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Review

The Nuclear Pore Complex in Cell Type-Specific Chromatin Structure and Gene Regulation

Jiayu Sun,[1,2] Yuming Shi,[1,2] and Eda Yildirim[1,2,*]

Nuclear pore complex (NPC)-mediated nucleocytoplasmic trafficking is essential for key cellular processes, such as cell growth, cell differentiation, and gene regulation. The NPC has also been viewed as a nuclear architectural platform that impacts genome function and gene expression by mediating spatial and temporal coordination between transcription factors, chromatin regulatory proteins, and transcription machinery. Recent findings have uncovered differential and cell type-specific expression and distinct chromatin-binding patterns of individual NPC components known as nucleoporins (Nups). Here, we examine recent studies that investigate the functional roles of NPCs and Nups in transcription, chromatin organization, and epigenetic gene regulation in the context of development and disease.

Highlights

NPCs act as platforms to organize the structure, positioning, and 3D conformation of chromatin within the nucleus.

NPCs and Nups can participate in gene regulation by physically interacting with the genome and cofunction with transcription factors, histone modifiers, or chromatin remodelers.

Nups are differentially expressed in various cell types and their gene regulatory functions impact cell type-specific transcription programs.

Altered genome regulatory functions of nucleoporins have been implicated in specific developmental defects and diseases, such as cancer.

Nuclear Architecture in Chromatin Structure and Gene Expression

Establishment of cell type-specific transcription programs relies on gene regulation and spatial genome organization during early mammalian development [1,2]. Specifically, pluripotent stem cells exhibit extensive changes in gene regulatory programs mediated by master pluripotency transcription factors as differentiation progresses [3]. These changes involve a transition of **chromatin** (see Glossary) from the less compact **euchromatin** to condensed and transcriptionally inactive **heterochromatin** through the activity of **chromatin-remodeling** proteins and chromatin-modifying complexes [4–6]. This transition is also accompanied by distinct features of nuclear architecture, including higher-order chromatin reorganization, changes in the nuclear position of pluripotency and differentiation-specific genes, and the subnuclear compartmentalization of gene regulatory factors at distinct nuclear regions [7–10]. Thus, cell state-specific changes in nuclear architecture are tightly linked to chromatin accessibility and gene regulation.

Nuclear architecture is organized by nuclear structural proteins that include the NPC and nuclear lamins, both of which have prominent roles in cell type-specific transcriptional regulation [11,12]. The NPC is an ~60–120 MDa macromolecular channel that is embedded in the **nuclear envelope.** It mediates **nucleocytoplasmic transport** of messenger ribonucleoprotein complexes (mRNPs), RNAs, and proteins, which are important for gene regulation and key cellular processes, including cell signal transduction and cell growth [13]. Notably, studies that started during the 2000s have provided evidence that, beyond their canonical role in nucleocytoplasmic transport, NPCs can provide a nuclear compartment that accommodates distinct chromatin structures and mediates transcriptional outputs [13–18]. At these compartments, specific NPC components either directly associate with chromatin or the NPC environment itself provides a binding platform, or scaffold, for transcription factors and proteins that mediate **epigenetic** mechanisms. Such proteins include **histone modifying**, chromatin-remodeling, and **chromatin architectural proteins,** which influence gene expression or chromatin organization [19]. In this review, we compare and contrast NPC-mediated gene regulation and chromatin structure in

[1]Department of Cell Biology, Duke University School of Medicine, Durham, NC 27710, USA
[2]Duke Cancer Institute, Durham, NC 27710, USA

*Correspondence:
eda.yildirim@duke.edu (E. Yildirim).

yeast and metazoans and examine recent advances that provide molecular insights into the functional relevance of individual NPC components in cell type-specific genome function during development and disease.

