Multiple facets of nuclear periphery in gene expression control

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Nuclear pore complexes play a central role in controlling the traffic between the nucleus and the cytoplasm. Progress during the last decade has highlighted nuclear periphery components as novel players in chromatin organization, gene regulation, and genome stability. For instance, lamins associate with repressive chromatin while nuclear pores tend to associate with active chromatin. Interestingly, nucleoporins (Nups) act not only at the nuclear periphery but also in the nucleoplasm. Here we provide an overview of the latest findings and discuss the functional importance of nucleoporin association with specific genes, their role in transcriptional memory, the coupling of transcription and mRNA export, and genome integrity.

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Introduction
Chromosomes are highly organized within the nucleus and reside in specific functional subnuclear compartments in interphase cells [1]. These compartments are not membrane delimited and instead arise from the clustering of DNA regions with specific proteins, creating microenvironments that can favor or impede particular cellular processes such as transcription regulation or replication. Thus, the three-dimensional (3D) nuclear architecture provides a fundamental level for the regulation of gene expression (for review see [2,3]).

The nuclear envelope (NE) surrounds and defines this complex nuclear architecture. It consists of two membrane bilayers, perforated by nuclear pores, which control the traffic in and out of the nucleus. The NE is also associated with perinuclear proteins including membrane-associated or transmembrane proteins and the nuclear lamina. In higher eukaryotes, the inner nuclear membrane is associated with a network of intermediate filament proteins called lamins, which help to maintain the spherical geometry of nuclei. These play an important role in many fundamental processes such as NE assembly/disassembly during mitosis, gene expression, DNA replication and nuclear pore complex (NPC) positioning (for review see [4]).

The overall structure of the NPC is evolutionarily conserved and is a large protein complex of about 60 MDa embedded in the NE [5–7]. The primary function of the NPC is to mediate selective bidirectional transport between the nucleus and the cytoplasm [8,9]. NPCs are composed of approximately 30 different nucleoporins (Nups) [9,10] that fall into two broad categories: firstly, scaffold Nups and secondly, peripheral Nups. The scaffold Nups form the NPC core. On the other hand, the peripheral Nups, many of which contain phenylalanine-glycine (FG) repeats, are responsible for establishing the permeability barrier [11] and mediating nuclear trafficking [12]. Interestingly, several Nups are mobile and dynamically shuttle between the nucleoplasm and the NPC [13–15]. In this review, we highlight the role of nuclear periphery components in transcriptional control (Table 1).

Gene silencing at the nuclear periphery
Classical cytological studies revealed that heterochromatin has a tendency to associate with the nuclear periphery, raising the possibility that proximity to the NE facilitates silencing (for review see [16]). In Saccharomyces cerevisiae, for example, telomeres form clusters at the nuclear periphery [17]. Although tethering to the nuclear periphery has been shown to promote silencing, moving to the nuclear periphery is neither necessary nor sufficient for silencing [18,19].

Observations in higher eukaryotes also suggest a repressive role of the NE. For example, in human cells, gene-poor chromosomes tend to localize at the nuclear periphery as well as the inactive X chromosome or Barr body [20,21]. Several loci were found to localize near the NE in their inactive state and to change their nuclear localization upon induction of transcription. For example, the IgH locus moves away from the nuclear periphery in B cells concomitant with the initiation of V(D)J recombination [22]. Similarly, when the CFTR (cystic fibrosis transmembrane conductance regulator) gene is inactive, it preferentially associates with the nuclear periphery while in its actively transcribed state it associates with euchromatin in the nuclear interior [23].
Table 1

