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Journal of Cell Science 123, 1989-1999

doi:10.1242/jcs.053694

Summary

It is now well established that the position of a gene within the nucleus can influence the level of its activity. So far, special emphasis has been placed on the nuclear envelope (NE) as a transcriptionally silent nuclear sub-domain. Recent work, however, indicates that peripheral localization is not always associated with repression, but rather fulfills a dual function in gene expression. In particular, in the yeast *Saccharomyces cerevisiae*, a large number of highly expressed genes and activated inducible genes preferentially associate with nuclear pore complexes (NPCs), a process that is mediated by transient interactions between the transcribed locus and the NPC. Recent studies aimed at unraveling the molecular basis of this mechanism have revealed that maintenance of genes at the NPC involves multiple tethers at different steps of gene expression. These observations are consistent with tight interconnections between transcription, mRNA processing and export into the cytoplasm, and highlight a role for the NPC in promoting and orchestrating the gene expression process. In this Commentary, we discuss the factors involved in active gene anchoring to the NPC and the diverse emerging roles of the NPC environment in promoting gene expression, focusing on yeast as a model organism.

This article is part of a Minifocus on exploring the nucleus. For further reading, please see related articles: 'The nuclear envelope at a glance' by Katherine L. Wilson and Jason M. Berk (*J. Cell Sci.* **123**, 1973-1978) and 'Integrating one-dimensional and three-dimensional maps of genomes' by Natalia Naumova and Job Dekker (*J. Cell Sci.* **123**, 1979-1988).

Key words: Nuclear pore complex, NPC, Transcription, mRNA export, mRNA surveillance, Yeast

Introduction

The positioning of a gene within the nucleus is nonrandom and can influence its expression level. In particular, the nuclear periphery has well-documented effects on transcription. Indeed, heterochromatin and developmentally repressed genes are often found in association with the nuclear lamina in metazoan cells. In yeast, peripheral localization promotes silencing of telomeres and the mating type loci because of the enrichment of silencing factors in this nuclear subcompartment (Andrulis et al., 1998; Andrulis et al., 2002; Finlan et al., 2008; Gartenberg et al., 2004; Guelen et al., 2008; Hediger and Gasser, 2002; Hediger et al., 2002; Makatsori et al., 2004; Pickersgill et al., 2006; Reddy et al., 2008; Taddei et al., 2004; Taddei et al., 2009). Thus, the presence of repeats that sequester factors limiting for heterochromatin formation appears to be responsible for creating a repressive environment at the nuclear periphery. However, high-resolution images of mammalian nuclei showed early on that the heterochromatin patches at the nuclear periphery are interrupted by light euchromatin staining at nuclear pore complex (NPC) locations. These observations provided the basis of the 'gene gating' hypothesis speculating that compact chromatin associates with the nuclear membrane, whereas actively transcribed chromatin associates with nuclear pores, contributing to efficient nuclear mRNA export (Blobel, 1985). The presence at the nuclear periphery of DNase-I-hypersensitive chromatin in vertebrate cells was another early sign that active genes might associate with the nuclear envelope (NE) (Hutchison and Weintraub, 1985). The confirmation of the existence of active chromatin at the nuclear periphery only recently emerged in studies of budding yeast: it was discovered that, not only does a set of highly transcribed genes associate with NPC components, but a number of inducible genes (including *GAL* genes, *SUC2, INO1* and *HXK1*) are also specifically recruited to the nuclear periphery upon transcriptional activation (Brickner and Walter, 2004; Casolari et al., 2004). Live-cell four-dimensional (4D) imaging experiments revealed that recruitment is paralleled by a change in the sub-nuclear distribution of genes and a reduction in their mobility, resulting in constrained movement along the NE. The limited sliding movement of activated genes along the NE suggested that gene tethering is mediated by transient interactions between the transcribed locus and pore components. It should be noted, however, that association with the nuclear periphery is not obligatory for gene activation (Cabal et al., 2006; Taddei et al., 2006).

Nuclear pores have also been associated with increased gene expression in species other than budding yeast. Notably, in *Drosophila melanogaster*, dosage compensation is achieved by doubling the transcript levels of X-linked genes in males and coincides with X-chromosome enrichment at the nuclear periphery, probably through an interaction with NPC proteins (Mendjan et al., 2006). In addition, the six heat-shock protein 70 (*hsp70*) gene loci have been reported to contact the NPC before and after transcription activation, and this association is necessary for efficient gene expression and mRNA export (Kurshakova et al., 2007b). Moreover, the malaria parasite *Plasmodium falciparum* undergoes antigenic variation to evade host immune responses through switching of variant surface proteins encoded by the *var* gene family. These genes are positioned at the nuclear periphery independently of their transcription state, but relocalize to two distinct peripheral sites when activated. This dual localization is essential for repression as well as for the switch between repressive and activated states, presumably because activation involves repositioning to a peripheral location permissive for transcription

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(Duraisingh et al., 2005; Ralph et al., 2005). In mammalian cells, the gene encoding interferon-γ is constitutively associated with the nuclear periphery, even when primed for expression during, for example, T-helper-cell differentiation (Hewitt et al., 2004). Another example in mammalian cells is the β-globin locus, which localizes at the nuclear periphery at the time of activation and moves to the nuclear interior at a later stage (Ragoczy et al., 2006). However, it is not yet known whether the sub-peripheral compartment permissive for transcription in *Plasmodium* and mammalian cells corresponds to the NPC, neither has it been shown that this localization contributes to high expression levels.

Together, these data are in agreement with the visionary gene-gating hypothesis and imply that the nuclear periphery is divided into at least two distinct parts involved in the functional organization of chromatin: a repressive zone at the nuclear membrane and a transcription-permissive zone in the sub-compartment that is defined by nuclear pores.

NPCs form aqueous channels that span the NE, and function as gatekeepers of all macromolecular trafficking between the nucleus and the cytoplasm (Box 1). Their essential role in transport indicates that NPCs have a crucial role in regulating gene expression by controlling the access of transcription factors to the genome as well as by regulating mRNA export (Capelson and Hetzer, 2009). However, recruitment of genes to the NPC suggests that, beyond their core function as gatekeepers, nuclear pores in addition contribute to chromatin organization and transcriptional regulation and thereby impact the two endpoints of nuclear gene expression: the production of mRNA and its eventual export to the cytoplasm for translation. Consistent with the reversible maintenance of expressed genes at the NPC, there is growing evidence that the gene-gating mechanism involves multiple tethers at different stages of gene expression. These contacts are maintained through chromatin-to-pore and nascent mRNA-to-pore interactions. In this Commentary focused on yeast, we summarize the known tethers implicated in gene anchoring and explore how the NPC environment promotes and coordinates various gene expression events.