NPC Structure and Characteristics of Its Components

NPCs comprise ~30 nucleoporin proteins (**Nups**) that are structurally organized into a cylindrical assembly with eightfold symmetry [20,21]. The NPC structure is divided into structural and peripheral elements (Box 1). The three key structural elements are the cytoplasmic ring, inner pore/central ring, and nuclear ring. The peripheral elements comprise the nuclear basket, which extends into the nucleoplasm, and the cytoplasmic filaments, which face the cytoplasm [22–25]. Formation of the NPC relies on the preassembly of subcomplexes that bind to each other in multiple copies. Among these subcomplexes, the inner ring and so-called 'Y-complex' constitute the NPC scaffold [23,26–28]. Crystal structure analyses of the Y-complex suggested that its components are conserved across eukaryotes [29]. Interestingly, studies that examined the NPC assembly and cell type-specific composition provide further evidence that a subgroup of peripheral and inner ring Nups exhibit dynamic association with the NPCs and are differentially expressed in distinct cell types during development [30,31]. For example, NUP210 has been implicated in cell differentiation in myogenic and neuronal lineages [32] and cell homeostasis in immune cells [33]. By contrast,NUP153 is highly expressed in mouse embryonic stem cells (ESCs), and altered NUP153 expression was causally linked to loss of ESC pluripotency [34]. Both highly mobile Nups (e.g., NUP50, NUP153, and NUP98) and inner ring components (e.g., SEC13 and NUP62) are among Nups that associate with chromatin at the NPC and within the nucleoplasm [35–39]. These Nups have been proposed to impact transcription of early development and cell cycle-specific genes [38,40–42]. The existence of soluble Nups suggests that they impact transcription directly by associating with the transcription machinery or chromatin-regulatory factors, or indirectly by regulating targeting of mRNPs from sites of transcription to the NPCs for export. Future studies are necessary to dissect these mechanisms.

NPCs as Platforms Regulating Active Transcription in Yeast

It has long been proposed that NPCs have active roles in organizing open chromatin and function as gates of the nucleus, at which efficient transcription–translation coupling is achieved, a hypothesis referred to as the 'gene gating hypothesis' [43]. Early electron micrographs of mammalian nuclei inspired this hypothesis by showing that chromatin is organized differently along the nuclear lamina compared with the NPCs [44]. Specifically, condensed heterochromatin was detected lining the nuclear periphery with the exception of regions near the NPCs. These observations suggested the NPC environment as a potential subnuclear compartment for transcriptional activation. Beyond this function, the gene gating hypothesis also proposes that the NPC participates in organizing chromatin into interchromatin compartments that aid formation of so-called channels between the NPCs and transcription sites. As discussed later, work with

Box 1. The NPC Structure

The NPC is constructed by the self-assembly of multiple copies of ~30 Nups into a structure with eight semisymmetric spokes and an axis perpendicular to the plane of the nuclear envelope [22]. A significant number of Nups organize into stable subcomplexes that form the major building blocks of the NPC. The high-resolution structure of the yeast NPC was recently resolved [88,89], revealing differences between human NPC [90,91] and yeast NPCs. Although the eightfold symmetry is preserved, yeast NPC contains 16 copies of most Nups, while human NPCs contain double or more of the amount of Nups, resulting in drastic differences in their overall size. The selectivity of transport through the NPCs is primarily mediated through dynamic interactions between transport factors and Nups that contain phenylalanine (F)-glycine (G)-rich regions called FG domains [92]. Two current theories for the specificity of transport through the central pore are that, unless a transport factor binds, the FG domain either imposes a polymer brush that pushes back macromolecules or that the FG domains interact with each other to form a matrix blocking passage of larger molecules.

Glossary

Cell fate: a developmental program along which a cell progresses to reach its specific differentiated state or that of its daughter cells. Cell fate commitment results in changes in both gene expression and chromosome organization.
Chromatin: a complex of DNA, histones, and other proteins that comprise the chromosomes in eukaryotic cells.
Chromatin architectural proteins: proteins that help determine the 3D configuration and arrangement of chromatin within the nucleus.
Chromatin looping: a basic unit of interphase chromosome organization in which regions on the same chromosome are fixed in close physical proximity, allowing for interactions between genes and distal regulatory elements.
Chromatin remodeling: the dynamic rearrangement of nucleosomes along DNA to change the compactness of chromatin.
Dosage compensation: mechanisms used to equalize sex chromosome-linked gene expression between members of different biological sexes. For example, in therian mammals, one X chromosome in XX females will be singled out for inactivation during embryonic development.
Epigenetic: gene expression regulation that arises from factors other than the genetic sequence that can be inherited by daughter cells.
Euchromatin: less compact chromatin that is often enriched for actively transcribed genes.
Gene positioning: subnuclear location of a genetic loci relative to the nuclear periphery or a nuclear compartment.
Heterochromatin: compact chromatin in which genes are generally transcriptionally silenced.
Histone modifier: an enzyme that catalyzes post-translational modification, such as methylation or acetylation of histones, resulting in altered gene expression or chromatin structure.
Imprinted gene: a gene the monoallelic expression of which is determined by the parent of origin.
Insulator proteins: a protein the binding of which results in blocking of long-range genomic interactions through the formation of DNA–protein complexes.

