Chapter 4
Nuclear Pore Complex in Genome Organization and Gene Expression in Yeast

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Abstract The nuclear pore complexes (NPCs) are large, evolutionarily conserved multiprotein channels embedded in the nuclear envelope of all eukaryotes cells. NPCs mediate macromolecular import and export from the nucleoplasm and cytoplasm by an active signal-dependent process. Recent research indicates that the NPCs play many additional roles in gene function and spatial organization of the genome. This chapter highlights our current understanding of NPC in genome-related functions in budding yeast. In yeast, Nups physically interact with a large number of highly expressed genes and active inducible genes. The repositioning of inducible genes to the NPCs leads to stronger expression and is regulated through multiple regulatory strategies including cell cycle regulated phosphorylation of Nup1. Many inactive or poised genes also interact with Nups. The interaction of recently repressed *GAL1* and *INO1* with the NPC is necessary for transcriptional memory. Retention at the NPC for these genes lead to an altered chromatin structure that primes them for rapid transcriptional reactivation. Thus, interactions with the NPC influences the spatial organization of the genome and impacts transcription.

Keywords Chromosomal spatial organization · nuclear pore complex · yeast nucleoporins · transcription control · interchromosomal clustering · transcriptional memory · cell cycle regulation · repositioning of inducible genes · regulation of gene recruitment and clustering · aging

4.1 Introduction

A membrane-bounded nucleus is a defining feature of all eukaryotic cells. The nucleus contains the majority of the genetic material in the cell and isolates nuclear from cytoplasmic functions. The nucleus is delimited by a double lipid

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M.A. D'Angelo (ed.), *Nuclear Pore Complexes in Genome Organization, Function and Maintenance*, https://doi.org/10.1007/978-3-319-71614-5_4

bilayer membrane called the nuclear envelope (NE) and communication between the cytoplasm and nucleus is mediated by the nuclear pore complex (NPC). The NPC regulates the bidirectional exchange of macromolecules, export of specific RNA molecules, and selective transport of regulatory factors. Thus, the NPC is a critical mediator of cellular processes between the nucleus and the rest of the cell.

Within the nucleus, eukaryotic genomes are organized spatially and some nuclear functions are compartmentalized. Each chromosome occupies a distinct "territory" and can position it's chromatin into subnuclear compartments where loci can cluster with co-regulated regions or interact with stable nuclear structures (Sexton and Cavalli 2015). The spatial position of individual genes often reflects their transcriptional states (Pombo and Dillon 2015). In metazoans, chromosomes fold back onto themselves forming distinct non-overlapping globular territories (Cremer et al. 2006). Transcriptionally active regions tend to position at the edges of the territories in the inter-territorial space. Soluble factors such as transcriptional regulators and RNA polymerase II are non-uniformly distributed within the nucleus (Bartlett et al. 2006). The nucleolus, for example, is a subnuclear compartment that serves as the site for ribosome biogenesis (Boisvert et al. 2007). The nucleolus concentrates factors involved in rRNA production and ribosomal biogenesis (Andersen et al. 2005). Thus, both chromatin and soluble factors are spatially organized within the nucleus.

The organization of chromatin is also dynamic; developmental and physiological signals that alter gene expression also alter chromatin organization (Peric-Hupkes et al. 2010; Randise-Hinchliff et al. 2016). This suggests that the spatial organization of the genome within the nucleus contributes to gene regulation. However, the mechanisms and functional significance of the nuclear organization are not fully understood. What is clear is that stable nuclear structures bind to certain chromosomal regions, imparting organization and influencing transcriptional regulation (Meldi and Brickner 2011; Taddei and Gasser 2012). For example, in metazoans, the nuclear lamina, a filamentous network of lamins and lamin-associated proteins at the nuclear periphery, associates with large, transcriptional repressed regions of the genome (Luperchio et al. 2014). Because the nuclear lamina associates with chromatin modifying proteins and transcriptional repressors, it has been proposed that it is a transcriptional repressive environment (Gruenbaum and Foisner 2015).