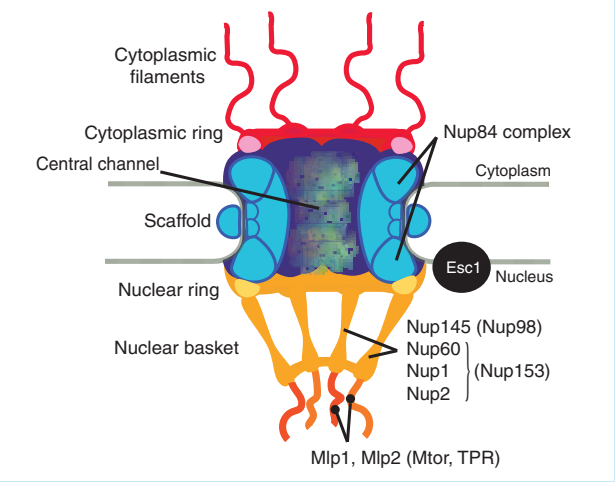
NPCs and transcription activation

Gene recruitment to the NPC relies on transcription activation

When gene-to-pore recruitment was discovered, one goal was to understand the mechanism that drives the genes toward the NPC in coordination with transcription activation. Visual inspection of gene trajectories revealed that, when repressed, specific loci are very dynamic, randomly exploring a broad volume of the nuclear interior (Cabal et al., 2006; Taddei et al., 2006). Gene-NPC gating, therefore, probably relies on the kinetics of these Brownian (i.e. random) movements and the increased affinity of activated genes for nuclear pores, resulting in them becoming tethered at this location when randomly entering the NPC environment. RNA fluorescence in-situ-hybridization analyses on *GAL* gene transcripts revealed that, within a cell population, most genes that undergo NPC recruitment are highly expressed (Cabal et al., 2006), suggesting that the mRNA export machinery connects the nascent mRNA and the NPC. However, these experiments also revealed that some genes were transcribed but not associated with the nuclear periphery, indicating that mRNA production, if required, is not sufficient for stable localization at the NPC, nor is gene-NPC association an absolute requirement for the transcription of these genes in yeast. In line with this observation, induction of the glucose-repressed *HXK1* gene through an alternative pathway (using a LexA-targeted VP16-activation domain) resulted in the loss of perinuclear anchoring without affecting *HXK1* transcription. Therefore, initial steps of gene recruitment to the NPC might depend neither on the transcription process per se nor on mRNA production, but probably more on upstream specific activation events (Taddei et al., 2006).

Transcription activation in eukaryotes usually starts with the binding of activators at upstream activating sequences (UAS), which promote chromatin remodeling at the more downstream promoter. Subsequent assembly of the pre-initiation complex (PIC) at the TATA box directs transcription initiation by RNA polymerase II (RNA Pol II). At *GAL* genes, the Gal4 activator binds to the UAS and recruits the SAGA co-activator complex upon cellular activation in galactose-containing growth medium. Using a new

Box 1. Schematic view of NPC structure and composition



Although its mass increases from yeast (60 kDa) to vertebrate (120 kDa), the overall NPC structure is evolutionarily conserved (Alber et al., 2007). It displays an eightfold symmetry around the central axis and comprises an NE-embedded scaffold surrounding a central transport channel. The NPC also comprises two rings from which extend the cytoplasmic filaments and nuclear basket. The NPC consists of multiple copies of about 30 distinct conserved proteins called nucleoporins. FG-nucleoporins (such as Nup1, Nup2, Nup60 and Nup145), which directly interact with transport receptors through their FG-repeat domain, are thought to create a continuous surface that extends from the cytoplasmic filaments through the central channel to the nuclear basket (Suntharalingam and Wente, 2003). Although it is associated with the NPC, Nup2 is dynamic and shuttles between the NPC and the nucleoplasm (Dilworth et al., 2001). Nup145 and Nup60 are involved in the anchoring at the nuclear basket of Mlp1 and Mlp2 (Feuerbach et al., 2002), two related filamentous proteins that are involved in mRNP surveillance (Galy et al., 2004). Nup1 serves as a docking site for TREX2, the NPC-associated mRNA export machinery (not shown) (Fischer et al., 2002). The Nup84 complex, which is located on both sides of the NPC, has been shown to be involved in NPC structure, and overlaps in function with Nup60, Mlp1 and Mlp2 (Palancade et al., 2007). Esc1 is located at the inner nuclear membrane and contributes to NPC structure and function (see text for references). The scheme primarily describes the yeast NPC proteins, and the names of equivalent proteins in *Drosophila* or mammals are indicated in parentheses.

method designed to map the genomic interaction sites of the nucleoporin Nup2 in vivo through fusion of this nucleoporin to micrococcal nuclease (known as chromatin endogenous cleavage, ChEC), Schmid et al. reported that Nup2 association with the activated *GAL1* locus occurs at the promoter and requires the UAS and TATA-box elements as well as the UAS-binding activator Gal4 (Schmid et al., 2006). This study also showed that neither the histone acetyltransferase SAGA co-activator complex recruited by Gal4, nor TBP (TATA-binding protein), nor active transcription are required for Nup2 interaction with promoters, suggesting that gene-NPC association is mediated by events preceding transcription initiation. However, this study did not include a proper negative control to map the chromatin sites hypersensitive to soluble micrococcal nuclease. Furthermore, a quantitative peripheral localization of the genes was not performed, leaving open the possibility that binding of Nup2 to chromatin could occur owing to non-NPC-associated soluble Nup2 (Dilworth et al., 2001; Dilworth et al., 2005). Consistently, the contribution of Nup2 in the recruitment of activated genes to the NPC is subject to controversy (Cabal et al., 2006). However, in perfect agreement with the previous findings, recruitment of *INO1* to the nuclear periphery occurs prior to the rapid accumulation of mRNA and is not affected by global inactivation of RNA Pol II (Brickner et al., 2007). Moreover, using genomic deletions, we found that transcription-induced repositioning of the *GAL2* gene primarily depends on the promoter and not on the coding and 3′UTR sequences (Dieppois et al., 2006). Therefore, although peripheral gene recruitment might function to couple transcription and mRNA export, an initial anchoring event of some genes appears to rely primarily on early activation events rather than on transcription per se (Fig. 1).