yeast has yielded important evidence for NPC–genome interactions as well as identifying protein factors that mediate these interactions. Some aspects of these mechanisms are also detected in metazoans. However, accumulating evidence suggests that distinct mechanisms govern NPC- or Nup-mediated gene regulation in metazoans likely due to the complexity of their genome and the diversity of transcription programs that impact development.

In support of the gene gating hypothesis, an early genome-wide study in yeast provided evidence that several Nups, as well as specific NPC-associated proteins, preferentially associate with genes that exhibit high transcriptional activity [45]. These genes include *GAL1*, *GAL2*, *HXK1*, *INO1*, *TSA2*, *HSP104*, *SUC2*, and *MFA2*, which, upon activation, show preferential positioning to the nuclear periphery [46–51]. **Gene positioning** to the periphery might impact gene expression beyond promoting mRNA export. Using the *GAL* and *HXK1* loci, it was shown that Nups can directly associate with gene elements, including the promoters [18], and impact **transcriptional memory** [49] (Box 2). Interestingly, active transcription at the *GAL* genes is not required for these loci to position to the periphery, suggesting that the NPC association represents an early event of gene activation, instead of being a result of mRNA production. Evidence from a follow-up study revealed that, although NPC association is not necessary for transcriptional activation of *HXK1*, relocalization of the locus to the NPCs increases its expression level [48]. Based on these data, NPC–genome associations were proposed to confer a specific environment at which gene expression is fine-tuned based on the specific cellular states.

One of the well-characterized molecular mechanisms that control transcriptional regulation at the NPCs involves the histone Spt-Ada-Gcn5 acetyltransferase (SAGA) complex, which associates with the promoters of the target loci and coordinates transcription and mRNA transport with the mRNA export complex (TREX-2) [17,52]. Recent findings showing interaction between TREX-2 and the MEDIATOR complex [a regulator of RNA polymerase II (Pol II) recruitment] further strengthens the argument that NPCs can provide a favorable environment for recruitment of active **transcriptional machinery** in yeast [53].

The genetic information for NPC recruitment may reside in genes themselves, a phenomenon exemplified by the yeast *INO1* locus. Genetic analyses of the *INO1* promoter sequence led to the identification of two *cis*-acting DNA elements called Gene Recruitment Sequences I and II (GRS I and II), which function redundantly to target the *INO1* locus to the NPC [51,54]. Importantly, these elements are sufficient to also target genomic loci other than *INO1* to the nuclear periphery. These genes include *TSA2*, *HSP104*, *GAL1-10*, *HIS4*, and *PRM1*, which were also found to contain specific GRSs that are necessary for their recruitment to the NPC [51,54,55]. Examination of the spatial positioning of these sequences genome wide showed that GRS I and GRS III,

Nuclear envelope: the lipid bilayer that envelopes the genetic material of the eukaryotic nucleus; also known as the nuclear membrane.
Nucleocytoplasmic transport: the bidirectional transport of macromolecules, such as mRNA and proteins, between the nucleus and cytoplasm.
Nucleoporin proteins (Nups): the constituent proteins of the NPC.
Transcriptional machinery: a complex comprising RNA polymerase, transcription factors, chromatin remodelers, and histone modifiers that is created at regions that are transcriptionally regulated.
Transcriptional memory: an epigenetic phenomenon in which the transcription response of a gene is changed in response to repeated exposure to a stimulus.