<table>
<thead>
<tr>
<th>Yeast</th>
<th>Files</th>
<th>Mammals</th>
<th>Location</th>
<th>Functions</th>
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<tbody>
<tr>
<td>Mlp1</td>
<td>Megator (Mtor)</td>
<td>TPR</td>
<td>Nuclear basket</td>
<td>Mtor is also present in the nuclear interior [80] Associates with active genes [28,39,40]** Mlp1/2 play a role in control of exported mRNA [81] TPR functions in the mitotic spindle checkpoint [82] Mlp1p is implicated in transcription memory [57]** TPR is required for the formation of heterochromatin exclusive zones [53]**</td>
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<tr>
<td>Nup1</td>
<td></td>
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<td>Nuclear basket</td>
<td>Phosphorylation of Nup1 is required for peripheral targeting of active INO1 and GAL1 genes [63]</td>
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<td>Nup60</td>
<td>Nup154</td>
<td>NUP155</td>
<td>Core</td>
<td>Drosophila Nup154 does not associate with chromatin [48]**</td>
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<td>Nup170</td>
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<td>Nic96 associates with transcribed genes [28] Mammalian NUP93 interacts with inactive chromosomal regions [52]</td>
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<td>Nic96</td>
<td>CG7262</td>
<td>NUP93</td>
<td>Core</td>
<td>Nic96 associates with transcribed genes [28] Mammalian NUP93 interacts with inactive chromosomal regions [52]</td>
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<td>Nsp1</td>
<td>Nup62</td>
<td>NUP62</td>
<td>Central channel</td>
<td>Nsp1 associates with genes that are moderately transcribed [28] Drosophila Nup62 associates with active genes [48]**</td>
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<td>Nup84</td>
<td>Nup107</td>
<td>NUP107</td>
<td>Core</td>
<td>Nup84 subcomplex mediates transcriptional activation [85] Nup84 subcomplex acts as a coordinator of SUMO-dependent repair pathway [74]<strong>. Nup154c associates with genes that are not highly transcribed [28] Drosophila Nup88 associates with inactive genes [47</strong>,48]**</td>
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<td>Nup145C</td>
<td>Nup96</td>
<td>NUP96</td>
<td>Nuclear ring</td>
<td>Dynamic nucleoporin [46] Drosophila Gp210 does not associate with chromatin [48]**</td>
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<td>Nup82</td>
<td>Nup88/Mbo</td>
<td>NUP88</td>
<td>Cytoplasmic filaments</td>
<td>Mobile nucleoporin [84] Drosophila Nup88 associates with inactive genes [48]**</td>
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<td>Pom152</td>
<td>Gp210</td>
<td>GP210</td>
<td>Trans-membrane</td>
<td>Mobile nucleoporin [84] Drosophila Gp210 does not associate with chromatin [48]**</td>
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Genome-wide studies performed both in Drosophila melanogaster and in human cells have revealed that lamin-bound genes are generally transcriptionally silent, late replicating and lack active histone marks. Interestingly, lamin-associated genes can be released from lamin upon transcriptional activation suggesting that perinuclear association promotes silencing [24**,25**]. All together these findings support the long-standing classical view of heterochromatin domains residing close to the nuclear membrane, and that the interaction of genes with lamins generally leads to gene repression (Figure 1).

Gene activation at the nuclear periphery

Several lines of evidence indicate that the nuclear periphery has a dual role in gene regulation, since it is not only involved in creating a repressive compartment but also promotes high levels of gene induction (Table 1). Already in 1985, the ‘gene gating’ hypothesis suggested...
that active genes associate with NPCs to increase the efficiency of nuclear export of transcribed RNA [26]. Support for this hypothesis comes from several studies done in S. cerevisiae. Among the first reports linking the NPC to gene activity showed that nucleoporin Nup2p functions as a boundary and blocks the spreading of heterochromatin into a reporter gene. Importantly, the insulation of the reporter gene from the surrounding heterochromatin involved its physical tethering to the NPC via Nup2 [27]. Later a genome-wide (ChIP-on-chip) approach demonstrated that Nups associate preferentially with transcriptionally active genes [28]. Nups have also been shown to associate with promoters of active genes [29]. Furthermore, a number of inducible genes including INO1, GAL1, HXK1 or HSP104 are targeted to the nuclear periphery (NPCs) upon activation [28,30,31] (Figure 1). One explanation is that gene–NPC association might be particularly important for inducible genes such as galactose and heat shock controlled promoters, which require rapid and high expression levels and export, which could be facilitated by their positioning closer to the NPCs. The chromatin remodeling complex SAGA controls expression of stress inducible genes and is connected to the mRNA export machinery by one of its components Sus1 that binds to the NPC [32].