However, the nuclear periphery is not exclusively associated with transcriptionally silent heterochromatin. Electron microscopy shows decondensed euchromatin positioned adjacent to NPCs (Belmont et al. 1993). In yeast, repressive regions and NPCs form distinct, non-overlapping foci (Taddei et al. 2004). This suggest that beyond its vital role in nucleo-cytoplasmic transport, the NPC may interact with active regions of the genome. Indeed, in yeast, flies, worms and mammalians, NPC components interact with hundreds to thousands of active genes (Brickner and Walter 2004; Casolari et al. 2004; Brown et al. 2008; Ahmed et al. 2010; Kalverda et al. 2010; Rohner et al. 2013). In yeast, these interactions occur at the nuclear periphery (Ahmed et al. 2010). However, in flies and mammals, such interactions can occur at both the NPC and with soluble nuclear pore proteins, in the nucleoplasm (Capelson et al. 2010; Kalverda et al. 2010). Interaction with

nuclear pore proteins promotes stronger transcription, alters chromatin structure and limits the spread of silencing (Ishii et al. 2002; Dilworth et al. 2005; Ahmed et al. 2010; Kalverda et al. 2010; Light et al. 2010, 2013; D'Urso et al. 2016). In yeast, interaction with the NPC can also lead to interchromosomal clustering of co-regulated genes (Brickner and Brickner 2012; Brickner et al. 2016; Randise-Hinchliff et al. 2016). Additionally, recently repressed genes bound at the NPC are poised for faster reactivation (Brickner et al. 2007; Brickner 2009; Tan-Wong et al. 2009; Light et al. 2010; Botstein and Fink 2011). Thus the NPC plays an important role in both the spatial organization of the nucleus and transcriptional regulation.

Here we review our current understanding of the mechanism and functional significance of the interaction of the NPC with the budding yeast genome. Research in yeast has provided significant conceptual and mechanistic insight into chromosomal organization and its effects on gene regulation. These discoveries have stimulated work in metazoan systems, which has revealed that these mechanisms are largely conserved.

4.2 Spatial Organization of the Yeast Genome

Budding yeast, *Saccharomyces cerevisiae*, has served as an outstanding model for understanding fundamental cell and molecular biology of eukaryotic cells (Taddei et al. 2010; Botstein and Fink 2011). However, budding yeast has several nuclear features that contribute to chromatin organization that are distinct from higher eukaryotes (Taddei et al. 2010; Zimmer and Fabre 2011). The primary difference is that budding yeast undergoes a closed mitosis; the NE does not break down during mitosis. During interphase, the centromeres of the 16 relatively small chromosomes (230–1,500 kb) remain tethered to the spindle pole body (SPB). The SPB, functionally analogous to the microtubule organizing center, is embedded in the NE and is positioned opposite the nucleolus (McBratney and Winey 2002). Chromosome arms emanate away from the SPB towards the opposite pole of the nucleus, where telomeres cluster as well. The 32 telomeres form a small number of foci at the NE by FISH, reflecting their inter-chromosomal clustering (Hediger et al. 2002). Since centromeres remain tethered through interphase, there is a strong determinant for the spatial position of chromosomal regions (Duan et al. 2010; Zimmer and Fabre 2011). In other words, short chromosome arms are unable to explore the same nuclear volume as longer arms. Consistent with this notion, telomeres of chromosomes having short arms (< 300 kb) cluster together near the SPB and telomeres of chromosomes having longer arms cluster together near the nucleolus (Duan et al. 2010). This organization is known as the Rabl configuration and is not specific to yeast. It was first observed by Carl Rabl in 1885 in epithelial salamander larvae and later in *Drosophila melanogaster* embryos and in many cereal species (Marshall et al. 1996; Parada and Misteli 2002). Despite yeast possessing unique features, the morphology and mechanisms that influence the spatial arrangement of yeast chromosomes have been important to understanding genomic organization in all eukaryotes.