Box 2. Regulation of Transcriptional Memory by Nucleoporin Proteins

Transcriptional memory is an epigenetic phenomenon in which the transcriptional response of a gene is changed in response to repeated exposure to a stimulus and is critical for fine-tuning cell signal transduction during development. NPCs have been proposed to be sites at which the transcriptional memory of genes is established. For example, the inducible yeast genes *GAL1*, *GAL2*, *HXK1*, *INO1*, *TSA2*, *HSP104*, *SUC2*, and *MFA2* relocate to the NPCs upon induction [46–51] and maintain their position at NPCs following repression, which subsequently contributes to their rapid transcription upon reactivation. Transcriptional memory has also been attributed to the gene-looping role of Nups, such as the yeast TPR homolog Mlp1. Chromatin looping mediated by Mlp1 promotes rapid Pol II recruitment and subsequent transcription at NPCs [62]. Another mechanism by which NPCs confer transcriptional memory includes regulation of Pol II pausing. In yeast, NUP100 is required to tether the *INO1* locus to the NPC and mediates transcriptional memory at the locus, which relies on incorporation of the histone variant H2A.Z and Pol II pausing at the promoter proximal region [49,93]. This mechanism is conserved in human cells, whereby NUP98 maintains transcriptional memory at interferon gamma (IFN-γ)-inducible genes by mediating recruitment of paused Pol II and H3K4me2 at promoters, which facilitates faster transcription initiation [85].

which were identified within the *HSP104* promoter, cluster at the NPCs [56], supporting the idea that these genetic elements aid specific intra- and interchromosome interactions contributing to the 3D genome organization.

NPCs in Regulation of Chromatin Organization

Although several studies with yeast show that the NPC components physically interact with actively transcribed genes and open chromatin structures [45,57,58], NPCs are also required to tether and maintain silent chromatin, such as the telomeres and subtelomeric genes, through associating with chromatin-remodeling proteins. For example, Mlp1 and Mlp2 (yeast homologs of mammalian peripheral nucleoporin TPR) are required to tether telomeres to the nuclear periphery and repress telomeric genes by interacting with the telomere silencing factor, Yku70 [15]. A recent study provided more insights into this mechanism by showing that Nup170p binds to subtelomeric genes and mediates their silencing by cooperating with the ATP-dependent chromatin structure remodeling (RSC) complex and the silencing factor, Sir4p [59]. Interestingly, the RSC complex is required for the formation of proper nuclear envelope morphology and localization of NPCs [60,61].

The NPCs participate in establishing **chromatin looping** between distal regulatory elements and promoters. Such regulation is facilitated by Mlp1 in yeast, which preferentially binds to the 3′ end of actively transcribed genes and brings the 5′ end and the 3′ end of a gene into close proximity [46]. This mechanism was later determined to be critical for transcriptional activity at the inducible genes *HKX1* and *GAL1* [62]. Consistent with these findings, recent work with *Drosophila* detected NUP98-mediated enhancer–promoter looping of ecdysone-responsive genes, which are differentially regulated during fly developmental transitions [63]. Chromatin looping at these loci was shown to be critical for the establishment of transcriptional memory. Interestingly, upon ecdysone induction, NUP98 physically interacts with the chromatin architectural protein, CCCTC-binding factor, CTCF, a well-characterized **insulator protein** and the master organizer of 3D chromatin conformation [64], further supporting a functional role for NPCs in 3D genome organization (Figure 1A).

Roles for NPCs in organizing the structure and positioning of chromatin are also reported in mammalian cells. By utilizing poliovirus-induced changes in heterochromatin organization in HeLa cells, it was discovered that the NPC basket protein TPR is necessary for heterochromatin organization and that depletion of TPR resulted in elimination of heterochromatin-free regions at NPCs [65]. Consistent with this finding, recent work identified TPR to be necessary for the formation and maintenance of heterochromatin organization in cells that exhibit oncogene-induced senescence [66]. In this system, increased NPC density and TPR function were shown to be responsible for heterochromatin reorganization. NUP153 is another mammalian Nup that is involved in chromatin organization. It was shown that NUP153 controls establishment of heterochromatin domains in interphase cells by recruiting Repo-man (CDCA2), a protein phosphatase 1 (PP1)-targeting subunit protein, which subsequently mediates chromatin remodeling [61,64]. These data collectively argue that, in metazoan cells, chromatin-remodeling complexes act as intermediary molecules linking NPC to chromatin architecture and organization.