What is the functional significance of NPCs–gene interaction? In yeast, targeting of certain genes to the nuclear periphery seems to involve nascent RNA transcripts [33–35]. However, since GAL1 or HSP104 gene association with the NPC can be disrupted without affecting expression levels, perinuclear localization of these genes may be a consequence rather than a cause of transcriptional activation [30,35]. Therefore, a model has been proposed where the GAL1 gene is first activated and then is ‘gated’ at the nuclear periphery [30]. In contrast, targeting of the INO1 gene to the nuclear periphery is not dependent on transcription [36], but is instead
controlled by DNA ‘zip codes’ in the promoter that enhance transcription [37**].

Given that most of the current evidence supporting ‘gene gating’ has been obtained in budding yeast, it leads one to question the evolutionary conservation of this phenomenon. In *Drosophila* the scenario appears more complex. For example in Schneider (SL-2) cells, Hsp70 genes not only localize nonrandomly at the nuclear periphery under nonheat shock conditions but their position also remains peripheral upon induction [38]. This peripheral localization is lost upon Xma-2 or E(y)2 depletion, however, Hsp70 mRNA levels are only reduced by 50% indicating that the peripheral localization of Hsp70 genes is not necessary for their expression. As further evidence for the role of the nuclear pore components in the regulation of active chromatin, Nup153, and Megator (Mtor) have been found to copurify with MSL complex members, which participate in the X chromosome transcriptional hyperactivation during dosage compensation in *D. melanogaster* [39]. The male X chromosome was shown to be enriched in nucleoparin associated regions (NARs) that frequently reside closer to the nuclear periphery [40**]. Future studies will reveal how general functions of these proteins impact on specific processes such as X chromosome regulation.

In vertebrate cells, the DNaseI sensitive chromatin localizes preferentially at the nuclear periphery [41]. More recently it was shown that at the time of activation, the β globin locus is localized at the nuclear periphery and only moves into the nuclear interior at a later time point [42]. Support for this model comes from the study of repositioning of the Th2-specific transcription factor loci during Th1 differentiation [43]. Furthermore, the IFNγ position is localized at the nuclear periphery even under induced conditions, arguing strongly that the nuclear periphery cannot be an indiscriminately repressive environment.

**Role of nuclear pore components off the pore**

The spatial restriction of chromatin movement in the interphase nucleus [44,45] makes it highly unlikely that all genes need to relocalize to the NPC to be activated. Therefore, the coupling of transcription and export might be conserved in metazoan Nups at intranuclear active sites, where they can serve as a platform for coregulated assembly of transcription machinery and mRNA export factors. Support for this hypothesis comes from the fact that some Nups are mobile and that the dynamics of mammalian Nup98 and Nup153 is dependent on active transcription [14,15,46], possibly establishing a functional connection between sites of production of mRNAs and NPCs. One important question is therefore whether the chromatin–Nup interaction reported in yeast to happen at the NE can also take place in the nuclear interior.