4.3 Composition of NPC

The yeast NPC is one of largest and most complex proteinaceous assemblies in the cell, consisting of approximately 400 proteins with a mass of 66 million Daltons (Aitchison and Rout 2012). The NPC is composed of approximately 30 nucleoporins (Nups), each of which are present in multiple copies (usually 8 or 16), reflecting the eight-fold symmetry of the structure. Specific groups of Nups contribute to repetitive subcomplexes that form the NPC (Aitchison and Rout 2012). Based on structure, motifs, and locations, Nups can be classified into distinct groups (Fig. 4.1). Furthermore, many Nups bind dynamically to the NPC, cycling on or off or associating only during certain phases of the cell cycle (Dilworth et al. 2001; Makhnevych et al. 2003; Tran and Wente 2006). Thus, the exact number and definition of Nups is uncertain.

The NPC is a highly conserved structure and the majority of Nups have structural conservation that has been extrapolated to the last common eukaryotic ancestor (Brohawn et al. 2008; Neumann et al. 2010). However, due to a recent whole-genome duplication during *Saccharomyces* evolution, followed by gene divergence and loss, several Nups that are encoded by single genes in vertebrates exist as paralogous pairs in *S. cerevisiae* such as Nup116/Nup100 (Nup98 in vertebrates), Nup157/Nup170 (Nup155 in vertebrates), and Nup53/Nup59 (Nup3 in vertebrates; (Aitchison and Rout 2012). Also, the metazoan cytoplasmic filament Nups, Nup358 and Aladdin, are absent in yeast and the nucleoplasmic yeast Nup60 is absent in vertebrates (Wu et al. 1995; Cronshaw et al. 2002; Hoelz et al. 2011).

The yeast NPC, compared to the vertebrate NPC, is also both significantly smaller (66MDa compared to 125MDa) and less abundant in the NE (200 compared to 2,500–5,000) (Reichelt et al. 1990; Rout and Blobel 1993; Grossman et al. 2012). In metazoan organisms, NPCs are disassembled and reassembled

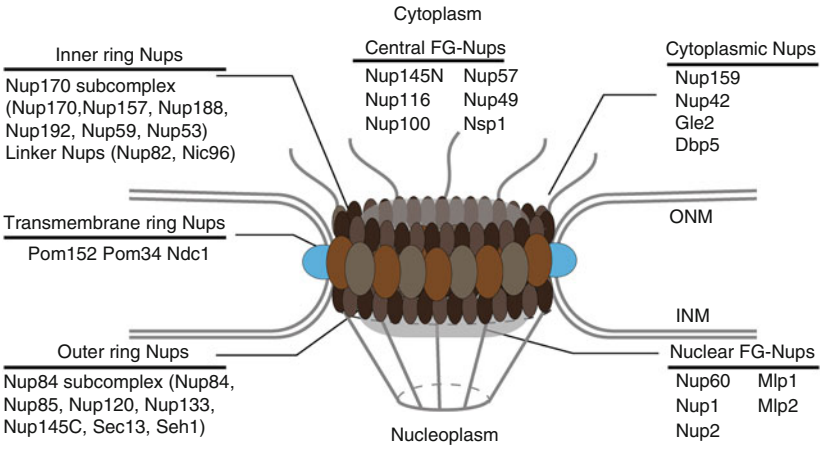

Fig. 4.1 Overall structure of the yeast nuclear pore complex (NPC). Nups are classified into distinct groups by structure and location

during mitosis while in yeast, due to a closed mitosis, the NPC remains assembled through the life cycle of the cell. Besides these differences, the core structure and function of the NPC is conserved between yeast and metazoans.

