis of their residence times at NPCs[49]; subcomplexes range from highly dynamic (red) to fairly stable (blue). Individual NUPs within the NPC are labelled, and NUPs in bold contain repeat domains rich in phenylalanine and glycine amino acids (FG repeats). GP210, nuclear pore membrane glycoprotein 210; POM121, nuclear envelope pore membrane protein POM121; RAE1, ribonucleic acid export 1.

Gene regulation by the nuclear periphery

The proteins and structures of the nuclear periphery actively participate in facilitating the organization of the genome within the nucleus. The following sections of this Review focus on the roles that the NPCs and the nuclear lamina play in regulating the genome.

Nucleoporins regulate gene expression

NPC structure and function. The NPC is the major gateway for transport between the nucleus and the cytoplasm (FIG. 2). The mammalian NPC is a modular assembly of ~30 distinct NUPs that form ~120 MDa structures (reviewed in REF.[9]). NUPs can be functionally divided into two classes (FIG. 2). The first class of NUPs represents the structural framework of the NPC and is not directly involved in nuclear transport. Many of these structural NUPs are very stable within the NPC assembly[40], and a subset of these essentially do not turn over for the entire lifespan of a cell[41,42]. The second class of NUPs contain unstructured domains rich in phenylalanine and glycine amino acids (FG repeats) that function directly in nuclear transport by binding soluble transport receptors. The conformational flexibility of the FG-repeat NUPs is key to their role in nuclear transport[43] but may also allow these proteins to conform to a wide array of binding partners and mediate other functions. Importantly, many of these NUPs shuttle dynamically on and off the NPC[40].

Evolution and specialization of the NPC. Although the overall structure of the NPC is highly conserved, the physical size of the NPC has roughly doubled from yeast to humans[9], and NUP genes are among the fastest-evolving genes in the genome[44]. Recent work suggests that the functional repertoire of the NPC has expanded in mammals, as in many cases the depletion of specific NUPs does not have an impact on nucleocytoplasmic transport but does have tissue-specific effects on development and differentiation. For example, the NPC protein NUP133 is dispensable for embryonic stem (ES) cell proliferation but is essential for differentiation into neural lineages[45]. By contrast, high expression of NUP153 in ES cells[46] and in neural progenitor cells[47] promotes pluripotency, and NUP153 downregulation accompanies differentiation. Furthermore, NUP50 (REF.[48]) and NUP210 (REF.[49]) are required for muscle differentiation but are dispensable in proliferating myoblasts. These and other examples of tissue-specific requirements for NUPs are suggestive of transport-independent functions. In this section, we review recent work that indicates that NUPs also have the ability to regulate the genome.

NPC–genome contacts regulate gene expression at the nuclear periphery. Early studies in yeast demonstrated that NPCs bind to the genome[50,51] and that gene tethering to the NPC promotes both the activation of genes in response to stimulus[52,53] and the stable repression of ribosomal and subtelomeric genes[54]. Elegant work in yeast has determined that Nup100, which is homologous to human NUP98, is required for tethering activated genes to the NPC[55]. Transcription factors and chromatin-modifying enzymes also cooperate to promote tethering of activated genes to the yeast NPC[56].