NPCs as Scaffolds for Compartmentalized Gene Regulation in Metazoans

The metazoan NPCs may function as scaffolds for the assembly of transcription complexes or provide specialized microenvironments for compartmentalized gene expression. In line with this idea, transcription factor MYC (c-Myc) was recently shown to be recruited to the nuclear periphery and to associate with the NPC basket proteins NUP153 and TPR in mitogen-activated cells that exhibit high proliferation [67] (Figure 1B). Interestingly, activation of MYC through serine 62 phosphorylation or PIN1-mediated isomerization promoted MYC association with the NPC

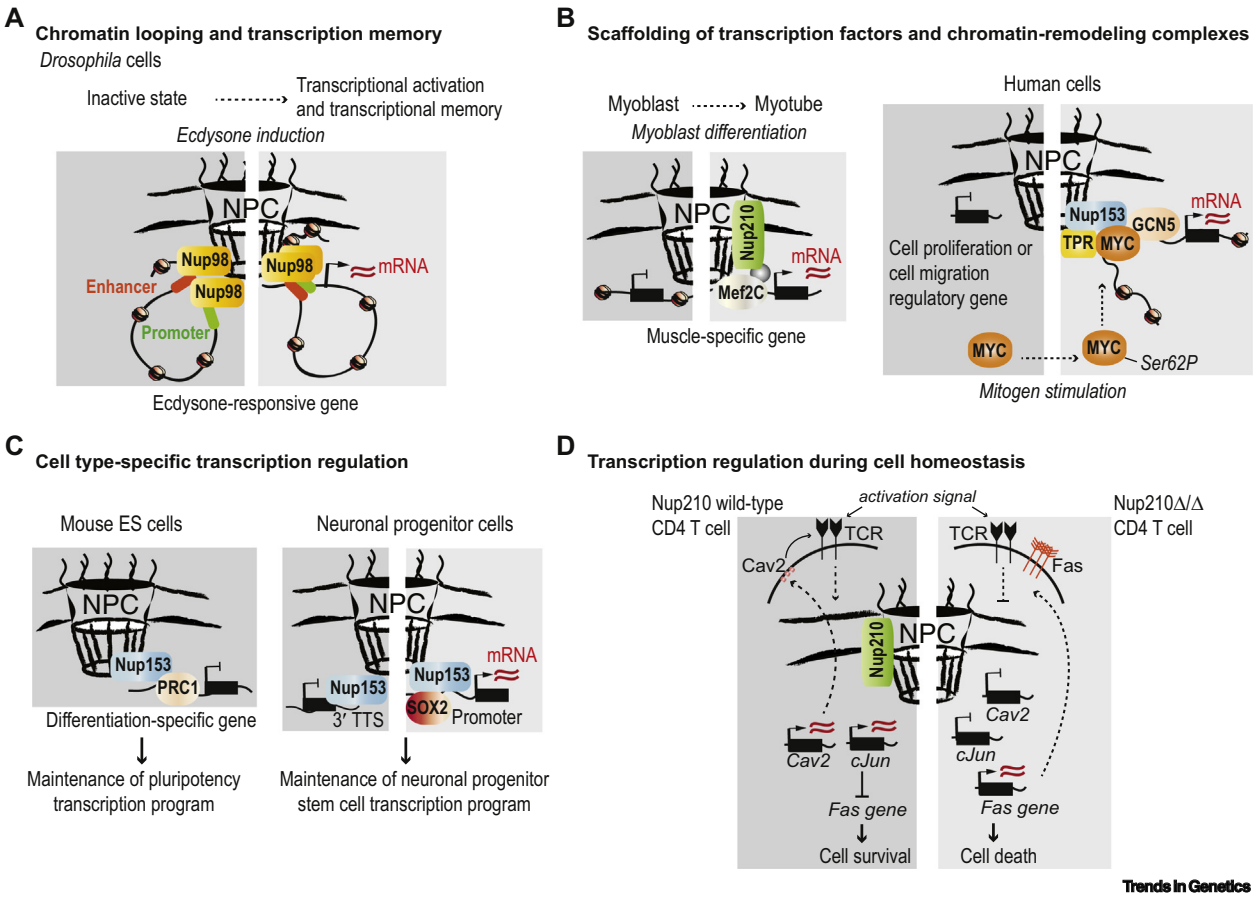

Figure 1. Nuclear Pore Complexes (NPCs) and Individual Nucleoporin Proteins (Nups) Mediate Cell Type-Specific Chromatin Structure and Transcription through a Range of Mechanisms in Metazoans. Schematic showing the range of molecular mechanisms that involve NPC or individual Nups in (A) chromatin looping, (B) scaffolding of transcription factors and chromatin regulatory proteins, (C) cell type-specific transcription regulation, and (D) regulation of transcription programs to maintain cell survival. Abbreviations: ES, embryonic stem; TCR, T cell receptor; TTS, transcription termination site.

