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NUCLEAR PORES IN GENOME ARCHITECTURE AND ENHANCER FUNCTION

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Abstract

Nuclear genome architecture relies on interactions between the genome and various nuclear scaffolds. One such nuclear scaffold is the nuclear pore complex (NPC), which in addition to its nuclear transport function, can interact with underlying chromatin. In particular, NPCs have been recently reported to associate with a number of enhancers and super-enhancers in metazoan genomes, and select NPC components have been shown to promote the formation of specific genomic loops. Here we provide a brief overview of current models of enhancer function, and discuss recent evidence that NPCs bind enhancers and contribute to topological genome organization. We also examine possible models of how gene and enhancer targeting to NPCs may contribute to tissue-specific genome architecture and expression programs, including the possibility that NPCs may promote phase separation of transcriptional compartments.

Introduction

The complexity of multicellular organisms relies on specific and highly regulated transcriptional programs that control activation of genes in a cell and developmental time dependent manner. To achieve such specificity, gene expression is controlled by the orchestrated action of *cis*-regulatory elements, such as enhancers, that are located at large distances away from the transcription start sites (TSSs) [1]. Gene expression is also influenced by the nuclear architecture of the genome, or how the genome is folded and arranged inside the nuclear space [2–5]. Nuclear architecture includes both long-range interactions between distant genomic loci and the interactions of loci with nuclear scaffolds and structures. One of the most pronounced nuclear scaffolds is the nuclear envelope (NE), which includes nuclear lamina and nuclear pore complexes (NPCs). NPCs are large protein assemblies, whose classically defined cellular function is to allow and regulate selective nuclear-cytoplasmic transport [6]. NPCs consist of approximately 30 conserved components, termed Nucleoporins (Nups). A susbset of Nups, classified as stable, comprise the NE-embedded NPC core structure, while other Nups, classified as dynamic, associate with the NPC core in an on-and-off manner [7,8]. A recurring protein domain found in the majority

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of dynamic Nups is the extended array of phenylalanine-glycine (FG) repeats, which is known to mediate the selective permeability transport barrier of NPCs [9–12].

In addition to transport, multiple studies have implicated NPCs in gene regulation via chromatin binding, and thus in functioning as nuclear scaffolds for genome organization [13–15]. As discussed below, recent evidence has also shown that nuclear pore complexes target a number of enhancers and super-enhancers in metazoan genomes [16–18]. These findings have highlighted the notion that NPC-genome interactions contribute to long-range folding of the genome, to enhancer functions and to tissue-specific transcriptional programs. Here we provide a brief overview of the current models of enhancer function, and of the recent evidence that NPCs bind enhancers and participate in nuclear architecture of the genome. We then discuss possible models as to what functional purpose is carried out by gene and enhancer targeting to NPCs.

Mechanisms of genome organization

Enhancers consist of short DNA segments that are found some distance away from promoters and that can direct tissue-specific gene expression via recruitment of sequence-specific transcription factors (TFs) (refs) [19]. In addition to their functional definition, enhancers can often be recognized through a set of chromatin characteristics, such as enrichment of H3K27 acetylation, H3K4 mono-methylation, binding of the p300/CBP histone acetyl transferase and non-promoter binding of RNA Pol II (refs) [1,20,21]. In some cases, multiple enhancers are found in close genomic proximity, resulting in a cluster of closely spaced enhancers that has been termed locus control region (LCR) or super-enhancer (SE). Besides the chromatin characteristics found in enhancers, SEs also show strong enrichment for coactivators, specifically BRD4 and the Mediator complex, and exhibit particularly high levels of H3K27 acetylation (refs) [20,22]. Generally, SEs and LCRs are believed to bring a coalition of transcription factor (TFs) and cofactors to the target promoter, delivering a unique regulatory signature that will determine the appropriate functional outcome for the cell type and the time in development.