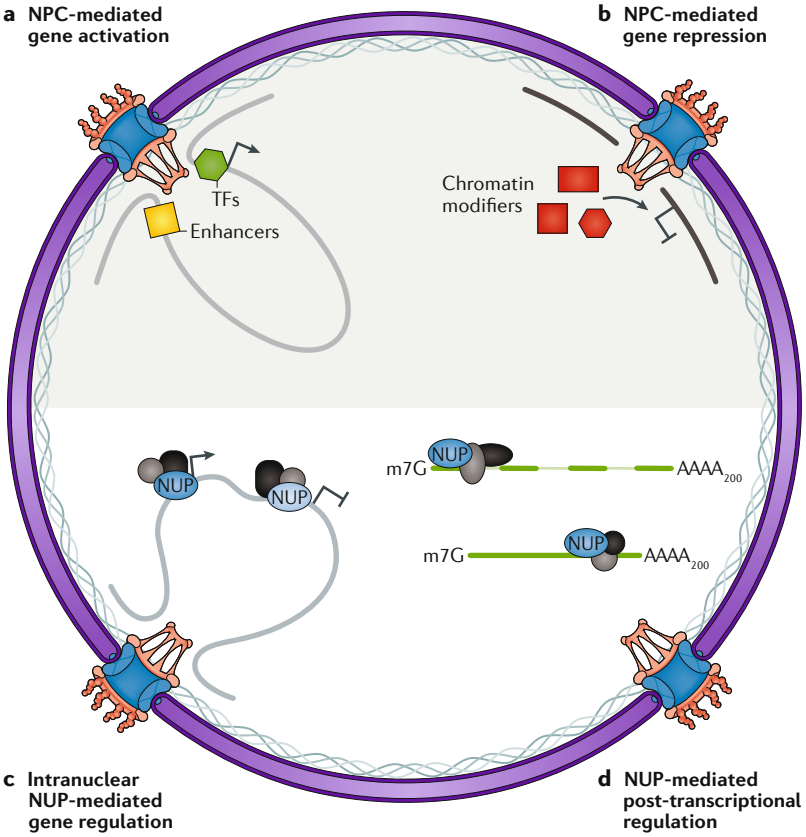

Fig. 3 | **NPC-mediated gene regulation. a** | Nuclear pore complexes (NPCs) bind enhancers at the nuclear periphery to facilitate enhancer–promoter looping for gene activation. NPCs also recruit specific transcription factors (TFs) to the nuclear periphery to control gene profiles involved in cell fate. **b** | NPCs recruit chromatin modifiers to the nuclear periphery to mediate gene repression at specific genomic loci, including ribosomal, subtelomeric and early differentiation genes. **c** | Nucleoporin proteins (NUPs) bind the genome within the nucleus and interact with an array of proteins, including histone modifiers and RNA helicases, to regulate gene expression. **d** | NUPs can regulate gene expression through post-transcriptional processes, such as mRNA splicing and stability. ATP-dependent RNA helicase A (DHX9) has been shown to facilitate NUP-mediated mRNA splicing, but other proteins involved in these processes still remain to be identified. m7G, 7-methylguanosine.

Chromatin immunoprecipitation followed by sequencing (ChIP–seq). An assay that combines chromatin immunoprecipitation with DNA sequencing to identify protein–DNA interactions within the genome.

DNA adenine methyltransferase identification (DamID). An assay that fuses *Escherichia coli* DNA adenine methyltransferase with a protein of interest to induce adenine methylation in proximity to the fusion protein. Adenine-methylated DNA fragments represent protein-binding sites within the genome.

Work in metazoan systems also implicates NPCs in mediating both gene activation and repression. For example, in pluripotent cells, NUP153 binds to both transcription start sites[46,47] and termination sites[47], promoting silencing of some targets and activation of others. During muscle differentiation, NUP210 scaffolds the transcription factor myocyte-specific enhancer factor 2C (MEF2C) at the NPC, promoting activation of its target genes[57].

Furthermore, NPCs contribute to higher-order chromatin organization in metazoans. As mentioned above, dense heterochromatin is a prominent feature of the nuclear periphery, whereas regions of less-compact euchromatin underlie NPCs[12] (FIG. 1). The formation of these heterochromatin exclusion zones beneath NPCs has been shown to depend on the NPC component nucleoprotein TPR[58], suggesting that NPCs actively maintain a domain of euchromatin at the nuclear periphery. In *Drosophila melanogaster*, NUP98 mediates enhancer–promoter looping of poised inducible genes at the NPC[59]. This arrangement potentiates transcriptional activation in response to stimulus by the hormone ecdysone[59]. In human cells, NPCs bind to super-enhancers, which are regulatory structures that drive the expression of key genes that specify cell identity[60]. These NUP-associated super-enhancers localize to the nuclear periphery, and ablation of NPC tethering results in super-enhancer disruption and transcriptional dysregulation. Super-enhancers bound and regulated by NPCs vary across cell types, but in each context they regulate groups of genes involved in defining cellular identity[60], suggesting that NPC association with these regions could be important for maintaining cellular fate.

It remains unclear what factors dictate whether NPC–genome interactions activate or repress transcription, but analyses of genome binding by chromatin immunoprecipitation followed by sequencing (ChIP–seq) and DNA adenine methyltransferase identification (DamID) both indicate that NUPs show affinity for distinct regions of chromatin[50]. This observation suggests that mechanisms exist to specialize the interaction of individual NUPs and/or NPCs with the genome. Given that most NUPs have no demonstrated ability to bind directly to DNA or to chromatin, NUP-associated transcription factors[47,56,57], chromatin-modifying complexes[46] and other unidentified factors likely mediate the distinct chromatin-binding preferences of NUPs.