The cylindrical structure of the NPC is organized with eight-fold symmetry around a central transport channel and pseudo two fold symmetry between the cytoplasm and the nucleoplasm (Fig. 4.1) (Hoelz et al. 2011). The NPC is composed of two main functional regions; a central core and peripheral structures. The NPC core consists of coaxial inner, outer, and transmembrane rings surrounding a central channel, approximately 40 nm in diameter (Hoelz et al. 2011). The core is built from scaffold Nups (outer ring Nups, linker Nups and inner ring Nup), membrane-embedded ring Nups, and central FG-Nups. The core scaffold defines the shape and dimensions of the NPC (Kampmann and Blobel 2009). These Nups are structurally related to vesicular coat proteins and have been proposed to catalyze the formation of the sharply curved pore membrane (Devos et al. 2004). The pore membrane domain harbors three transmembrane proteins, Ndc1, Pom152 and Pom34, that interact with the core proteins and anchor the NPC within a pore in the NE. Finally, 11 Nups rich in phenylalanine-glycine (FG) repeats, are natively unstructured domains that form the permeability barrier of the NPC channel and serve as docking sites for transport receptors (Alber et al. 2007). The peripheral structures are made up of asymmetrical filaments that extend into either the cytoplasm and nucleoplasm. The cytoplasmic filaments are composed of Nup159, Nup42, Gle2 and Dbp5 and function in mRNP remodeling (Okamura et al. 2015). The nuclear basket forms the peripheral structure within the nucleus. It consists of filaments of FG Nups: Nup60, Nup1, Nup2, Mlp1, and Mlp2 (Hoelz et al. 2011). The nuclear basket functions in transport but an accumulating body of evidence also connects the nucleoplasmic basket to transcriptional regulation, modulating chromatin structure and organization of the genome.

4.4 Nuclear Pore Complex Interacts with the Genome

In addition to its role in regulating nucleo-cytoplasmic transport, the NPC also contributes to transcription and the spatial organization of the genome within the nucleus. Nuclear pore components directly interact with transcriptional regulators, mRNA export factors and chromatin (Table 4.1; (Steglich et al. 2013). The interactions with chromatin provide anchor points along the nuclear periphery to spatially organize and compartmentalize the genome. Using chromatin immunoprecipitation (ChIP) coupled to DNA microarray analysis (ChIP-chip), the interactions of Nups and NPC-associated factors were mapped genome-wide in yeast (Casolari et al. 2004, 2005). For a majority of the NPC components, genomic occupancy strongly correlated with transcriptional activity (Casolari et al. 2004). This included the nuclear basket components Nup2, Nup60, Mlp1 and Mlp2, the scaffold components Nic96 and Nup116, and the karyopherins Xpo1 and Cse1. These Nups also preferentially bound to genes involved in glycolysis and protein biosynthesis (Casolari

Table 4.1 Summary of nucleoporins in genome-related functions in yeast

Name	Location	Functions*
Nup 1	Nuclear basket	• Cell cycle phophorylation of Nup1 is required for periperhallocalization and interchromosomal clustering of *GAL*1 and *IN01* genes • Physically interacts with TREX-2 complex
Nup2	Nuclear basket	• Association with active genes • Required for peripheral localization of *GAL*1, *IN01* and tDNA genes • Required for peripheral localization of recently repressed *IN01* • Role in chromatin boundary activity • Physically interacts with H2A.Z as well as *IN01* and *GAL*1 gene promoters
Nup60	Nuclear basket	• Association with active genes • Required for peripheral localization and clustering of *GAL*1 and *IN01* genes • Required for peripheral localization of recently repressed *IN01*
Mlp1	Nuclear basket	• Association with active genes • Physically interacts with the SAGA complex and Ulp1 • Required for transcriptional memory of *GAL1* • Required for interchromosomal clustering of *GAL*1 gene
Mlp2	Nuclear basket	• Association with active genes • Physically interacts with Ulp1 • Required for peripheral localization of *GAL*1 and *IN01* genes
Nup100	Central FG-Nups	• Physically interacts with *GAL1* • Required for transcription memory and peripheral localization of recently repressed *IN01*
Nup116	Central FG-Nups	• Association with active genes • Physically interacts with *GAL1*
Nic96	Inner ring Nups	• Association with active genes
Nup170 subcomplex	Inner ring Nups	• Required in tethering and silencing of ribosomal and subtelomeric genes
Nup84 subcomplex	Outer ring Nups	• Required for peripheral localization of recently repressed *IN01*

*Refer to text for citations.

et al. 2004). Thus, certain active chromatin regions position and physically interact with the NPC (Table 4.1).