Several studies, however, revealed that the SAGA co-activator complex, which acts at a later stage during transcription activation (Larschan and Winston, 2001), might represent a key factor in promoting gene recruitment to the nuclear periphery. This story began with the identification of Sus1 (Rodriguez-Navarro et al., 2004), a small protein that physically interacts with the transcriptional co-activator SAGA through the H2B de-ubiquitylating subcomplex composed of Sgf73, Sgf11 and Ubp8 (Kohler et al., 2006; Kohler et al., 2008). In addition to interacting with SAGA, Sus1 also interacts with the transcription and export complex 2 (TREX2), a complex composed of Sac3, Thp1 and Cdc31 that is associated with the NPC and is involved in mRNA export (Fischer et al., 2004; Fischer et al., 2002; Gallardo et al., 2003) (also discussed below). The proteasomal protein Sem1 is a versatile protein that has recently been described as an additional component of TREX2 and that stabilizes this mRNA-export complex at the NPC (Faza et al., 2009). The crystal structure of Sus1 complexed with Sac3 and Cdc31 has recently been obtained (Jani et al., 2009). The co-sedimentation of a fraction of Thp1 and Sac3 with SAGA components suggested the existence of a SAGA-TREX2 supercomplex, which is proposed to facilitate mRNA export by physically tethering the activated gene locus to the NPC (Rodriguez-Navarro et al., 2004). This view was confirmed by several groups, who established that the SAGA components Sgf73, Ada2 and Sus1, the TREX2 components Sus1, Thp1 and Sac3, as well as their NPC anchor, the nucleoporin Nup1, are required for detectable gene-NPC association by microscopy (Cabal et al., 2006; Chekanova et al., 2008; Kohler et al., 2008) (Fig. 1).

More recent studies revealed that loss of the SAGA component Sgf73 affects the integrity of both SAGA and TREX2, reducing the recruitment of Sus1 to both complexes. Together with gene localization experiments, these observations indicate that SAGA and TREX2 act synergistically to recruit Sus1 to chromatin, and to promote gene-NPC anchoring (Jani et al., 2009; Kohler et al., 2006; Pascual-Garcia et al., 2008). In light of these findings and because of the earlier observation that the interaction of Nup2 with *GAL* gene promoters does not require SAGA components (Schmid et al., 2006), it is conceivable that gene repositioning to the NPC occurs mainly in two steps: the interaction of specific promoter-bound transcription activators with nucleoporins mediates the initial binding to the NPC, whereas the subsequent recruitment of SAGA might stabilize gene-NPC anchoring by interacting with TREX2 at a minimum. Moreover, chromatin immunoprecipitation (ChIP) experiments revealed that the NPC-associated protein Mlp1 binds to the UAS of active genes through direct interactions with SAGA components (Luthra et al., 2007). These data, and the fact that Mlp1 is required for efficient gene-NPC anchoring (Dieppois et al., 2006; Tan-Wong et al., 2009), suggest that – in addition to TREX2 – the interaction between Mlp1 and SAGA contributes to stabilizing gene-NPC associations during and following the early transcription activation process (Fig. 1).

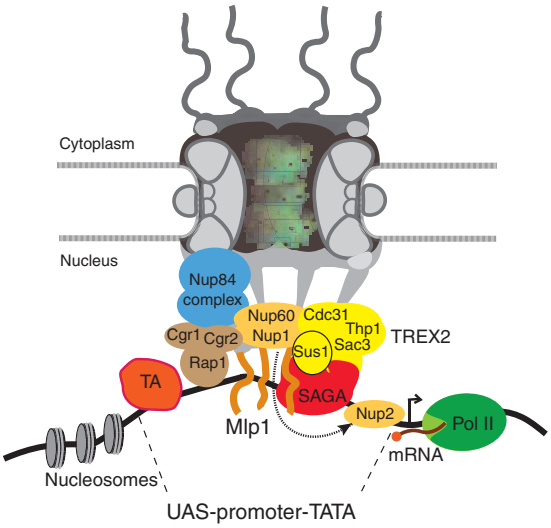


Fig. 1. Gene gating at the NPC during transcription activation. Genes become tethered to the nuclear periphery during transcription activation. Nup2 was shown to associate with active promoters and might participate in initial steps of gene-NPC tethering following activation by early transcription activators (TA) or chromatin remodellers (Dilworth et al., 2005; Ishii et al., 2002; Schmid et al., 2006). In addition to the proposed tethering mediated by Nup2, genes are tethered to the NPC through interaction of the SAGA co-activator complex with the NPC-bound TREX2 complex (consisting of Sac3, Thp1, Sus1 and Cdc31) (Cabal et al., 2006; Fischer et al., 2004; Fischer et al., 2002; Rodriguez-Navarro et al., 2004). The nuclear basket Mlp proteins might also contribute to gene anchoring, as they bind the promoter of active genes through interactions with SAGA subunits (Dieppois et al., 2006; Luthra et al., 2007). The nucleoporins Nup1 and Nup60 are the docking sites of TREX2 and Mlp1, respectively (Feuerbach et al., 2002; Fischer et al., 2002). It is proposed that the NPC Nup84 promotes gene activation by recruiting the Rap1-Gcr1-Gcr2 complex, facilitating its loading onto promoters of genes that reach the NPC (Menon et al., 2005). NPC association is a consequence of transcription activation that might contribute to optimal gene expression. However, NPC tethering is not necessary for gene activation.

Role of the NPC in transcription activation

Several studies have highlighted a role of the NPC in transcription by showing that artificial tethering of genes to the NPC favors their expression (Brickner and Walter, 2004; Ishii et al., 2002; Menon et al., 2005; Taddei et al., 2006). In agreement with a conserved role of gene anchoring, recent studies in *D. melanogaster* revealed that the Sus1 homologue ENY2 is required for the association of the *Hsp70* gene cluster at the nuclear periphery, that it interacts with transcription factors and the mRNA export machinery, and that peripheral localization is necessary for efficient *Hsp70* gene expression (Kurshakova et al., 2007b). To explain this phenomenon, the idea of a 'reverse recruitment mechanism' emerged, which speculated that the NPC represents a platform containing the pre-assembled transcriptional machinery (Menon et al., 2005). One of the proposed models is that the Nup84 nucleoporin complex binds to the Rap1-Gcr1-Gcr2 activation complex and facilitates its recruitment to Rap1-controlled genes that relocate to the NPC early during transcription activation in yeast (Casolari et al., 2004; Menon et al., 2005) (Fig. 1). The fact that Mlp1 interacts with promoters and several transcription regulators, including SAGA components (Luthra et al., 2007), also suggests a direct role for Mlp proteins in modulating the recruitment and/or activity of SAGA at the UAS of NPC-associated genes. Additional studies in *D. melanogaster* indicate a link between dosage compensation and the NPC. Indeed, the binding of the MSL (male-specific lethal) complex all along the unique male X chromosome results in a twofold upregulation of all X-linked genes and correlates with X-chromosome localization at the nuclear periphery, possibly through an interaction of MSL with Nup153 and Mtor (homologous to yeast Nup60 and Mlp1/2, respectively) (Mendjan et al., 2006) (Box 1). However, these two pore proteins reversibly associate with the NPC (Rabut et al., 2004; Zimowska et al., 1997), and whether peripheral localization is essential for X-chromosome upregulation is under debate (Grimaud and Becker, 2009). Moreover, recent studies in *D. melanogaster* show that several other loosely associated NPC components (including the FG-nucleoporins Nup98, Nup50 and Nup62) contribute to the expression of developmentally and cell-cycle-regulated genes through binding to these loci in the nucleoplasm, away from the nuclear periphery (Capelson et al., 2010; Kalverda et al., 2010). Finally, in mammals, recent mechanistic studies show that leukemogenic chimeric proteins that contain the DNA-binding domains of specific transcription factors fused to FG-nucleoporin-repeat domains are able to transactivate or transrepress target genes. These activities correlate with the ability of FG-repeats to associate with the histone acetyltransferase CBP/p300 (Kasper et al., 1999; Wang et al., 2007) or the histone deacetylase HDAC1 (Bai et al., 2006), and provide additional evidence of a role for FG-nucleoporins in modulating chromatin structure and function, probably away from the NPC environment. Thus, genes might relocate to pores in the relatively small yeast nucleus. However, in the larger metazoan nuclei, mobile nucleoporins might move towards genes to regulate their expression within the nucleoplasm.