Enhancer-promoter communication is encompassed in a higher-order layer of regulation, determined by the organization of the genome in nuclear space. Data from microscopy studies and the development of molecular methods for capturing the spatial organization of the genome, such as genome architecture mapping (GAM) [23] or experiments based on DNA-ligation approaches (chromosome conformation capture (3C) technique and its subsequent maturation) [24], have revealed that the genome is spatially compartmentalized into topologically associated domains (TADs) [25–27]. One of the functional purposes of TADs is believed to be the limitation of enhancer activity to a particular genomic region – such that the activity of enhancers is constrained to cognate promoters within the same TAD, while being insulated from regulatory activity in neighboring domains [28,29]. In this manner, enhancer-promoter loops and other smaller scale loops represent sub-TAD structures, contained within a larger self-interacting TAD. The boundaries of TADs are stabilized by the binding of architectural proteins and by the presence of highly transcribed genes and repetitive elements [30]. The interruption of these boundaries can lead to TAD

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disorganization and spurious contacts between previously isolated domains, which has been linked to irregular gene expression, developmental pathologies and disease [31,32].

Models of enhancer-promoter communication

How distally located enhancers can convey regulatory information to its promoters has become a topic of intense study. The most established and supported model of enhancer-promoter communication proposes that chromatin loops bring enhancers and promoters into close physical proximity, and that such enhancer looping drives transcriptional activation [2,5,33]. Although put forward decades ago, most of the molecular evidence for this model has come from locus-specific and genome-wide 3C methods. One of many examples of enhancer looping is the mouse *β-globin* SE, also known as locus control region (LCR), which consists of four regions with enhancer activity [34]. The LCR is located 50 kb away from the *β-globin* gene cluster and can loop over to contact its target promoters in erythroid cells (where the β-globin gene is active) but shows no interaction with the promoters in cells from other lineages [35]. Importantly, loop formation appears to be sufficient to activate transcription, as demonstrated by force-tethering the self-association domain of the transcriptional cofactor Ldb1 to the *β-globin* promoter, which induced both looping of LCR and transcriptional activation in pro-erythroblast cells [36]. Recent studies have also shown that individual elements of the *β-globin* SE can aggregate to form a hub that can accommodate multiple loops or target promoters simultaneously [37]. Enhancer-promoter looping has also been observed for a variety of active genes controlling a wide aspect of biological processes, such as the *CFTR* gene, which codes for cystic fibrosis transmembrane protein, [38] or the proto-oncogene *c-Myc* locus [39], among many others.

On the other hand, recent evidence has challenged the looping model as the only model to explain communication between enhancers and promoters. This evidence has come primarily from imaging of *in situ* labeled gene loci, using fixed or live microscopy. For example, induction of the *sonic hedgehog morphogen (Shh)* gene in neural progenitor cells is controlled by the action of distal brain enhancers that appear to move further away instead of closer to the activated *Shh* gene [40]. The authors propose that instead of looping over, the *Shh* enhancers may drive regional chromatin decompaction to create an active environment. Interestingly, this regulatory method seems to be cell type specific, since expression of *Shh* in the limb buds is regulated by a distant limb enhancer that does appear to loop out the intervening chromatin to contact its promoter [41]. Similarly, the spatial organization of the key pluripotency regulator *Sox2* and its essential *Sox2 Control Region (SCR)* SE was investigated using live-cell microscopy in mouse embryonic stem cells (mESCs) [42]. In this study, *Sox2* and *SCR* also show no evidence of increased spatial proximity upon activation, suggesting that newly formed enhancer-promoter contacts do not drive Sox2 transcription. Furthermore, a genome-wide detection of distal promoter-interacting regions in multiple cell lines revealed that while some enhancer-promoter loops are very dynamic in response to lineage commitment signals, other enhancer contacts remain stable during cell differentiation [43]. In agreement with this observation, 4C experiments in *Drosophila* embryos suggested that transcriptional changes during *Drosophila* development mainly occur in the context of pre-formed chromatin loops [44]. These data indicate that the enhancer-promoter looping event does not always result in concurrent gene activation, but

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instead may be formed beforehand to allow subsequent transcription or as the *Shh* study suggests [40], may not be a continuous requirement for transcription.

To reconcile these conflicting conclusions on the significance of enhancer looping, one intriguing scenario that has been proposed is a situation where enhancers such as *SCR* establish a large isolated compartment for transcription [33,40,42]. Within such compartments, enhancers and promoters are engaged in preferential communication but do not have to be in constant physical contact. This model is supported by recent studies, which propose that coactivators form phase-separated condensates at SEs of key cell-identity genes [45–48]. Such phase-separated regulatory clusters are thought to be sustained by cooperative interactions between enhancers' bound factors and the transcriptional machinery. These clusters exhibit properties of selectively permeable liquid droplets, resulting in spatial compartmentalization that assures the concentration of the transcriptional apparatus, thus providing robust transcription. Supporting the idea of transcriptional phase separation, these studies demonstrate that the low complexity domains (LCDs) of TFs can phase separate with co-activators frequently found at SEs, such as the Mediator complex and BRD4 [45,47,48]. It has been suggested that multiple properties of SEs can be successfully explained by the phase separation model [49].