Interaction with Nups does not always correlate with transcription. The genomic occupancy of Nsp1, Nup84, Nup145 and Nup100 had no correlation with expression (Casolari et al. 2004). Thus, the NPC interacts with both active and inactive regions of the genome. The differences in the observed binding profiles for nuclear pore components may either reflect functional distinct molecular interactions with NPC or distinct NPC molecular composition. In support of the idea that different NPCs might be compositionally distinct, Mlp1, Mlp2, Ulp1 and Pml39 are associated with only a subset of NPCs (Zhao et al. 2004; Palancade et al. 2005).

Many inducible genes reposition from the nucleoplasm to the nuclear periphery and physically interact with the NPC in response to different environmental stimuli. For example, the *GAL* genes (*GAL1*, *GAL2*, *GAL7* and *GAL10*) in glucose are transcriptionally repressed and are localized in the nucleoplasm with sub-diffusive constrained movement (Casolari et al. 2004; Cabal et al. 2006). In contrast, in galactose, the *GAL* genes become transcriptionally induced and reposition to the nuclear periphery with more constrained diffusion (Cabal et al. 2006; Brickner 2007). At the nuclear periphery, *GAL* genes physically interact with Nup116, Mlp1, Nup60, Nup2, Cse1, XpoI and Nup100 (Casolari et al. 2004). This interaction depends on gene activity and the transcriptional activator Gal4 and occurs in the gene promoter (Schmid et al. 2006). In strains lacking Nup2, Nup1, Nup60 or Mlp2, *GAL1* remains nucleoplasmic in galactose media (Brickner et al. 2007; Brickner et al. 2016). Furthermore, the Gal genes are not the only region of the genome that is recruited to the NPC in galactose. When media is shifted to galactose, large scale rearrangements occur, repositioning many chromosomal regions to the nuclear periphery through multiple anchor points (Dultz et al. 2016).

Gene recruitment to the NPC has been observed in many environmental stimuli such as nutrient shifts (*INO1, HIS4, HXK1, SUC2*), osmotic stress (*CTT1*, *STL1*), heat shock (*TSA2*, *HSP104*) and mating pheromone treatment (*PRM1*, *FIG2, FUS1*; (Brickner and Walter 2004; Casolari et al. 2005; Dieppois et al. 2006; Taddei et al. 2006; Sarma et al. 2007; Ahmed et al. 2010; Regot et al. 2013; Guet et al. 2015; Randise-Hinchliff et al. 2016). The *INO1* gene (encoding inositol 1-phoshate synthase) repositions to the nuclear periphery upon activation during inositol starvation. The repositioning of *INO1* requires many Nups including Nup1, Nup2, Nup60, Nup157, Nup42, Gle2, and Mlp2 (Ahmed et al. 2010). Interaction of *INO1* and *GAL1* promotes stronger transcription by increasing the fraction of cells that respond to the inducing signal (Brickner et al. 2007; Texari et al. 2013; Brickner et al. 2016).