Nucleoporins regulate gene expression within the nucleus. In higher eukaryotes, individual NUPs have acquired spatially and functionally distinct roles in different nuclear domains (FIG. 3). In principle, any NUP that associates dynamically with the NPC[40] could take on additional roles within the nucleoplasm. Many NUP-bound loci are found within the nuclear volume when visualized by fluorescence in situ hybridization (FISH), indicating that these interactions can occur independently of the NPC. For example, NUP98 binds genes both at the NPC and within the nucleoplasm[61,62], and association within the nucleoplasm correlates with high levels of target gene activation[62]. Similarly, NUP153 associates with genes both within the nucleus and at the NPC[46,60], although whether gene binding has distinct effects on gene activity in each of these domains is less clear. Other NPC components, including NUP50, NUP88, SEC13 and TPR, also bind to the genome within the nuclear interior[48,61,63,64]. These interactions regulate the expression of genes involved in differentiation, development, cell cycle progression and the antiviral response[61,63–65]. Recent advances in our understanding of the mechanics of NUP-mediated transcriptional regulation within the nucleus have particularly focused on NUP98.

NUP98 binding on the genome coincides with marks of transcriptional activity, including RNA polymerase II association, nuclease accessibility and H3K4me3 (REFS[59,61,62,66]). NUP98 binding is enriched at transcription start sites, at times to an extent comparable to known transcription factors[66], and the depletion of NUP98 potently downregulates target gene expression[61,66], suggesting that NUP98 functions as a transcriptional co-activator. NUP98 can also maintain

Fluorescence in situ hybridization
(FISH). A fluorescence microscopy technique used to visualize specific genomic regions within the nucleus using fluorescently tagged DNA probes designed to hybridize to the region of interest.

Transcriptional memory
The state in which genes are poised for rapid transcriptional reactivation after an initial stimulus.

previously expressed genes in a poised state in order to amplify expression in response to future stimuli (such as a nutrient or hormone), a phenomenon known as transcriptional memory. This transcriptional memory has been observed both at the NPC[59] and within the nucleoplasm[67].

However, NUP98 has no demonstrated ability to bind to DNA[68], implying that another factor (or factors) recruits it to the genes that it regulates. In *D. melanogaster*, enzymes that catalyse transcription-promoting chromatin modifications have been implicated in recruiting NUP98 to chromatin. Specifically, the binding of NUP98 to chromatin requires the Non-Specific Lethal (NSL) complex, which mediates H4K16 acetylation[69], a chromatin mark that antagonizes chromatin compaction[70]. Chromatin-bound NUP98 can then promote the expression of genes targeted by the H3K4 histone-lysine *N*-methyltransferase trithorax[69]. Similarly, in mammalian cells, NUP98 binds to the histone-lysine *N*-methyltransferases SETD1A and SETD1B and promotes the deposition of H3K4me3 (REF.[66]). This interaction is subverted in leukaemias caused by translocations of the *NUP98* gene[71]. These fusions lack the NPC-targeting carboxyterminal domain of NUP98 but retain the ability to interact with chromatin modifiers[72]. Emerging evidence indicates that NUP98 fusions drive leukaemia by hyperactivating genes that promote sustained proliferation of haematopoietic progenitors[73]. These findings highlight broad interdependencies between NUP98, chromatin modifiers and gene activation that are only just beginning to be understood.

The roles of NUPs in gene regulation have further expanded with the recent evolution of a hominoid-specific NUP variant, soluble nuclear envelope pore membrane protein POM121 (sPOM121), which cooperates with NUP98 to bind the genome in human cells[74]. Importantly, sPOM121 lacks an NPC-anchoring domain and is instead found exclusively within the nucleoplasm. sPOM121 recruits the NUP107–160 complex and potentially other factors to NUP98 genome binding sites. As sPOM121 expression is readily detectable in every human tissue, in many cases at levels surpassing full-length POM121 (REF.[74]), sPOM121 could have a major role in NUP-mediated gene regulation.

Post-transcriptional regulation of gene expression by NUPs. Gene expression is initially driven by transcription, yet there are many regulatory processes between the transcription of nascent RNA and mRNA translation that can modulate gene expression[75]. Evidence that transcription, splicing and RNA export are coupled at the NPC provided early credence to the idea that NUPs also regulate gene expression after transcription[17,76]. Roles for NUP98 have recently been uncovered in mRNA splicing and stability. For example, NUP98 interacts with the ATP-dependent RNA helicase A (DHX9) and co-binds to specific mRNA transcripts to regulate their splicing[77]. Moreover, NUP98 seems to protect specific p53-induced transcripts from degradation by the exosome[78]. It remains unclear whether NUP98 can bind directly to mRNAs for post-transcriptional gene regulation or whether NUP98 works in concert with other proteins to regulate this process. However, these discoveries highlight the increased functionality of NUPs beyond transport and transcription regulation.