Another view is that the NPC favors gene expression by regulating the activity of transcription factors through specific post-translational modification. This possibility is supported by the observation that, upon cellular activation in galactose-containing medium, the yeast glucose repressor Mig1 is removed from the promoter of glucose-repressed genes through phosphorylation and is subsequently exported into the cytoplasm. This phosphorylation specifically occurs at the nuclear pore, indicating a role for the NPC in transcriptional regulation through post-translational modification of repressors (Sarma et al., 2007). Along the same lines, it is known that Ulp1, the main small ubiquitin-like modifier (SUMO) protease in yeast, is mainly localized at the NPC through interaction with Mlp proteins (Zhao et al., 2004). We recently obtained evidence in favor of a model whereby the NPC might regulate the activity of sumoylated transcription factors at the nuclear periphery by restricting Ulp1 localization at the pore (G.D., unpublished data).

Role of the NPC in chromatin insulation and loop formation

The NPC is a highly stable structure (D'Angelo et al., 2009) that directly contacts active chromatin in the mainly silenced environment of the nuclear periphery. This raises the possibility that NPCs might favor gene expression because they are involved in protecting active domains from invading heterochromatin. In a screen for chromatin boundary activities in *S. cerevisiae*, Ishii et al. reported that artificial tethering of a partially silenced mating-type locus to the NPC – through association on both sides with Nup2 or the NPC-associated transport receptors Mex67, Cse1 or Los1 – was sufficient to block the spreading of heterochromatin and to activate the silenced locus, presumably through the formation of a protected gene loop (Ishii et al., 2002). As neighboring loci are also recruited to the NPC upon gene activation, it is possible that pore proteins naturally function as boundaries. Interestingly, it was proposed that the boundary activity displayed by Nup2 is mediated by an interaction between Nup2 and Prp20, a Ran guanylyl-nucleotide exchange factor (RanGEF) (Dilworth et al., 2005). Prp20 preferentially occupies silent chromatin and becomes excluded upon transcription activation (Casolari et al., 2004), suggesting it is involved in the transition between inactive and active chromatin states. Furthermore, Prp20-bound histones show a pattern of modifications that is typical for both heterochromatin and euchromatin, indicating that Prp20 delimits transcriptionally active and silenced regions (Dilworth et al., 2005). As Prp20 favors the production of RanGTP required for cargo release into the nucleus, one view is that Prp20 facilitates the dissociation of chromatin remodelers or transcription factors from their import receptors in the vicinity of the pore, thereby promoting transcription activation in this location. Additional genetic interactions provided evidence of a role in this process for the H2A variant Htz1, which has been proposed to act as a boundary factor in yeast (Dilworth et al., 2005; Meneghini et al., 2003) (Fig. 2). In agreement with a conserved function of the NPC as an insulator, the Su(Hw)-dependent insulator of the gypsy transposon often associates with the nuclear periphery and requires ENY2, the *D. melanogaster* Sus1 homologue, for its barrier activity (Kurshakova et al., 2007a), indicating a structural and active role of the NPC in isolating chromatin domains.

Interestingly, genome-wide mapping indicated a preferential association of Nup2 and Mlps with the 5′ and 3′ ends of active genes, respectively (Casolari et al., 2005; Schmid et al., 2006), suggesting that the gene extremities are brought close to each other at the pore. The NPC could, therefore, establish active chromatin domains by arranging DNA in a loop conformation, a hallmark of 'genuine' insulator function (Burgess-Beusse et al., 2002; Gerasimova et al., 2000; Ishii et al., 2002; Noma et al., 2006; Xu et al., 2004). Accordingly, chromatin loops connecting the 5′ and 3′ ends of genes are formed upon transcription activation of a number of genes through interactions between