basket and facilitated formation of a transcriptionally permissive environment that included the SAGA complex component, GCN5 acetyltransferase, and MYC target genes. Transcription of several cell proliferation and migration genes depend on this mechanism. These data argue that spatial scaffolding of MYC by NPCs is critical for its downstream function as a transcriptional regulator.

The ability of NPCs to form specific subnuclear compartments argues that NPCs could establish subnuclear compartments at which distinct transcriptional outputs are generated based on the positioning of alleles to or away from the NPCs. Genomic imprinting is an epigenetic phenomenon through which monoallelic gene expression is achieved based on parent of origin [68]. *Kcnq1ot1* is an **imprinted gene** and encodes a noncoding RNA transcript that is expressed antisense to the *Kcnq1* gene [69]. In mouse extraembryonic endoderm stem cells, NUP107, NUP62, and NUP153 were shown to be required for the recruitment of the paternal *Kcnq1ot1* locus to the nuclear periphery and maintenance of *Kcnq1ot1* transcription [70]. It has been proposed that the histone-modifying protein, EZH2, and cohesin, which is a master regulator of chromatin 3D organization [71], mediate *Kcnq1ot1* transcription at the NPC. Nevertheless, more detailed analysis is necessary to dissect the interplay between Nups and imprinted gene regulation as well as to

determine whether and how this mechanism is utilized during gene expression at other imprinted loci.

An intriguing question is whether nucleoplasmic Nups can also form specific nuclear hubs and/or structures that can serve as platforms for transcription or chromatin regulation. Evidence supporting this view was reported in 2002 showing that GLFG repeats of NUP98 are required to cluster nucleoplasmic NUP98 into a novel structure termed the 'GLFG body' [35]. Moreover, analyses of genome-wide interaction maps of NUP98, NUP62, and NUP50 showed that they co-localize within the nucleoplasm [39]. Interestingly, a recent study identified a variant of the transmembrane Nup, POM121, which lacks the membrane-anchoring domain, localizing to the nucleoplasm while retaining interaction with NUP98 [72]. Based on this evidence, it is plausible that gene regulation by specific Nups exists within the nucleoplasm, independent of NPC association and in a co-regulatory fashion [39]. Establishment of cell lines in which endogenous Nup loci are tagged or conditionally targeted will be critical for these studies, because overexpression of Nups often associates with expression patterns that unlikely to inform physiological associations.

Nucleoporin-Mediated Gene Regulation in Establishment of Cell Type- and Cell State-Specific Transcription

In higher eukaryotes, several Nups exhibit chromatin association at genomic sites with distinct chromatin structures. The functional significance of these associations in the context of development are only beginning to be understood. In *Drosophila*, NUP153 and TPR were identified as factors that associate with the Dosage Compensation Complex (DCC), which is responsible for mediating doubling of transcription output of X-linked genes in male flies, a **dosage compensation** phenomenon called X-hyperactivation [57,73]. Depletion of either NUP153 or TPR in this system leads to repositioning of the male X chromosome away from the nuclear periphery and downregulation of X-linked genes, suggesting a causal role for NUP153–chromatin interactions in transcription activation of the X chromosome. Later studies revealed that Nup–chromatin associations are more diverse. Specifically, a subset of Nups, including SEC13, NUP98, NUP88, NUP62, and NUP50, bind to specific regions of the fly genome at which development and cell cycle regulation-specific genes reside [38,39]. Interestingly, some of these interactions were detected away from the NPC, supporting earlier observations that nucleoplasmic Nup pools might participate in gene regulation independent of the NPC structure. Nevertheless, whether these associations are indeed NPC independent is yet to be elucidated.