Although several NUPs have been functionally linked to gene regulation, many of these NUPs exist as stable subcomplexes within the context of the NPC (FIG. 2), and it remains unclear which cohort of binding partners specific NUPs might be associated with when binding the genome. For example, NUP153 binds to NUP50 and the transport factor importin-α to function in nuclear transport[79], but interdependencies between NUP50 and NUP153 in gene regulation have not been defined. Similarly, NUP98 and ribonucleic acid export 1 (RAE1) function together to promote mRNA export[80], but it remains unclear whether they cooperate in gene regulation. The ability of additional NUPs to dynamically dissociate from the NPC and associate with chromatin remains to be explored. One potentially interesting candidate is ELYS, which may be unique among NUPs in its ability to bind directly to nucleosomal DNA in vitro[22]. Overall, many open questions about the extent and mechanism of gene regulation by NPCs and by individual nucleoporins remain.

Gene regulation by lamins

The nuclear lamina, which underlies the nuclear envelope and is interspersed with NPCs (FIG. 1), is composed of four proteins in mammalian somatic cells: the A-type lamins, lamin A and lamin C, and the B-type lamins, lamin B1 and lamin B2 (REF.[8]). Lamins assemble into nonpolar, bundled filaments that provide support against strain and that scaffold the genome[8,81]. A-type lamins are expressed at low levels in pluripotent cells and at higher levels in differentiated tissues, whereas at least one B-type lamin is expressed in every cell type[82,83]. The nuclear lamina functions in close concert with a cohort of associated proteins, including proteins of the nuclear envelope and non-membrane-bound nuclear proteins. Many nuclear lamina-associated proteins are expressed only in selected tissues, perhaps contributing to specialized functions of this structure[7]. The nuclear lamina is essential for organogenesis, as *Lmnb1*-null and *Lmnb2*-null mice each die at birth with defects in brain, lung and diaphragm development[84,85], whereas *Lmna*-null mice exhibit cardiac and muscular defects and die within days of birth[86]. Mutations to the lamin genes, most prominently to *LMNA*, cause at least 15 distinct diseases, which are known as laminopathies (reviewed in REF.[87]). This Review focuses on the functions of the nuclear lamina in gene regulation.

Lamina association confers gene repression. A dense layer of heterochromatin underneath the nuclear lamina is a prominent feature of the nuclear periphery in most cells[11] (FIG. 1). DamID[88] with lamin B1 fusion proteins has been extensively employed to identify the regions of the genome that are in close proximity to the nuclear lamina[26,89,90]. These lamina-associated domains (LADs), which exhibit a median size of 500 kb (REF.[86]), exhibit heterochromatic features, including low gene density, low transcriptional activity and late replication timing[89].

In mammalian cells, up to one-third of the genome and 10% of coding genes are found within LADs[91,92]. They are enriched with repressive histone modifications, including H3K9me2, H3K9me3 (REFS[29,89]) and H3K27me3 (REFS[89,93]), and are sparsely populated with active chromatin marks such as H3K4me[89]. In addition to gene-poor LADs that are consistent across tissues, a subset of LADs known as variable LADs (vLADs) contain lineage-specific genes[90]. Genes found in vLADs are often irrelevant to the functions of the cell type in which they are found[91,94], suggesting that vLADs maintain cell identity by restricting gene expression.

Do LADs represent an intrinsically repressive nuclear domain, or do they result from gene inactivity? When a reporter gene is inserted within a LAD, its expression is lower than the same reporter residing in the nuclear interior[95,96]. Sequences derived from LADs can home to the lamina and undergo transcriptional repression when randomly integrated into the genome[26,93], suggesting that this process is actively mediated. Finally, changes to genome–lamina association usually correlate with changes in gene expression during differentiation[91]. Taken together, these findings indicate that the residence of genomic regions within LADs correlates with lower gene activity. By analysing the features of genomic regions in close proximity to the lamina, the mechanisms through which LADs form and mediate gene silencing have begun to emerge.

Cooperation of chromatin readers, writers and tethers at the nuclear lamina. Much of what we know about LADs has been defined by analysing the genomic regions in close proximity to lamin B1 (REFS[89,91,97]). How do different lamin isoforms contribute to LAD organization? Lamin isoforms generally exhibit very similar genome binding profiles[98], although the A-type lamins also interact with euchromatin within the nucleoplasm[99]. By moving between binding sites within the nucleus and at the nuclear lamina, A-type lamins could potentially modulate LAD organization[100]. Intriguingly, depletion of A-type lamins is sufficient to disrupt LAD tethering in differentiated cells, although these cells also express B-type lamins[93,101]. It remains unclear whether A-type lamins can directly mediate LAD interactions or whether the disruption of A-type lamins displaces associated proteins that also participate in LAD tethering.