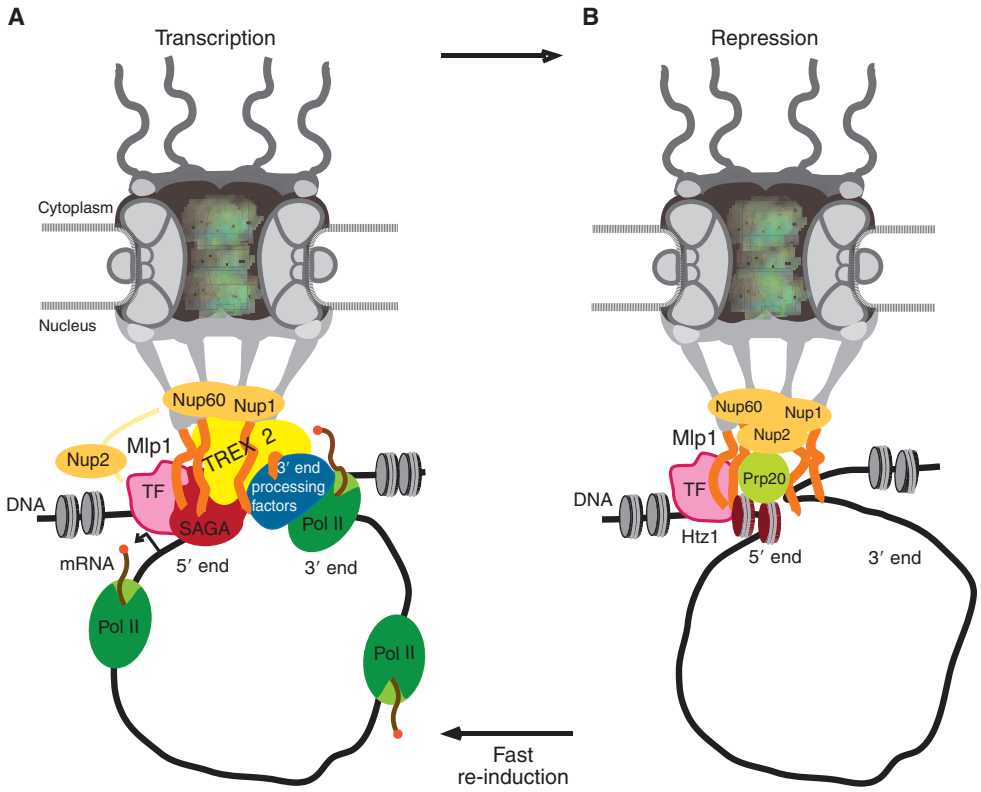


Fig. 2. Gene looping and transcriptional memory at the NPC. (**A**) Gene tethering and loop formation during active transcription. Activated genes adopt a loop conformation through interactions between transcription factors and mRNA-processing factors located at the promoter and 3′ end of genes, respectively (Ansari and Hampsey, 2005; O'Sullivan et al., 2004; Perkins et al., 2008; Singh and Hampsey, 2007). Mlp1 contributes to gene-NPC anchoring (Dieppois et al., 2006) and might be directly involved in stabilizing the loop scaffold through binding the promoters (via SAGA) as well as the 3′ ends of active genes (Casolari et al., 2005; Luthra et al., 2007; Tan-Wong et al., 2009). (**B**) In repressive conditions, Mlp1 is involved in memory gene loops (MGLs) by maintaining the gene loop at the NPC for 1 hour after removal of RNA Pol II (Tan-Wong et al., 2009). Upon reinduction, transcription initiates more rapidly due to the retention of transcription factors (TF) in the loop scaffold. Notably, formation of gene loops is not always linked to transcription memory (Tan-Wong et al., 2009). In addition, the NPC displays boundary activity that involves Prp20 interactions both with the chromatin of repressed genes and with Nup2 at the NPC (Dilworth et al., 2005; Ishii et al., 2002). Prp20 might facilitate Htz1 incorporation into promoter nucleosomes at early stages of repression. Fast reactivation involves Htz1 insulator activity and/or Htz1-dependent maintenance of the gene in the NPC optimal environment for transcription (Brickner et al., 2007; Meneghini et al., 2003).

mRNA transcription and mRNA 3′-processing factors (Ansari and Hampsey, 2005; O'Sullivan et al., 2004; Perkins et al., 2008; Singh and Hampsey, 2007). These loops were proposed to enhance expression by facilitating recycling of RNA Pol II to the 5′ end of the gene after each round of transcription. Interestingly, Tan-Wong et al. recently showed that Mlp1 associates with both the 5′ and 3′ ends of NPC-recruited genes, and is required for their maintenance in a loop conformation after transcriptional repression, rather than for initial loop formation upon transcriptional activation (Tan-Wong et al., 2009). Thus, the chromatin-Mlp1 interactions that occur upon gene gating might stabilize the previously formed loop structure between the promoter and the 3′ end, even after the removal of RNA Pol II from the template. A current model proposes that the loop scaffold maintained by interaction of chromatin with the NPC might retain transcription factors, allowing faster loading of RNA Pol II and thereby kinetically enhanced transcription activation upon gene re-induction after short-term repression, a phenomenon that has subsequently been termed MGL for memory gene loop (Tan-Wong et al., 2009). The importance of 3′UTR sequences in the NPC tethering of some genes might reflect their role in loop stabilization (Abruzzi et al., 2006; Taddei et al., 2006). A role for gene loops in transcription memory was confirmed by the Hampsey lab (Laine et al., 2009); however, gene loops do not necessarily result in transcription memory (Tan-Wong et al., 2009) (Fig. 2).

Along this same line of thought, a study by Brickner et al. (Brickner et al., 2007) proposed that the recruitment of active genes to the NPC facilitates Htz1 incorporation into promoter nucleosomes, although these findings have been the subject of controversy (Tan-Wong et al., 2009; Zacharioudakis et al., 2007). Htz1, in turn, was suggested to retain the gene at the NPC and to facilitate fast transcriptional reactivation, either through its insulator activity or through maintaining the gene in an activation-optimal environment over several cell divisions (Brickner et al., 2007).

Gene-NPC anchoring and coordination of mRNP biogenesis during transcription elongation

The export machinery is recruited by the RNA Pol II elongating complex

The observation that gene-to-pore localization enhances gene expression suggests that the NPC also facilitates the coordination of the multiple mRNA maturation steps that are required before mRNA export can occur. Although transcription elongation might

not be determinant for the gene-gating process, NPC association might influence mRNA biogenesis and enhance the coupling of 3′-end processing with mRNA ribonucleoprotein particle (mRNP) release and exit through nuclear pores. In eukaryotes, the C-terminal domain (CTD) of RNA Pol II tightly links mRNA transcription and processing by recruiting factors involved in 5′-end capping, splicing, 3′-end cleavage and polyadenylation to nascent transcripts, resulting in the formation of mRNPs that are competent for export. The yeast heterodimeric export receptor Mex67-Mtr2 (TAP-p15 or NFX1-NXT1 in mammals) mediates the translocation of mature mRNPs through the NPC via direct interactions with the TREX2 component Sac3 and FG-nucleoporins lining the pore. Notably, Mex67 binds to mRNA poorly and requires adaptor proteins such as Yra1 (also known as REF), the DEAD-box ATPase Sub2 or the mRNA-binding protein Npl3 to associate with mature mRNPs. Over the past few years, our understanding of mRNP biogenesis and the pathway leading to Mex67 recruitment has expanded significantly, and the existence of tight interconnections between transcription, mRNA-processing steps and packaging into export-competent mRNP complexes has become evident. In particular, several studies revealed that many export factors, including Sub2, Yra1 and Mex67, are recruited to the transcription elongation machinery prior to their loading on the mRNP, establishing a functional and physical link between transcription and mRNA export through the NPC (for a review, see Iglesias and Stutz, 2008; Kohler and Hurt, 2007; Stutz and Izaurralde, 2003).