Enhancers at NPCs and the roles of Nups in nuclear architecture

Multiple studies over the last two decades have uncovered functional roles of Nups in gene regulation via chromatin binding [50]. Recently, several reports have demonstrated that enhancers and SEs are frequently targeted to nuclear pores [16–18], and that NPC components contribute to formation of long-range genomic contacts [16,51–53]. In *Drosophila,* comparison of Nup ChIP-seq datasets to distribution of the H3K27acetyl mark and to genome-wide enhancer maps [54] have revealed the targeting of Nups to a subset of promoters and enhancers in multiple cell types [16]. In particular, a dynamic NPC component Nup98 was found to bind promoters and enhancers of *Hox* genes and of genes induced by a steroid hormone ecdysone [16]. In mammalian cells, two separate reports have similarly identified association of SEs and LCRs with NPCs and specific Nups [17,18]. First, DamID mapping of stable Nup93 and dynamic Nup153 revealed extensive binding of these Nups to tissue-specific SEs in human cells [18]. For instance, in U2OS cells, nearly half of the classified SEs were found to be targeted by Nup153. Depletion of Nup153 resulted in gene expression changes that were particularly severe for SE-regulated targets, providing evidence for the potential functionality of this binding [18].

Second, a dCas9-based proteomics approach, which aimed to identify protein composition of regulatory elements via sequence-specific guide RNAs, has revealed multiple components of the NPC as highly abundant at LCR enhancers of the human *β-globin* gene cluster [17]. This approach yielded several known regulators of enhancer function, such as GATA1 and chromatin remodeling complexes, but also the dynamic Nups discussed above, Nup98 and Nup153. Targeting of Nup153 and Nup98 to LCR enhancers was further confirmed by ChIP-seq analysis of both Nups, and depletion of either Nup lead to a down-regulation of globin genes in primary erythroid cells. Nup98 and Nup153 were also found to be highly enriched at multiple erythroid SEs [17], providing further evidence for the frequent association

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between SEs and Nups. Together, these studies suggest that complex enhancer landscapes such as those in LCRs, SEs and *Hox* gene clusters, are preferentially associated with nuclear pores.

Although Nup153 and Nup98 can interact with chromatin at the NPC or in the nuclear interior [55–58], the Nup-enhancer contacts were found to preferentially occur at the NE-embedded NPCs [16,18]. For both mammalian Nup153-SE contacts and *Drosophila* Nup98-enhancer contacts, this was demonstrated by FISH and supported by chromatin binding of a stable NE-embedded Nup, Nup93. Interestingly, Nup98 targeting to promoters and enhancers of ecdysone-inducible genes occurred at the NPC regardless of the transcriptional state of these genes [16]. Similarly, the mammalian *HoxA* cluster was found to exhibit ChIP signal of Nup93 in cells where *HoxA* is normally silenced, and this localization was found to depend on Nup93 [59,60]. These findings suggest that NPCs can target silent or poised genes and enhancers, and highlight the role of NPCs as stable binding scaffolds for developmentally regulated targets.

The recurrent detection of enhancer targeting has suggested architectural roles for Nups, and multiple lines of evidence have now demonstrated a functional relationship between Nups and topological looping, particularly at the more local sub-TAD level. Depletion of *Drosophila* Nup98 was found to destabilize enhancer-promoter loops that are induced by addition of ecdysone [16]. This functional role of Nup98 in loop formation parallels similar findings in yeast, where transcriptional 5’−3’ looping of galactose-inducible genes was found to depend on another NPC component Mlp1/2 [53,61]. Moreover, inter-chromosomal clustering and long-range interactions between distant co-induced genes were found to involve binding to the NPCs [51,52]. Interestingly, in the fly system, Nup98 and NPC targeting have also been repeatedly linked to boundary elements. Genome-wide studies have revealed enrichment of DNA binding motifs of insulator proteins among NPC-genome contacts [62] and a high level of ChIP-seq overlap as well as a physical interactions between Nup98 and insulator proteins such as CTCF and Su(Hw) [16]. Genome-wide comparison of various ChIP-seq datasets to Hi-C data has identified Nup98 as one of the factors enriched at the bases of sub-TAD architectural loops containing enhances in fly cells [63]. Furthermore, Nup98 binding was also observed at a subset of TAD boundary regions, suggesting that association with nuclear pore proteins may result in stronger boundaries [64]. These reports support the notion that NPCs function in architectural folding of the genome, perhaps especially in the establishment of sub-TAD structures or certain TAD boundaries, and contribute to formation and stabilization of enhancer-promoter loops.