Although there is some evidence that lamins can bind directly to chromatin[102,103], nuclear lamina-associated proteins also contribute to LAD assembly. For example, LBR, which is localized to the inner membrane of the nuclear envelope, associates with lamin B1 (REF.[104]) and exhibits a very similar genome binding profile to lamin B1 (REF.[60]). Given that LBR also binds to H3K9me2-modified and H3K9me3-modified chromatin via HP1 (REF.[105]) and to H4K20me2-modified chromatin through its Tudor domain[106], this protein could function as a chromatin 'reader' at the nuclear lamina. The ability of LBR to bridge interactions between the lamina and chromatin has clear functional importance as it is required to tether heterochromatin to the nuclear periphery in developing tissues[25]. LBR and lamin A/C trade this role through development[107,108]; developing tissues rely on LBR for peripheral heterochromatin tethering, whereas differentiated tissues seem to rely on lamin A/C[25]. It may be that a lamin A/C-associated protein mediates heterochromatin tethering in differentiated tissues, but such a functional partner remains to be identified.

Another group of proteins that participate in LAD organization are the LEM-domain family of nuclear envelope proteins, named for the founding members lamina-associated polypeptide 2 (LAP2), emerin, and MAN1 inner nuclear membrane protein[109]. LEM domains interact with the DNA cross-bridging protein BAF[109], which brings these nuclear envelope proteins into close contact with chromatin. Emerin tethers muscle differentiation genes to the nuclear periphery, helping to maintain muscle progenitors[110,111], whereas LAP2β limits the differentiation capacity of cardiac muscle progenitors by a similar mechanism[26]. A number of other, less well-characterized nuclear envelope proteins, such as nuclear envelope transmembrane protein 23 (NET23), NET39, transmembrane protein 38A (TMEM38A) and wolframin (WFS1), seem to have supporting roles in genome organization across differentiated tissues[112,113].

Chromatin reading and editing enzymes cooperate with nuclear envelope protein tethers to maintain LADs. LAP2β and emerin recruit the histone deacetylase HDAC3 to the nuclear lamina to mediate gene repression in progenitor cells[30,114]. Disruption of histone-lysine *N*-methyltransferases that deposit H3K9me2 (EHMT2; also known as G9a)[115], H3K9me3 (SUV39H1)[116] or H3K27me3 (EZH2)[93] moderately affects LAD gene positioning and activity. Furthermore, pharmacological inhibition of histone deacetylation, H3K9me2 or H3K27me3 causes striking decompaction of LADs[101], suggesting that chromatin state promotes the tight clustering of repressed LAD domains in relation to each other as well as their close association to the lamina. When spread over the kilobase to megabase scales of an entire LAD, these interactions may cooperatively promote chromatin compaction and reinforce gene repression. Consistent with this idea, LAD regions of a chromosome cluster tightly together at the nuclear periphery of individual cells, whereas non-LAD regions on the same chromosome are more diffuse[101]. This compaction may limit transcription factor access and decrease the likelihood that a LAD resident gene will be expressed[100] (FIG. 4). The recently described ability of heterochromatin domains to exist in a separate phase suggests an alternative means for LAD regions to coalesce and limit transcription factor access[117,118]. Overall, nuclear lamina anchoring, reinforcement of repressive chromatin marks and compaction of LAD regions cooperate to stably maintain cell type-specific gene expression profiles.

The reach of lamins extends within the nucleus. Although the nuclear lamina is a prominent feature of the nuclear periphery, the A-type lamins are also found within the nuclear volume[99]. Whereas the B-type lamins preferentially associate with heterochromatin at the nuclear periphery, the A-type lamins bind to both heterochromatic and euchromatic regions[99,119]. The abundance of A-type lamins within the nucleoplasm is tuned by a variety of factors, including the expression levels