The interaction of the genome with the NPC is regulated through the cell cycle. Active genes such as *GAL1*, *INO1* and *HSP104* relocalize from the nuclear periphery to the nucleoplasm during S-phase (Brickner and Brickner 2010, 2012). This regulation of peripheral localization is due to oscillating Cdk-mediated phosphorylation of Nup1. Targeting of these genes to the NPC requires Cdk activity and either of two Cdk phosphorylation sites on Nup1. However, substitution of phosphomimetic aspartates in place of the phosphoacceptor residues at either position leads to localization at the periphery throughout the cell cycle and bypasses the requirement for Cdk activity (Brickner and Brickner 2010). Likewise, although tDNA genes encoding tRNAs are generally clustered in the nucleolus, during M phase, they reposition to the NPC (Chen and Gartenberg 2014). This coincides with the peak of tDNA expression. Loss of either Nup60 and Nup2 blocks recruitment to the NPC and leads to reduced transcription of tDNA genes during M-phase (Chen and Gartenberg 2014). Thus, in response to different environmental stimuli or cell cycle signals regions of the genome reposition to the NPC, enhancing transcription.

NPC-DNA interactions also play an important role in chronological aging in yeast, the process by which cells cease to divide after producing a fixed number of

daughter cells (Sinclair and Guarente 1997; Denoth-Lippuner et al. 2014). Aging is asymmetrically inherited; each generation the mother ages, but the daughter cell is born with full longevity (Sinclair and Guarente 1997). Extrachromosomal rDNA circles (ERCs) form spontaneously by homologous recombination within the rDNA locus and accumulate in older cells (Sinclair and Guarente 1997) and these ERCs have been proposed to serve as aging factors for several reasons (Denoth Lippuner et al. 2014). ERCs are asymmetrically inherited, accumulating and being retained in the mother cells. Artificially introducing ERC in daughter cells, or enhancing ERC formation in mother cells, shortens longevity (Sinclair and Guarente 1997). Conversely, reducing the rate of ERC formation increases lifespan (Defossez et al. 1999). Attachment of ERCs to NPC confine the DNA circles to the mother cell and preventing their inheritance (Denoth-Lippuner et al. 2014). Likewise, ERC association affects NPC inheritance to the daughter: ERC-bound NPCs are concentrated as an "NPC cap" in the mother cell and are retained, whereas unbound NPCs freely move into the daughter cell. The mechanism for this retention is not completely understood, however the SAGA complex is involved. Loss of SAGA complex components, such as Gcn5 and Spt3, cause DNA circles to dissociate from the NPC, spread into the daughter cells and lead to shorter lifespan (Denoth-Lippuner et al. 2014).

The NPC interacts with both active and repressed regions of the yeast genome, influencing its spatial organization, transcription and chronological aging. The role for Nups in regulating transcription may be evolutionarily conserved. In flies, mice and humans, expression of certain genes is enhanced by interaction with Nups (Brickner and Walter 2004; Casolari et al. 2004; Brown et al. 2008; Ahmed et al. 2010; Kalverda et al. 2010; Rohner et al. 2013). However, many inactive or poised genes also interact with Nups, so interaction with Nups or NPCs does not always correlate with transcription (Casolari et al. 2004; Brickner et al. 2007; Light et al. 2013). Below we discuss our current understanding of the impact of the NPC on transcriptional regulation, the molecular mechanisms that target genes to the NPC, how the interaction with the NPC leads to interchromosomal interactions and the role of the NPC in promoting epigenetic transcriptional memory in budding yeast.

4.5 Nups Influence Transcription

In 1985, Günter Blobel put forth an attractive "gene gating hypothesis," postulating that the interactions of active genes with NPCs might coordinate transcription with mRNA biogenesis and export out of the nucleus to limit mRNA diffusion rates (Blobel 1985). Indeed, interaction with NPC promotes stronger expression for inducible genes such as *INO1* and *GAL1* (Brickner et al. 2007; Ahmed et al. 2010; Brickner et al. 2016). Single molecule mRNA FISH suggests that this is due to an increase in the fraction of cells that induce these genes, rather than an increase in the amount of mRNA produced per transcription event (Brickner et al. 2016). It remains unclear if mRNA export is affected by this interaction. Promoter mutations that

block interaction of genes with the yeast NPC do not lead to nuclear accumulation of those mRNAs (Ahmed et al. 2010; Brickner et al. 2016). The yeast nucleus is small and mRNA export is rapid (Smith et al. 2015). Live cell imaging of mRNAs does not support the model in which mRNAs are directed to particular NPCs (Smith et al. 2015). Thus, although the transcription of genes is impacted by the interaction with the NPC, it is still unclear if post-transcriptional events are affected.