Using polytene chromosome stainings, it has been reported that various *Drosophila* Nups, including Nup98, Sec13, Nup50, and mAb414-positive Nups, associate with active loci that often localize in the nucleoplasm whereas Nup88 associates preferentially with inactive loci [47**.48**]. Furthermore, Nup153 and Mtor bind large domains (NARs) of about 10-500 kb called NARs that demarcate regions of open chromatin and active transcription [40**]. NARs were shown to be at the nuclear periphery as well as in the nucleoplasm [40**]. These findings support the idea of a NPC duality in gene regulation and suggest that Nups could target different sets of genes based on their transcriptional state [47**]. Since some Nups are found associated with the pore or located in the nucleoplasm, an alternative possibility is that different pools of the same Nup may exhibit different functions depending on their subnuclear location. Nup98 and Nup50 have been shown to be associated with both expressed and moderately transcribed genes [48**]. The bound-expressed genes generally localize inside the nucleus whereas the bound-poorly expressed genes localize at the periphery. Therefore, it has been proposed that nucleoplasmic NPC components activate the expression of internally localized genes whereas DNA–NPC interaction leads to gene silencing [48**]. These observations support a role of Nups in nuclear compartmentalization for gene expression regulation also in the nucleoplasm (Figure 1).

**Nucleoporins as regulators of the transcription process**

Several chromosomal translocations in acute myeloid leukemia (AML) result in fusion proteins containing the FG repeat part of NUP98 and members of the homeobox transcription factor family such as HoxA9. These oncogenic fusion proteins are able to activate or repress target genes within the nucleoplasm [49–51]. Interestingly, a genome-wide study of NUP93-chromatin association in vertebrate cells revealed changes in NPC–chromatin interactions based on histone modification status of chromatin [52].

The observations that the absence of some Nups such as Nup98 and Sec13 abolishes the recruitment of RNA PolII on target genes [47**], that the mobility of NUP98 and NUP153 is closely associated with ongoing transcription [14,15], and that loss of Nup153, Mtor or Nup98 affects global gene expression [40**.48**] indicate that Nups might function as direct or indirect regulators of the transcription process possibly by delivering transcriptional activators to genes that are expressed. Interestingly, in mammalian cells, TPR, a component of the nuclear basket, is required for the formation and maintenance of heterochromatin exclusive zones (HEZs) that could facilitate the access of large cargo, including transcription or transport associated complexes, to the NPC [53**] (Figure 1).
Furthermore, Nups have been implicated in different steps of transcription. For example, Nup98 and Sec13 recruitment precedes or coincides with RNA pol II recruitment on target genes. Their down-regulation leads to impairment in the recruitment of RNA pol II suggesting that these Nups are involved in the early stages of transcription initiation [47**]. This finding is in agreement with a previous yeast study that reports interaction between genes promoters and Nup2 and propose that contact with pores may be a general feature of gene activation [29]. Interestingly, Nup binding sites have also been mapped on the body of active genes [28]. These studies suggest that Nups may also regulate transcription elongation or the recycling of polymerases to the promoters for reinitiation [47**]. Future work will be instrumental in unraveling the possible molecular mechanisms by which Nups could influence transcription.

**Nucleoporins facilitating transcriptional memory**

In yeast, formation of a gene loop between the 5’ and 3’ end of a gene has been shown to play an important role in transcriptional regulation termed ‘transcriptional memory’ that enables past events to be ‘remembered’ [54,55]. Gene loops are dynamic structures whose formation is dependent on active transcription and components of the RNA processing machinery [54,55].

Tethering of genes at the NPC is reported to facilitate transcriptional memory [56] (Figure 1). Interestingly, Mlp1, a NPC component, plays a role in the maintenance of the gene loop structure [57**]. Consistent with this, Mlp1 displays a 5’/3’ distribution pattern on the HXK1 gene at time points coincident with gene loop formation [57**]. Therefore, one possible mechanism of how NPC localization could enhance gene expression is by inducing or stabilizing loop formation [57**]. Looping appears not to be a unique feature in yeast. The HIV provirus forms a loop between the 5’ long terminal repeat (LTR) and poly(A) signal, also in a transcription-dependent manner [58]. Dynamic promoter-terminator loops have been described for the breast cancer BRCA1 gene [55], and at the genes encoding the immunohistological marker CD68 in mammalian cells [59]. In D. melanogaster, looping of the HOX genes correlates with their repression and involves CTCF [60]. It is tempting to speculate whether Mtor (closest functional homologue of Mlp1) could also enhance gene expression by contributing to gene looping in Drosophila.