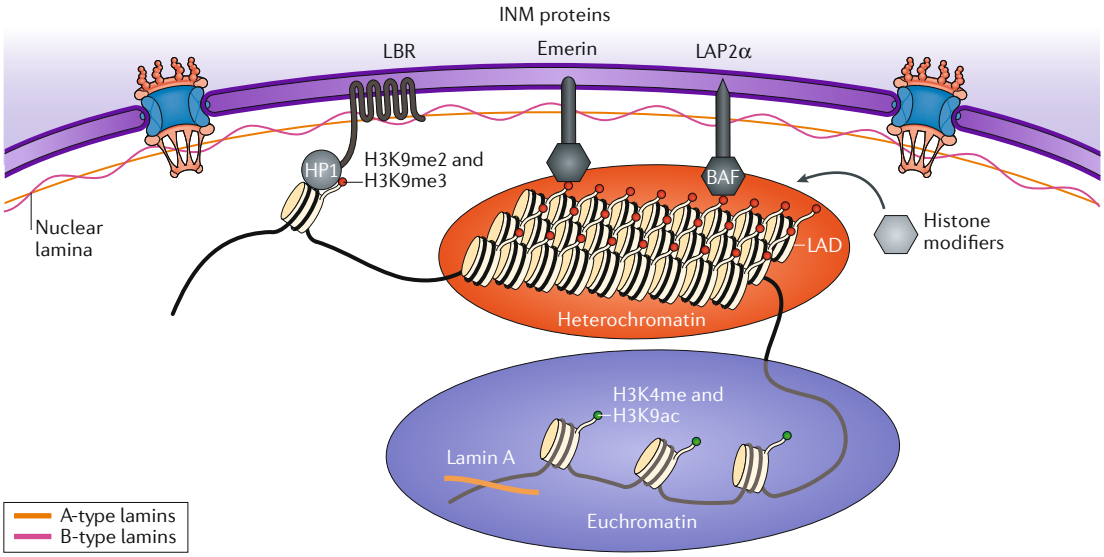

Fig. 4 | **Nuclear lamina-mediated genome organization.** The nuclear lamina underlies the inner nuclear membrane (INM) and is connected to the genome through lamin-associated proteins. Lamin-associated domains (LADs) are large, gene-poor regions of heterochromatin that interact with the lamina at the nuclear periphery and are often enriched in repressive histone modifications, such as histone 3 lysine 9 methylation (H3K9me) and histone 3 lysine 27 acetylation (H3K27ac) (red). Histone modifiers and transcriptional regulators have been suggested to cooperate with INM proteins to maintain LADs. A-type lamins, in association with lamina-associated polypeptide 2α (LAP2α), can also interact with regions of euchromatin within the nucleus that are enriched in active histone modifications, including H3K4me and H3K9ac (green). BAF, barrier-to-autointegration factor; H3K9me2, H3K9 dimethylation; H3K9me3, H3K9 trimethylation; HP1, heterochromatin protein 1; LBR, lamin B receptor.

of a nucleoplasmic binding partner, LAP2α[99,120], and phosphorylation events[121]. Depleting cells of LAP2α, and thus diminishing the levels of nucleoplasmic lamin A, allows derepression of some lamin-bound genes[99]. Nucleoplasmic lamins seem to limit the mobility of chromatin within the nuclear interior[122] and exert anti-proliferative and pro-differentiation effects[120,123].

Some disease-causative mutations deplete the nucleoplasmic pool of lamin A. For example, a dominant negative progeria-linked mutation causes lamin A to be constitutively lipid modified and associated with the nuclear envelope, which depletes A-type lamins from the nucleoplasm[124]. This nucleoplasmic depletion of A type lamins is accompanied by global disorganization of heterochromatin marks[125,126] and derepression of some genomic regions, including satellite DNA[125] and ribosomal DNA[127]. Because ribosomal DNA transcription controls the morphology, activity and composition of the nucleolus[128], deregulation upon lamin A disruption also affects the function and organization of this nuclear compartment[127]. Overall, nucleoplasmic lamins seem to perform important functions in regulating the activity of the genome and the organization of the nucleus that remain incompletely understood.

The ageing nucleus

Once nuclear organization is established, how is it maintained over time as cells age? The fundamental defining feature of ageing is a decline in the functional capacity of organs, which in turn results from incremental impairments in cellular homeostasis. Several hallmarks of ageing converge on the nucleus, and mutations to nuclear proteins cause premature ageing disorders[129], highlighting the nucleus as a weak point in cellular ageing. Nuclear ageing includes changes to the genome itself as well as to the protein structures that enclose and organize it. The ageing genome is subject to telomere shortening, disruptions to epigenetic modifications, increased transcriptional noise and accumulation of DNA damage[129]. Separately, declining protein homeostasis contributes both generally to cellular ageing and specifically to functional declines in nuclear structures such as the NPC[130]. The lifetime of the nucleus depends on the extent of cellular turnover in a tissue, and nuclei encounter distinct age-associated disruptions in rapidly proliferating cells (FIG. 5a) versus long-lived cells (FIG. 5b).

Nuclear remodelling in senescent cells

The function of many epithelial tissues depends on regular renewal. Nuclei in such proliferative tissues encounter challenges including progressive shortening of telomeres and accumulation of DNA damage and mutations[129], all of which may push cells towards the irreversible cell cycle arrest known as senescence. Although senescence is an important brake against tumorigenesis, senescent cells accumulate in ageing tissues and exert negative paracrine effects by secreting pro-inflammatory factors[131]. Senescence onset is accompanied by widespread changes to nuclear organization (FIG. 5a). Lamin B1 is degraded by autophagy[132,133], and peripheral lamin-associated heterochromatin becomes much less prominent[134,135]. Senescent cells exhibit dysregulated gene expression and derepression of repetitive elements[136]. Depletion of lamin B1 can drive cells into senescence prematurely[132], which

Satellite DNA
Highly repetitive non-coding sequences within heterochromatic regions of the genome.