Nuclear pores as hubs of transcriptional regulation – possible models

The findings described above demonstrate that nuclear pore proteins are frequently found at critical enhancer elements, and that select Nups contribute to expression and architectural states of their target genes. Based on this, we hypothesize 3 possible, non-mutually exclusive models for the chromatin-binding function of NPCs (Figure 1). These models offer potential cellular reasons for the involvement of nuclear pores in enhancer regulation, genome architecture and epigenetic maintenance of gene expression programs.

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Epigenetic stabilization of chromatin loops and states by Nups

NPC targeting and Nup98 in particular have been repeatedly linked to the phenomenon of transcriptional memory. Transcriptional memory or priming describes the enhanced response of previously activated genes to later rounds of activation, after a period of repression [65,66]. This ability of genes to respond more rapidly or robustly upon repeated exposure can be transmitted through cell divisions, i.e. epigenetically. Mechanistically, transcriptional memory has been shown to depend on a number of cellular players, but Nup98 has emerged as an evolutionary conserved factor required for transcriptional memory, necessary for priming of diverse inducible genes in yeast, flies and mammals [16,67]. As in other systems, Nup98 was found to be required for transcriptional memory of ecdysone-inducible genes in *Drosophila* culture cells [16]. Namely, depletion of Nup98 did not affect the transcriptional activation dynamics during initial exposure to ecdysone, but resulted in a less robust transcriptional response upon re-exposure. At the same time, Nup98 depletion also resulted in the loss of enhancer-promoter loops that were induced by initial ecdysone exposure [16]. This suggests that enhancer-promoter loops at ecdysone-inducible genes are not linked to concurrent transcriptional output, but instead may form as part of a memory complex to mark genes as recently activated. In support of this notion, enhancer-promoter loops and enhanced interactions of Nup98 with architectural proteins, induced by initial ecdysone exposure, were found to persist through the period of transcriptional repression [16].

These findings are also in line with previous reports, discussed above, which show that some enhancer-promoter loops do not correlate with transcriptional output. Thus, one possibility is that Nup98 and NPC targeting function in stabilization of enhancer-promoter loops, and that this stabilization is part of the overall process of epigenetic transcriptional memory that allows for more robust or more coordinated transcriptional responses in the future (Figure 1A). Another key pathway implicated in transcriptional memory is deposition of H3K4 methylation, particularly di-methylation (H3K4Me2), through the action of the conserved histone methylase COMPASS [67,68]. Transcriptional memory has been shown to rely on the deposition of H3K4Me2 mark at primed genes [67] and metazoan Nup98 homologues have been found to interact with H3K4 methylases Set1, MLL1 and Trithorax (Trx) [69–71]. It is currently unclear how H3K4 methylation and enhancer looping are integrated with each other, but both have been shown to depend on Nup98 and to function as components of epigenetic memory. In this manner, cells may utilize genome targeting to the NPC via Nup98 for assembly of a transcriptional memory complex, which includes a specialized chromatin and architectural state (Figure 1A).

Coupling of genome binding to nuclear transport

One of the initial ideas for the function of NPC-gene interactions was the “gene gating hypothesis”, which postulated that genes may be targeted to nuclear pores to couple the production and export of mRNAs [72]. This hypothesis has received biochemical support when the NPC-associated mRNA export complex TREX-2 was discovered to share a component, Sus1, with the transcriptional activator SAGA complex [73], and to physically interact with the Mediator complex [74]. NPC components are also involved in multiple steps of mRNA maturation, and in addition to export factors, interact with the quality control machinery that ensures retention of intron-containing transcripts [75]. On the other hand, it

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is difficult to explain how targeting enhancers, SEs and boundary elements to NPCs would integrate with mRNA export. Nonetheless, one function of gene targeting to the NPCs may be to streamline the mRNA biogenesis process by coupling multiple steps in transcription, chromatin regulation, mRNA processing and eventually, export (Figure 1B). In this manner, the reported interaction between Nups and the Mediator complex may play a role in both the stabilization of enhancer-promoter loops and the export of resulting transcripts.