THO, a tetrameric complex consisting of Hpr1, Tho2, Mft1 and Thp2, is associated with the elongating RNA polymerase, is important for mRNA export and has been implicated in the recruitment of mRNA export factors (Abruzzi et al., 2004; Chavez et al., 2000; Piruat and Aguilera, 1998; Strasser et al., 2002; Zenklusen et al., 2002). Indeed, early studies showed that the DEAD box ATPase Sub2, the adaptor Yra1 and Tex1 co-purify with THO to form TREX, a complex proposed to couple transcription and mRNA export (Strasser et al., 2002). It was shown that Hpr1 recruits Sub2 and it has been suggested that Sub2, in turn, binds Yra1 (Zenklusen et al., 2002). However, more recent work showed that Yra1 is initially recruited through Pcf11, the RNA Pol II CTD-binding subunit of the 3′ cleavage and polyadenylation factor. Instead, it was proposed that Sub2 facilitates the recruitment of Yra1 to the mRNP in a process linked to 3′-end formation and release of export-competent mRNPs from the transcription site (Johnson et al., 2009) (also discussed below).

Strikingly, the export receptor Mex67 was also found to associate with a variety of transcribed genes in an RNA-independent process (Dieppois et al., 2006; Gwizdek et al., 2006; Hobeika et al., 2007), suggesting that early co-transcriptional recruitment of Mex67 is not mediated by nascent RNA but involves interaction with components of the RNA Pol II elongating machinery. As Mex67 directly associates with the NPC, these observations raised the possibility that Mex67 participates in bridging activated genes to nuclear pores. Accordingly, gene-NPC gating is affected when Mex67 is compromised in its ability to associate with pores or active chromatin (Dieppois et al., 2006). Whereas the association of Mex67 with mature mRNPs is mediated by its N-terminal domain, the recruitment of Mex67 to transcribed genes mainly involves its C-terminal ubiquitin-associated domain (Mex67-UBA). Interestingly, Mex67-UBA directly interacts with THO via ubiquitylated Hpr1, and lack of Hpr1 ubiquitylation reduces

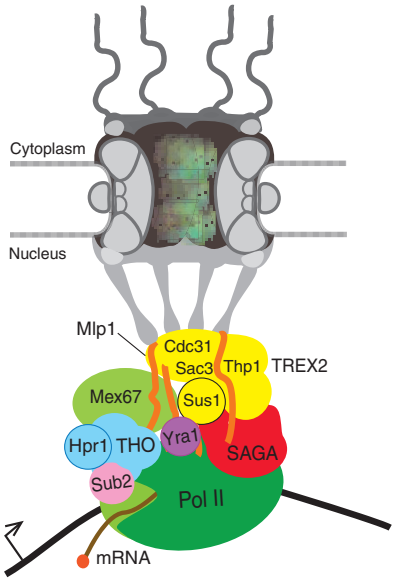


Fig. 3. Gene-NPC tethering during transcription elongation. Mex67 and Sus1 are loaded on the RNA Pol II elongation machinery, connecting the transcribed chromatin with the NPC (Dieppois et al., 2006; Pascual-Garcia et al., 2008). Mex67 is recruited through the ubiquitylated THO component Hpr1 (Gwizdek et al., 2006). Hpr1 also binds the ATPase Sub2 (Zenklusen et al., 2002). Sus1 loading on coding regions depends both on SAGA and TREX2, suggesting that these complexes function with Sus1 in tethering genes to the NPC both during initiation and elongation. Sus1 crosslinks with Mex67 and Yra1 on the transcription machinery, indicating that TREX2 could be recruited on the elongating RNA Pol II through interaction with these factors as well (Pascual-Garcia et al., 2008). Finally, it is conceivable that Mlp proteins contribute to gene-NPC gating during transcription elongation through interactions with RNA-Pol-II-bound factors and the nascent mRNP.

Mex67 co-transcriptional recruitment (Gwizdek et al., 2006; Hobeika et al., 2007). As Mex67-UBA interacts with other factors that are associated with the transcribing polymerase (Catherine Dargemont, Institut Jaques Monod, Paris, France), Mex67 might be recruited and promote gene-to-pore interaction during transcription elongation via sequential binding to several ubiquitylated partners (Fig. 3).

Reinforcing the view that transcription elongation events contribute to gene-NPC gating, it was reported that the export factor Sus1 is loaded on the elongating machinery through interaction with the phosphorylated CTD of RNA Pol II, and that it functions with SAGA and TREX2 not only during transcription initiation but also during elongation (Faza et al., 2009; Gonzalez-Aguilera et al., 2008; Govind et al., 2007; Pascual-Garcia et al., 2008; Wyce et al., 2007). Notably, TREX2 mutants exhibit defects in transcription elongation as well as enhanced transcription-dependent hyper-recombination. SAGA-TREX2-dependent NPC association might, therefore, also ensure sustained transcription processivity and genetic stability (Faza et al., 2009; Gonzalez-Aguilera et al., 2008; Pascual-Garcia et al., 2008). Interestingly, Sus1 also co-purifies with the export factors Yra1 and Mex67 in a manner dependent on phosphorylation of the CTD of RNA Pol II and contributes with Sac3 to the localization of Mex67 at the nuclear periphery (Fischer et al., 2002; Pascual-Garcia et al., 2008). Collectively, these observations suggest that Sus1 and Mex67 act in two partially redundant pathways to anchor genes at the NPC during elongation (Fig. 3).

Most mRNA export factors described thus far – including the export receptor Mex67 and mRNA-binding adaptor proteins, such as Yra1 and Npl3 – are initially recruited to active genes via components of the transcription machinery. A current view is that they are transferred to the mRNA at a later stage, presumably in coordination with 3′-end processing, polyadenylation and release of the mature transcript from the transcription site (Gilbert and Guthrie, 2004; Gwizdek et al., 2006; Iglesias and Stutz, 2008; Qu et al., 2009; Rougemaille et al., 2008) (also discussed below).

NPC association might contribute to the tight functional coupling between 3′-end processing and export

A connection between 3′-end formation and the NPC was revealed partly by the findings that the *HXK1* 3′UTR sequence is important for gene-NPC anchoring (Taddei et al., 2006) and that pore components preferentially interact with the 3′ end of active genes (Casolari et al., 2005). By contrast, we observed that the *GAL2* 3′UTR is not required for gene-NPC anchoring (Dieppois et al., 2006), indicating that the contribution of 3′UTR signals in gene-NPC association varies from gene to gene. Furthermore, different 3′UTR sequences differentially affect the retention of genes at the NPC upon transcriptional repression (Abruzzi et al., 2006). In this latter study, fusion of the wild-type *GAL1* 3′UTR to a reporter gene led to the accumulation of mRNAs in nuclear ‘dots’ at or close to the transcription site that persisted after transcription shut-off. The correlation between mRNA dot persistence and gene-NPC retention suggested that association with the nuclear periphery does not require ongoing transcription, and that mRNPs within dots contribute to the gene-nuclear periphery tether (Abruzzi et al., 2006; Chekanova et al., 2008).