It is becoming evident that interaction of the NPCs or individual Nups with transcription factors or proteins that coordinate epigenetic gene regulation affect mammalian **cell fate** determination and developmental processes. Transcriptional analyses across different cell types showed that expression of specific Nups, such as NUP98, NUP153, and NUP210, is differentially regulated. Specifically, identification of NUP98 chromatin interaction sites in human ESCs and neural progenitor cells revealed that NUP98–chromatin interactions are often detected within the nucleoplasm, but vary between these two cell types and are enriched at the neural developmental genes [41]. Furthermore, transcriptionally active genes associate with nucleoplasmic NUP98, while genes that undergo transcriptional induction associate with NPC-tethered NUP98 in neuronal progenitor cells. These data argue that NPC-associated Nups and nucleoplasmic Nups may represent functionally distinct populations or participate in different aspects of transcription regulation based on their protein partners.

In mice, NUP153 is expressed highly in mouse ESCs and neuronal progenitor cells. NUP153–chromatin interactions were proposed to be critical to suppress differentiation-specific genes in pluripotent ESCs [34] and mediate transcription programs favoring neuronal progenitor cell

maintenance [74]. In ESCs, NUP153 interacts with the Polycomb Repressive Complex 1 (PRC1) component, RING1B, which catalyzes the ubiquitination of Histone H2A and mediates epigenetic silencing of target genes during early development [75]. It was proposed that NUP153 and RING1B co-occupy ~30% of NUP153-binding sites and co-regulate transcriptional silencing at these sites, which partly explains the role of NUP153 in the maintenance of ESC pluripotency [34] (Figure 1C). In neuronal progenitor cells, NUP153–chromatin interactions overlap with sites that interact with the transcription factor, SOX-2 [74]. In this context, NUP153 behaves as a bimodal transcription regulator, which relies on its binding to promoters or 3′ transcription termination sites (TTS). NUP153 enrichment at the promoters has been linked to transcriptional activation ,while its 3′ TTS association has been linked to transcriptional silencing (Figure 1C).

NUP210 is another Nup that exhibits cell type specificity and function across species. Initial studies characterized NUP210 to be required for zebrafish skeletal muscle development and myoblast differentiation by regulating the expression of a subset of muscle-specific genes [76]. This regulation relies on NUP210-dependent recruitment of the MEF2C transcription factor and the NPC acting as a scaffold for physical interaction between NUP210, target loci, and the MEF2C transcriptional complex (Figure 1B). Recent work using a conditional knockout mouse model for NUP210 revealed a unique role for NUP210 downstream of T cell receptor (TCR) signaling that is critical for $CD4^+$ T cell homeostasis and survival [33]. It was proposed that NUP210-dependent transcription of Caveolin 2 (*Cav2*) and *cJun* is necessary for TCR activation and for blocking the expression of the FAS protein (a cell signaling protein that induces cell death). NUP210 deficiency leads to altered TCR signaling and loss of repression of Fab protein expression, triggering cell death in $CD4^+$ T cells (Figure 1D).

Diseases That Associate with Gene Regulatory Functions of Nups

Given that Nups participate in the regulation of chromatin structure and transcription during cell fate determination and development, it is not surprising that they have been implicated in diverse diseases, including neurodegenerative disorders, cardiovascular diseases, and cancer [77–80]. Although it is often difficult to determine whether it is the transport or gene regulatory function of Nups that underlies nucleoporin-related diseases, some studies have provided evidence linking the genome regulatory role of Nups to disease phenotypes. For example, NUP153 levels are elevated in the mdx mouse model of Duchenne muscular dystrophy, which presents cardiac defects in the form of cardiomyopathy [77]. Specifically, in mdx mice, NUP153 expression is mediated by protein acetylation and the NUP153 interaction with lysine acetyl transferases [81], P300, and PCAF was increased. The authors suggested that NUP153–KAT interactions mediate transcription of genes that associate with cardiomyopathy in mdx mice.