Another possible transport-related role for NPC-genome contacts is the connection to import of critical transcription factors (Figure 1B). Multiple transcription factors in yeast and metazoan systems have been shown to physically interact with NPC components or to mediate the targeting of specific genes to the NPC [76–78]. The ecdysone receptor (EcR), for instance, is thought to undergo nuclear translocation upon activation [79]. Reported binding of poised EcR-regulated genes at the NPC [16] may promote rapid targeting of EcR to its target genes upon nuclear entry. Another NPC component, the transmembrane Nup210, was recently shown to associate with a key transcriptional regulator of myoblast differentiation, Mef2C, and to physically bind its gene targets [77]. As Nup210 is required for muscle differentiation, this mechanism suggests that NPC-bound Nup210 functions to promote recruitment of Mef2C to its target genes to drive differentiation. And although no direct evidence exists for the functional coupling of import and gene binding at the NPC, it remains an intriguing possibility.

NPCs as phase-separated compartments for gene expression

One of the critical functions of FG domain-containing Nups (FG Nups) is to selectively regulate transport between the nucleus and the cytoplasm. The FG domains are LCD domains as they are highly repetitive, intrinsically disordered and show high conformational flexibility [12,80]. Extensive FG repeats are found in most dynamics Nups, including those located at the cytoplasmic fibrils, nuclear basket, and the nuclear pore channel. Selective transport is mediated through dynamic contacts between nuclear transport receptors and FG motifs [9,80]. Consistently with the intrinsically disordered nature of FG domains, higher densities of FG domains can phase separate *in vitro*, and the formation of FG hydrogels can recapitulate some of the selective transport properties of NPCs [9,80,81]. Both Nup98 and Nup153, discussed above, contain lengthy FG domains, and the FG domains of Nup98 homologs have been shown to phase separate from relatively dilute solutions [82].

Given these biochemical properties of FG Nups, and the recent evidence for the existence of SEs in phase separated compartments, discussed above, it is tempting to speculate that NPCs may help create a phase-separated compartment for SEs and other enhancer clusters (Figure 1C). NPCs can provide a high local density of FG domains that can seed or promote the formation of phase-separated environments around bound SEs and gene-enhancer clusters. This may be facilitated by the conserved interactions between FG-containing Nups and key regulators such as the Mediator complex, architectural proteins and Set1/Trx homologues. Such Nup-generated compartments may provide a way to concentrate regulatory molecules, promote an isolated environment for tighter transcriptional control and delimit a space for enhancer-promoter interactions. This phase-separating function of Nups is perhaps easiest to envision occurring at the actual NPCs, which offer a high local concentration of FG

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domains, but could theoretically also occur at nucleoplasmic binding sites of FG Nups such as Nup98 and Nup153. Moreover, such local environments can benefit from the function of Nups in regulation of selective permeability. It is conceivable that much like at the NPC transport channel, FG Nups may help set up a selectively permeable barrier to the entry of transcriptional and chromatin regulators. Currently, there is no direct evidence that phase-separating or permeability-regulating properties of Nups contribute to their transcriptional roles. But future experiments can address this question as well as the other models both *in vivo* and *in vitro,* elucidating the mechanistic role of the NPC in genome control.