One player implicated in transcriptional memory is the histone variant H2AZ. This factor has been shown to be required for the association of recently shut-off genes with nuclear periphery indicating that the chromatin state also plays a role in gene–NE interactions [36]. However, a recent study attributes the H2AZ function in general to GAL1 gene regulation rather than to transcriptional memory [61]. Interestingly, a DNA sequence called memory recruitment sequence (MRS) has been identified in the promoter of the budding yeast INO1 gene which mediates INO1 association with the NPC after transcriptional shut-off. The MRS is required for the incorporation of the histone variant H2A.Z, which is also necessary for INO1 transcriptional memory [37**]. Future studies will reveal whether sequence-dependent tethering could also be utilized by genes in other organisms.

**Factors contributing towards gene dynamics**

Multiple factors have been implicated in the relocation of active genes to the NPC, including transcriptional activators, mRNA processing and export factors, and distinct NPC subunits. The mechanisms allowing gene movement remain unclear. It is possible that intranuclear chromatin-binding Nups shuttle between genomic sites and the nuclear periphery thus acting as transport factors to target genes from one location to another.

Progression through the cell cycle is an important factor contributing to chromatin. In yeast active INO1 and GAL1 genes localize at the nuclear periphery during G1 and G2/M, but move to the nucleoplasm during S phase. Furthermore, phosphorylation of Nup1, a component of the NPC, by the cyclin dependent kinase (Cdk1) has shown to be necessary for targeting active INO1 and GAL1 to the nuclear periphery [63]. These findings suggest that post-translational modification of Nups could also play an important role for dynamic NPC–DNA interactions during the cell cycle.

Nuclear actin and myosin, as well as myosin-like and actin-related proteins have been proposed as candidates that could contribute to the organization of transcription in the interphase nucleus. Indeed, actin is found not only as part of the filamentous cytoskeleton, but also in various large chromatin modifying complexes that are exclusively nuclear [64–66]. Furthermore, actin-related proteins are also components of chromatin remodelers and are conserved from yeast to human [67]. Interestingly, actin-related protein Arp6, which is also a component of the chromatin remodeling complex SWR1, was recently shown to mediate localization of ribosomal protein genes to the nuclear periphery [68].

Interestingly, a recent genetic screen performed to comprehensively assess the role of essential factors in NPC localization, structure, and assembly into the NE has led to the identification of multiple components of the RSC chromatin remodeling complex including the essential ATPase catalytic subunits Sth1, RSC8, RSC58, and ARP9 in S. cerevisiae [69]. Consistently, several earlier reports also observed a link between NPCs and RSC [70,71].

These studies not only provide a functional link between the chromatin remodeling complexes and the nuclear
Concluding remarks
Recent progress has shed new light on the role of nuclear periphery components beyond nucleocytoplasmic transport. It appears that the nuclear pore components play an integral role in nuclear architecture, gene expression and genome stability by providing a supporting platform for tethering various molecules. This multifunctional platform serves to ensure the efficient control of gene expression at the transcriptional and post-transcriptional levels. Future studies will provide a better understanding of how components of the NPC execute their function on and off the pores.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


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See annotation to Ref. [25].


33. Abruzzi KC, Belostotsky DA, Chekanova JA, Dower K, Rosbash M: 3’-end formation signals modulate the association of genes with the nuclear periphery as well as mRNP dot formation. EMBO J 2006, 25:4253-4262.


This study identifies nucleoporins as a major class of global regulators of gene expression in Drosophila melanogaster.


This report identifies TPR protein as an essential determinant of the perinuclear organization, with a direct role in forming a morphologically distinct nuclear sub-compartment and delimited heterochromatin distribution.


Here, myosin-like protein 1 (Mlp1) is shown to be important for the formation of gene loop structures required for the transcriptional memory.