Nucleolus
A region within the nucleus where ribosomal RNA transcription, processing and assembly occur.

Senescence
The cellular state in which cells permanently exit the cell cycle but do not undergo cell death.

Autophagy
A process in which cellular material is recycled following degradation by the lysosome or vacuole.

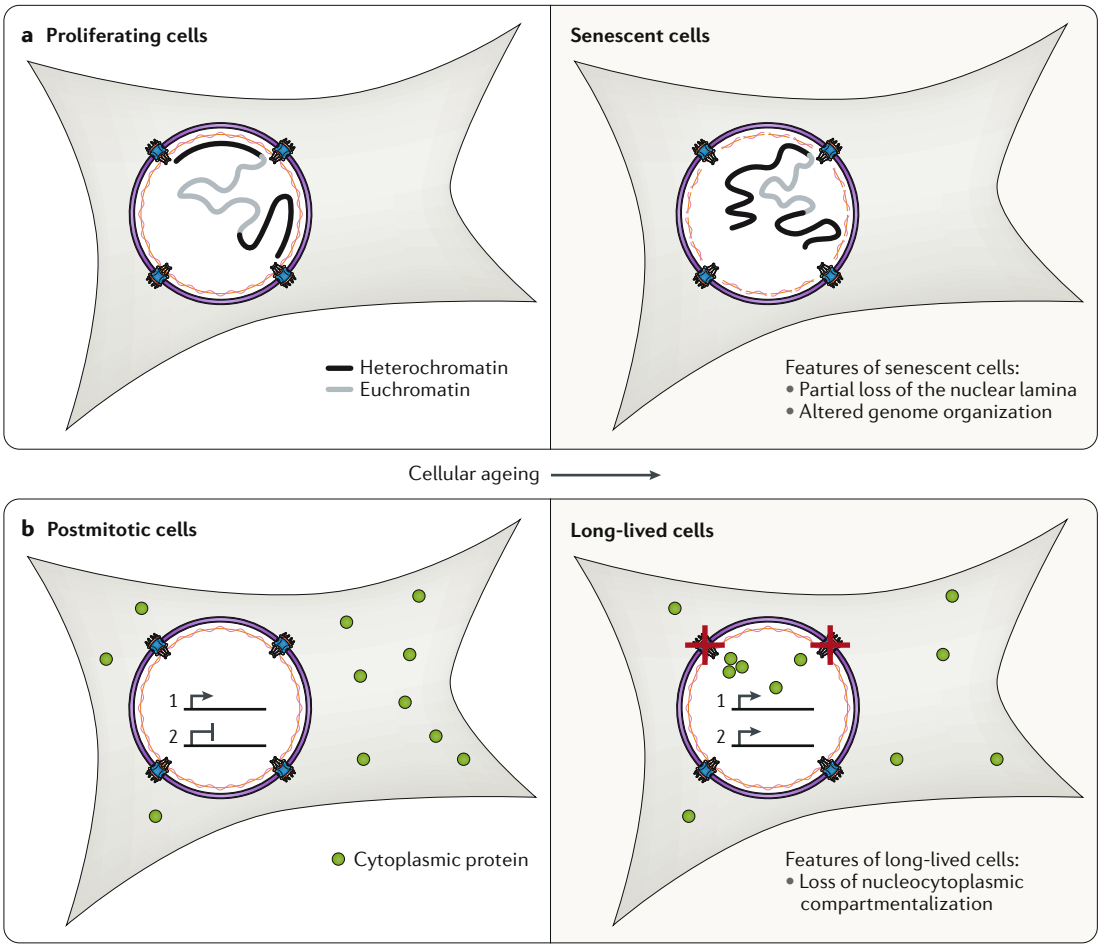

Fig. 5 | **Nuclear decline over cellular ageing. a** | Proliferating cells eventually exit the cell cycle and senesce as cellular ageing progresses. These senescent cells exhibit a disrupted lamina meshwork and altered chromatin organization. **b** | Postmitotic cells do not divide and must maintain their function over cellular ageing. However, the identification of proteins that undergo limited turnover in non-dividing cells suggests that these proteins accumulate damage that leads to nuclear decline. Two prominent characteristics of long-lived postmitotic cells are the loss of nucleocytoplasmic compartmentalization and increased transcriptional noise.

suggests that remodelling of genome–lamina contacts is an important event in senescence onset. Strikingly, the lamina can be so weakened in senescent cells that it no longer adequately protects the genome; naked chromatin fragments appear in the cytosol of senescent cells[137], where they promote the pro-inflammatory signalling that is characteristic of senescent cells by diverting innate immune response factors[138].