NUP98 has been the focus of cancer research because of its oncogenic role. Structural chromosomal rearrangements of NUP98 lead to the formation of oncogenic fusion genes, which have been implicated in several hematological malignancies, including acute myeloid leukemia [82]. Approximately 28 NUP98 fusion proteins have been identified. Nevertheless, the mechanisms that drive NUP98 fusion gene-mediated malignancies are not fully understood. One common characteristic among the oncogenic NUP98 fusion proteins is that they are formed of a portion of the NUP98 FG repeats and a portion of a transcriptional regulator protein including CBP/P300, histone methyltransferase complex, MLL [81,83], or DNA-binding proteins, such as HOX family members (e.g., HOXA9, HOXD13, and NSD1) [83,84]. These characteristics suggest that oncogenic properties of the NUP98 fusion proteins rely on their partners that drive expression of cancer-related genes.

Several studies in yeast and metazoans have addressed the molecular mechanisms that functionally and physically link NUP98 to histone methyltransferases, providing insights into the

molecular basis of NUP98–fusion protein-driven hematological malignancies. One of the earlier studies in yeast and mammalian cells showed that NUP98 (NUP100 in yeast) was associated with transcriptional activation and transcriptional memory [85]. In these systems, depletion of NUP98 led to altered Pol II pausing at the proximal promoter regions of NUP98 target genes, loss of histone 3 lysine 4 dimethylation (H3K4me2), and reduced transcriptional reactivation rates at loci that exhibit transcriptional memory. Notably, NUP98–chromatin interactions in the yeast genome were detected at the nuclear periphery, while NUP98–chromatin interactions in the mammalian genome were detected within the nucleoplasm. In fly, NUP98–chromatin binding associates with transcriptional activation, which relies on NUP98 interactions with the histone-modifying complexes, nonspecific lethal (MBD-R2/NSL) and mixed lineage leukemia (MLL/Trithorax). These associations are critical for transcription at the Hox loci during fly development [86]. These data suggested that the NUP98 fusion proteins also interact with the NSL and MLL complex in NUP98 fusion-driven hematological malignancies. Indeed, a recent study used the BioID method to tag NUP98-fusion proteins including NUP98-HOXA9 (NHA9), NUP98-HOXD13 (NHD13), NUP98-NSD1, NUP98-PHF23, and NUP98-TOP1, and demonstrated physical interactions between the fusion proteins and MLL1 and NSL complexes [78]. Importantly, depletion of MLL1 resulted in downregulation of transcription at genes that are co-occupied by MLL1 and NUP98-HOXA9, and reversal of oncogenic gene expression profiles. In support of these findings, NUP98 was shown to directly mediate recruitment of the Wdr82-Set1A/COMPASS complex, which is essential for Histone 3 Lysine 4 trimethylation (H3K4me3) during transcriptional activation in hematopoietic progenitor cells [87]. Whether this mechanism contributes to the AML phenotype detected in NUP98 fusion gene- mediated malignancies has yet to be studied.

Outstanding Questions

What are the mechanisms that underlie NPC- and Nup-mediated genome regulation? How are these mechanisms regulated during cell lineage specification?

Do NPCs promote the establishment of subnuclear compartments? What is the composition of these compartments?

What are the functional protein partners of Nups and how do they impact the gene regulatory function of Nups?

Do NPC-associating and nucleoplasmic Nups represent two functionally distinct populations with different roles in genome regulation?

How is the composition and function of NPCs regulated in a cell type-specific manner? Does NPC composition show heterogeneity in individual cells?

Which human diseases associate with the genome-regulatory function of Nups and NPCs?

Concluding Remarks and Future Perspectives

Well characterized as the nucleocytoplasmic transport channel, NPCs have been in the spotlight for their participation in genome regulation during establishment of cell type-specific chromatin organization and transcription programs. In yeast, NPCs provide a subnuclear environment to control transcription activation, promote transcriptional memory, and facilitate coupling between transcription and mRNA export. Dissecting mechanisms of how NPCs or individual Nups associate with gene regulation in higher eukaryotes is more challenging. This is because: (i) how the specificity of Nup–chromatin interaction sites is established is not well understood; (ii) protein factors that associate with Nups in different cellular states or cell types are not well defined; and (iii) several Nups exhibit dynamic dissociation and association with NPCs, arguing for a nucleoplasmic pool that is often difficult to distinguish from NPC-associated Nups (see Outstanding Questions). Addressing these challenges and providing molecular insights into how specificity between Nups with their target chromatin is achieved will be critical to gain insights into their gene regulatory role during mammalian development and disease.

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