The accumulation of mRNA in foci at or close to a locus being transcribed is generally associated with inefficient 3′-end processing (Brodsky and Silver, 2002; Hilleren et al., 2001; Jensen et al., 2001; Libri et al., 2002; Thomsen et al., 2003). This phenotype depends on the nuclear exosome, an evolutionarily conserved complex containing two 3′-5′ exoribonucleases, Dis3 (also known as Rrp44) and Rrp6, which function in mRNA surveillance by retaining and eventually degrading defective mRNAs (Hilleren et al., 2001; Houseley et al., 2006; Libri et al., 2002; Zenklusen et al., 2002). Accordingly, mRNA retention induced by the *GAL1* 3′UTR was drastically reduced in mutants lacking the non-essential nuclear exosome components Rrp6 and Lrp1, indicating that these factors act directly to accumulate the mRNA dot observed at certain wild-type genes (Vodala et al., 2008). More detailed analysis indicated that the exosome interferes with polyadenylation by the canonical poly(A)$^+$ polymerase Pap1, leading to the accumulation of improperly adenylated and export-incompetent transcripts, presumably because of their inability to recruit poly(A)$^+$-binding export factors (Vodala et al., 2008). Notably, the post-transcriptional retention of the *GAL1* gene at the NPC similarly depended on Rrp6

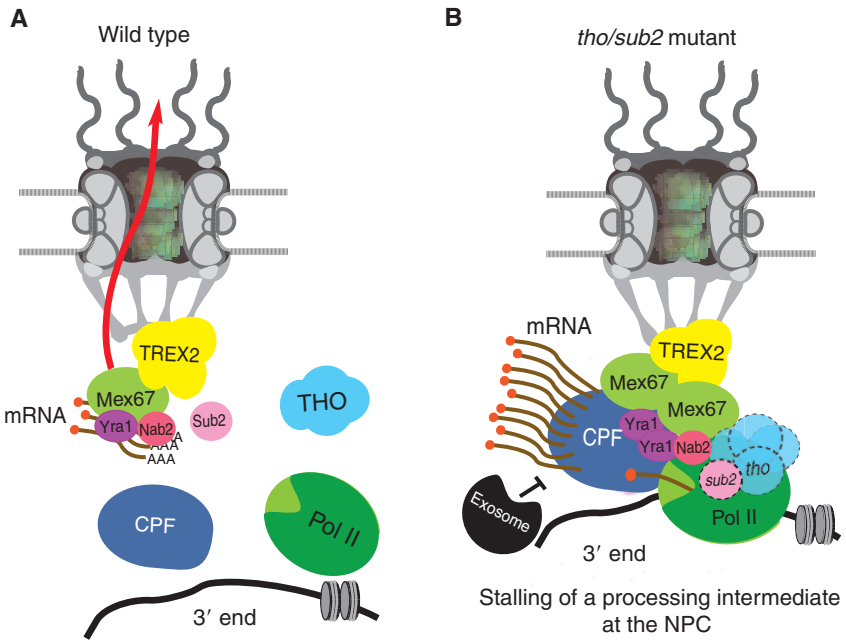


Fig. 4. Gene retention at the NPC during mRNP 3′-end processing and release. (**A**) In a wild-type setting, a series of highly dynamic and coordinated mRNP-remodeling steps occur after commitment to 3′-end processing and binding of cleavage and polyadenylation factor (CPF; also known as CF1A). These rearrangements trigger CPF dissociation and mRNP release from the transcription site. A current view is that the enzymatic activity of the DEAD-box ATPase and RNA helicase Sub2 is required for these rearrangements to occur (Rougemaille et al., 2008). Furthermore, the transfer of the export factors Mex67, Yra1 (also known as REF) and Nab2 from the transcription machinery to the mature mRNA might be coordinated with 3′-end processing and transcript release, ensuring that only mature mRNPs gain access to the pore channel (Gilbert and Guthrie, 2004; Iglesias and Stutz, 2008; Johnson et al., 2009). These reactions might take place in the context of, and be facilitated by, the NPC (Rougemaille et al., 2008). The red arrow represents mRNA export. (**B**) Loss of Sub2 or 3′-end-formation defects result in gene retention at the NPC (Rougemaille et al., 2008). In these situations, improperly processed mRNPs are recognized by the exosome, which inhibits their polyadenylation through Pap1 (Vodala et al., 2008). Lack of polyadenylation interferes with mRNP dissociation from the CPF and/or the gene, resulting in the accumulation of transcripts within a dot retained at or close to the transcription site. Block of mRNA release might create a roadblock and the stalling of 3′-end-processing and -elongation factors at the 3′ end of the gene (Rougemaille et al., 2008). Among the stalled factors, the NPC-associated proteins Mex67 and TREX2 might contribute to maintaining the gene at the NPC even after transcription repression (Abruzzi et al., 2006; Chekanova et al., 2008). Whether there is a direct involvement of the RNA dot in gene-NPC tethering is unclear, but this could be mediated by yet-unidentified mRNP-bound proteins.

and Lrp1, reinforcing the view that the exosome and/or the mRNAs sequestered at the transcription site contribute to bridge the locus to the pore (Vodala et al., 2008). Together, these data indicate that some 3′UTR sequences direct kinetically slower 3′-end processing events, delaying polyadenylation and mRNP release, thereby maintaining the gene at the nuclear pore in a process dependent on the exosome.

Importantly, THO-complex and Sub2 mutants display a similar mRNA dot phenotype associated with gene-NPC retention, consistent with a tight functional link between THO, Sub2 and 3′-end formation (Libri et al., 2002; Rougemaille et al., 2008; Saguez et al., 2008). More specifically, ChIP experiments in THO-complex and Sub2 mutants revealed the accumulation, at the 3′ end of activated heat shock genes, of a large stalled protein complex termed DCF (differential chromatin fractionation) containing 3′-end processing factors as well as Mex67 and nucleoporins. Gene localization experiments further showed that activated heat shock genes – usually not detected at the nuclear periphery in wild-type cells – become maintained at the NPC in THO-complex and Sub2 mutants. Consistent with a defect in 3′-end formation, both the accumulation of the stalled complex and gene association with the pore were dependent on the nuclear exosome (Rougemaille et al., 2008).