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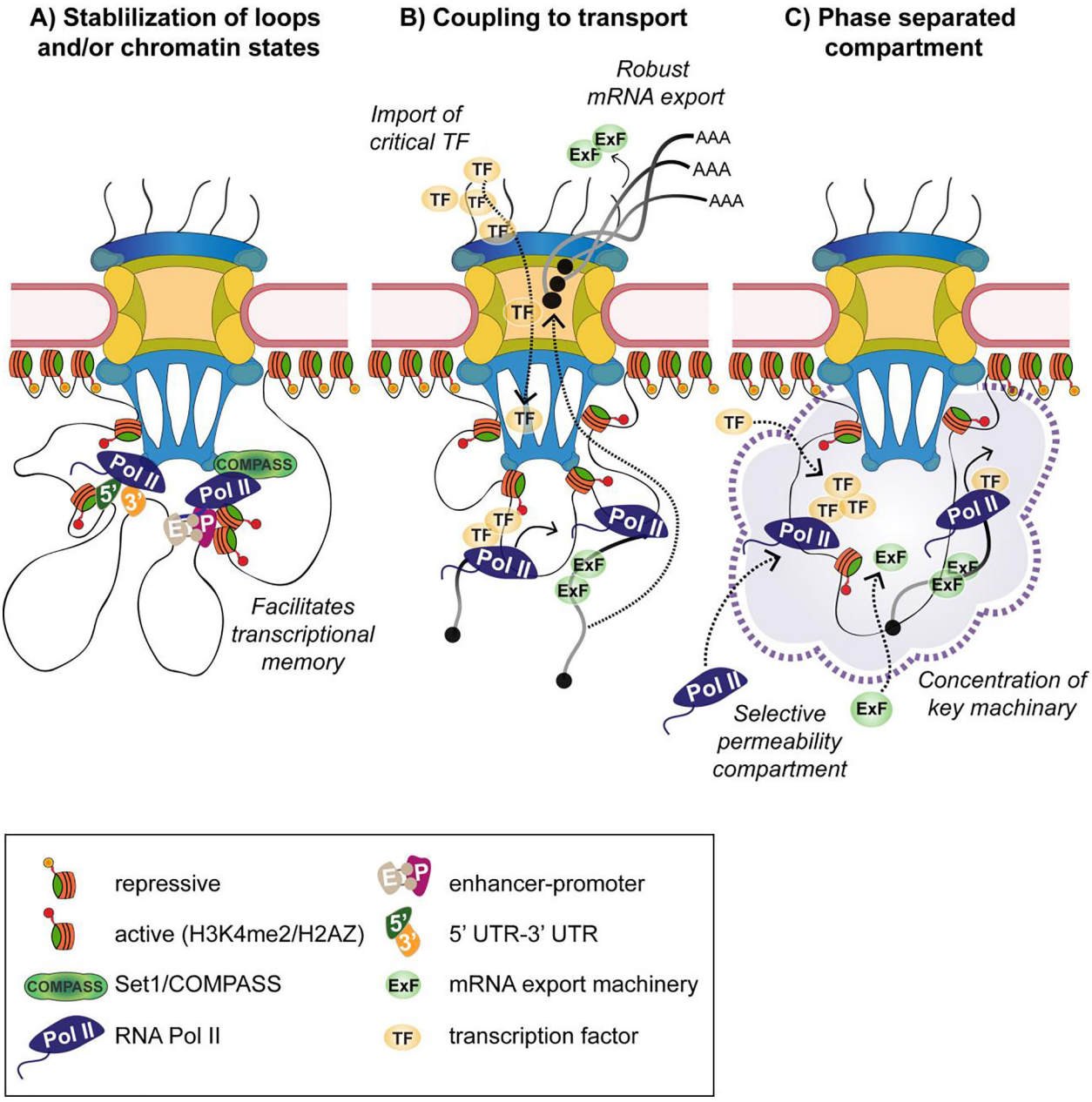

Figure 1. Possible non-mutually exclusive models for the genome-binding functions of NPCs.

A) Binding of inducible genes and their promoter and enhancer elements to the NPCs facilitates epigenetic maintenance of the activated state by stabilizing histone modifications, binding of the COMPASS complex and enhancer-promoter loops. In this manner, the NPC may function as a nuclear compartment/complex that aids in the interplay between these processes to promote transcriptional memory. **B)** Targeting active genes and enhancer units to NPCs may be coupled to transport-related functions of NPCs. Such transport-related functions may include the connection between transcription and mRNA export, to streamline the generation of mature mRNA, or the connection between the import of critical transcription factors and their targeting to NPC-bound genes, to promote the efficiency of gene targeting. **C)** Phase-separating properties of FG Nups at NPCs may promote phase

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separated transcriptional compartments at enhancers and super-enhancers and their target genes. Such compartments may help concentrate key chromatin and transcriptional regulators, and/or create isolated selectively permeable hubs, using known functions of FG Nups in regulation of selective permeability.