Nuclear decline in long-lived cell types

Although the cells of many tissues in the body are periodically renewed, organs including the heart[139] and brain[140] contain substantial numbers of cells that are born in early development and face a demand for lifelong performance. In such long-lived cell types, nuclear organization must be maintained for decades. Maintenance of the nuclear permeability barrier declines as neurons age, allowing cytoplasmic proteins such as tubulin to enter and accumulate within the nucleus[49,141] (FIG. 5b). Furthermore, nuclear proteins represent over half of the small cohort of proteins that are stable in neurons for years[42,142]. Over the lifetime of a cell, the accumulation of aberrant modifications to proteins can perturb critical processes. For example, eye lens crystallin is a long-lived protein that misfolds as a result of protein damage over time, causing cataracts[130]. It is similarly possible that long-lived histones, NUPs and lamins are vulnerable to damage, misfolding or aggregation and thus may underlie declines in nuclear integrity with age.

Recent reports indicate that declining nuclear integrity is accelerated in neurodegenerative disorders, including Huntington disease[143] and amyotrophic lateral sclerosis (ALS)[144]. Neurodegeneration-linked polypeptides seem to exert toxic effects on nucleocytoplasmic transport by sequestering the FG-repeat NUPs, including NUP98, that line the NPC channel[145,146]. Given the important roles of NUP98 in mediating gene activation, it is possible that expression of NUP98 target genes is also impaired when NUP98 is sequestered by toxic aggregates. Whether functional declines in other long-lived nuclear structures such as nucleosomes or the lamina also contribute to degenerative and age-associated diseases remains unexplored. Nonetheless, indications of nuclear integrity decline in ageing and age-linked

disease raise the possibility that the functional decline of long-lived nuclear structures promotes both cellular ageing and, beyond some threshold, neurodegeneration.

Conclusions and perspectives

Over the past decade, our view of the nuclear membrane has evolved from a passive membrane barrier to a dynamic interface that controls key regulatory aspects of cell division, differentiation and disease. Comparative genomics studies suggest an evolutionary trend towards increased complexity and multifunctionality at the nuclear periphery. The NPC is a remarkably ancient protein complex that first arose at least 1.5 billion years ago[147]. Lamins are evolutionarily younger, although they still represent the founding members of the intermediate filament protein family[147]. The lamina has increased in complexity through recent metazoan evolution, with developmentally controlled A-type lamins first appearing in *D. melanogaster*[148]. The expansion of lamin genes and splice isoforms has likely allowed for the diversification of lamin functions within distinct nuclear domains and across tissues, which remain to be fully elucidated. Surprisingly, nucleoporin genes are among the fastest-evolving genes[147], defying the expectation that proteins involved in critical cellular processes should be constrained by negative selection. The recently evolved ability of individual NUPs to associate with the genome within the nucleus has likely greatly extended the influence of the NPC in gene regulation and might reflect an evolutionary trend towards more complex patterns of gene expression and genome organization[149]. In particular, it is tempting to speculate that the recently evolved hominoid-specific soluble NUP variant sPOM121 may have contributed to a diversification in function through its ability to interact with both NUP98 and the nonameric NUP107–160 complex, which could nucleate distinct chromatin-associated NUP complexes within the nucleoplasm[74].

The nuclear lamina and the NPC both contribute to the organization of the genome and to transcription regulation. Whereas the net effect of lamina association is almost always gene repression[100], association with NPCs can either activate or repress genes. Association of a locus with the lamina does not induce its association with nearby NPCs[95], underscoring the independence of these modes of tethering. The factors that dictate whether NPC–genome interactions activate or repress loci remain to be determined. Moreover, it remains unclear how individual lamin isoforms and lamina-associated proteins contribute to scaffolding LADs. However, it is evident that lamina association is only one of several strategies for repressing gene activity, as of the thousands of genes that decrease in expression during ES cell differentiation, only hundreds become LAD residents[91].

It may be that nuclear lamina-directed genome organization is more important in differentiated cell types, as pluripotent cells lacking B-type lamins exhibit surprisingly modest defects in genome organization[85,150,151]. Compared with LADs, NUP-binding profiles are considerably narrower and cover a smaller and more variable portion of the genome. NUPs often bind at the level of individual regulatory elements associated with a specific gene, including enhancers, promoters and transcription start sites[47,59,60,66]. Unlike LADs, NUP interactions can be rapidly induced or disrupted by environmental stimuli[59,67].

Clearly, cells balance several strategies for organizing genomes, which may be further illuminated in the future by applying emerging approaches to additional systems. Single-cell HiC[27], DamID[92] and FISH[6,101] can reveal profound changes in the organization of individual cells that may be masked in population-wide analyses. These approaches will continue to advance our understanding of consistent versus variable properties of genome organization. Similarly, evaluating nuclear organization in primary cells, in 3D cultures and within tissues is likely to further define context-specific nuclear organization.

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