These observations support a model whereby Sub2 and the THO complex act in the context of the NPC to promote important mRNP-remodeling events following commitment to 3′-end formation. These rearrangements, which are probably stimulated by the ATPase activity of Sub2, might be required for optimal polyadenylation, dissociation of 3′-end processing factors and mRNP release from the transcription site (Fig. 4). Accordingly, in vitro, pre-mRNA substrates are poorly polyadenylated in extracts made from THO-complex and Sub2 mutants and are prone to degradation by the exosome (Saguez et al., 2008). Presumably, the THO-complex- and Sub2-induced rearrangements are coordinated with the transfer of export factors such as Mex67, Yra1 and possibly the poly(A)$^+$-binding protein Nab2 from the transcription machinery to the mature mRNA, allowing the mRNP to acquire full export competency and access to the pore channel. Notably, the early co-transcriptional recruitment of Mex67 via the THO complex might contribute to THO-complex–Sub2 stability and thereby influence completion of 3′-end processing and mRNP release. Indeed, the conditional *mex67-6* mutant, which is defective in co-transcriptional recruitment (Nahid Iglesias, EPFL, Lausanne, Switzerland), induces 3′-end formation and mRNA export defects (Gwizdek et al., 2006; Qu et al., 2009; Strasser and Hurt, 2000).

It is unclear whether the exosome directly participates in the formation of the stalled high-molecular-weight complex in THO-complex and Sub2 mutants, or whether it has a more indirect effect through promoting the build-up of improperly adenylated transcripts at the 3′ end of heat-shock genes (Rougemaille et al., 2008). Evidence suggests that accumulation of Mex67 and TREX2 in the stalled complex mediates interaction of chromatin with the NPC (Chekanova et al., 2008; Dieppois et al., 2006; Rougemaille et al., 2008), but a contribution of the retained RNA in gene anchoring cannot be excluded (Chekanova et al., 2008) (Fig. 4). The finding that cells defective for the THO complex or for Sub2 contain a stalled processing intermediate that is associated with pores raises the possibility that the ATPase activity of Sub2 is stimulated by a factor located at the NPC. Thus, one scenario could be that co-transcriptional loading of NPC-bound TREX2 and Mex67 contributes to the coordination of 3′-end processing and mRNA export by positioning Sub2 close to its activator. The nature of this activator and whether pore association is required for efficient 3′-end formation are interesting questions for the future.

mRNP quality control at the NPC

In addition to surveillance by the nuclear exosome, additional mRNA quality-control mechanisms at the NPC have been described in *S. cerevisiae*. The best characterized components of this surveillance system are the nuclear-pore-associated proteins Mlp1 and Mlp2 (Fasken et al., 2008; Galy et al., 2004; Grant et al., 2008; Green et al., 2003; Vinciguerra et al., 2005), the Mlp-bound downstream effector Pml39 (Palancade et al., 2005), the nucleoporin Nup60 (Galy et al., 2004; Lewis et al., 2007) and the NE protein Esc1 (Lewis et al., 2007). Mlp1, Mlp2 and Pml39 have been found to be involved in the retention of unspliced and malformed mRNPs at the nuclear periphery and to favor their degradation in the nucleus (Galy et al., 2004; Palancade et al., 2005; Vinciguerra et al., 2005). A current model proposes that mRNPs dock at the Mlp gate through direct interaction between the poly(A)$^+$-binding protein Nab2 and Mlp1 (Fasken et al., 2008; Grant et al., 2008; Green et al., 2003; Vinciguerra et al., 2005). Mlp1, in association with Pml39, would in turn act as a selective filter that either guides export-competent mRNAs towards translocation through the NPC or retains faulty mRNPs. The perinuclear Esc1 and Nup60 components serve predominantly structural roles in this pathway, as Esc1 functions in the correct assembly of the NPC nuclear basket (Lewis et al., 2007), and Nup60 directly tethers Mlp1 and Mlp2 and, therefore, Pml39 at the nuclear pore (Feuerbach et al., 2002; Palancade et al., 2005). Notably, Esc1, Nup60, Mlp1 and Mlp2 are all required to anchor the SUMO protease Ulp1 to the NPC, and Ulp1 is required for efficient nuclear pre-mRNA retention, raising the possibility that Ulp1-mediated de-sumoylation of mRNP-bound proteins is involved in mRNA-export surveillance (Lewis et al., 2007).

For the genes recruited to the nuclear periphery, Mlp-dependent surveillance might operate on chromatin-bound transcripts. Consistently, Nab2 is recruited co-transcriptionally (Nahid Iglesias, personal communication), suggesting that Nab2 interacts with Mlp1 prior to mRNA release. Moreover, Mlp proteins interact with chromatin of activated genes through RNA-dependent mechanisms (Casolari et al., 2005) and associate with mRNP factors that are recruited co-transcriptionally – such as Yra1, Mex67, Sub2 and Cbp80 (Vinciguerra et al., 2005). Following recognition by the perinuclear surveillance system, aberrant transcripts are eventually degraded by the nuclear exosome (Galy et al., 2004; Vinciguerra et al., 2005) and/or by Swt1, an evolutionarily conserved RNA endonuclease located at the pore (Skruzny et al., 2009). Genetic interactions between Swt1 and the transcription-associated THO and TREX2 complexes further support the possibility that this endonuclease acts on chromatin-associated transcripts that are generated in the vicinity of the pore.

Concluding remarks

Over the past few years, considerable efforts have been dedicated to deciphering the mechanism and physiological relevance of the gene-NPC-gating phenomenon. It turned out that gene anchoring is an active and highly dynamic process that involves multiple tethers during the whole transcription cycle, suggesting that NPC proximity is important for several steps of gene transcription. Favoring transcription activation, the NPC is involved in partitioning chromatin into silenced and active domains and

provides a platform for the loading and/or the regulation of transcription factors. To promote the correct processing and efficient export of mRNA, gene-NPC association facilitates co-transcriptional loading of export machineries that coordinate processing, packaging and release of functional mRNPs. Finally, the stable structure of the NPC might contribute to chromatin organization and the establishment of long-term epigenetic programs.

Interestingly, the NPC-associated Mlp proteins have been involved in mRNP quality control and retention of DNA loops. However, the interaction between these filamentous structures and a large panel of transcription, mRNP-processing and export factors suggests that Mlp proteins have a more general role in stabilizing gene-NPC association and in orchestrating gene expression events. Moreover, the role of Mlp proteins as regulators of the activity of the SUMO protease Ulp1 raises the possibility that Ulp1 contributes to gene regulation at the pore through de-sumoylation of chromatin-bound factors upon recruitment of activated genes to the nuclear periphery, an idea that awaits further exploration.

We thank Susan Gasser, Domenico Libri and members of the lab for the critical reading of the manuscript. We apologize to any of our colleagues whose work could not be cited because of space limitations.

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