NPCs may anchor and concentrate transcriptional regulators to promote expression, functioning as a transcriptionally active subnuclear compartment. Consistent with this notion, the kinetics of *GAL1* expression is enhanced by Ulp1 anchored at the NPC (Texari et al. 2013). Ulp1 is a SUMO protease that is maintained at the NPC by association with Mlp1 and Mlp2 (Zhao et al. 2004). Ulp1 enhances the rate of *GAL1* mRNA production by catalyzing the desumoylation and attenuation of two repressors, Tup1 and Ssn6 (Texari et al. 2013). Furthermore, many transcriptional activators and mRNA export factors bind directly to the NPC. For example, the multiprotein complex TREX-2, which is necessary for mRNA export, interacts with Nup1 and localizes to inner nuclear basket of the NPC (Fischer et al. 2002; Kohler and Hurt 2007). The SAGA complex, a transcriptional co-activator, is linked to TREX-2 through a common component, Sus1, and binds to the NPC directly through Mlp1 (Rodriguez-Navarro et al. 2004; Luthra et al. 2007). Finally, the Mediator complex, another transcriptional coactivator, also binds to TREX-2 (Schneider et al. 2015). Therefore, interaction of transcriptional regulators with the NPC might enhance expression of active genes at the NPC.

NPC components may also promote transcriptional repression. Loss of members of the Nup84 subcomplex (Nup84, Nup120, Nup133, and Nup145) detaches telomeres from the nuclear periphery and leads to loss of silencing of subtelomeric reporter gene (Therizols et al. 2006). Likewise, the Nup84 subcomplex participates in glucose-responsive repression of *SUC2* by physically interacting with Mig1 (Sarma et al. 2011). Finally, Nup170 is required for peripheral tethering and silencing of many ribosomal and subtelomeric genes through cooperation with chromatin remodeler RSC and Sir4 (Van de Vosse et al. 2013). These findings suggest NPC components can influence silencing.

One complication in understanding the effects of gene-NPC interactions on transcription is that null mutations can disrupt the spatial organization that is normally being exploited in a wild type cell. For example, the Ulp1 SUMO protease is maintained at the NPC by Mlp1 and Mlp2 and is normally important for promoting *GAL1* derepression (Texari et al. 2013). However, mutants lacking NPC basket components both block targeting of *GAL1* to the nuclear periphery and release Ulp1 into the nucleoplasm. This results in more rapid *GAL1* depression, which has been interpreted as a role for the NPC in negatively regulating *GAL1* (Green et al. 2012). However, in a strain lacking Mlp1 and Mlp2, normal regulation of *GAL1* is restored when Ulp1 is artificially anchored to the NPC (Texari et al. 2013). Thus, interpreting the effects of null mutations of NPC components can be complicated by the change in the spatial organization of NPC-associated factors. For that reason, mutations in *cis*-acting DNA elements that perturb the positioning of a gene in an otherwise normal nucleus can provide important

information about the function of NPC interactions (Ahmed et al. 2010; Light et al. 2010; Brickner et al. 2012, 2016). One caveat to this statement is that, in cases where the *cis*-acting DNA elements that control gene positioning are the same as the elements that control transcription, the effects of interaction with the NPC on gene expression have not been distinguishable from the effects on targeting (Randise-Hinchliff and Brickner 2016).

Finally, another function of the interaction of NPCs with chromatin may be to alter chromatin structure to insulate active and silent regions. Studies using a "boundary trap" identified several NPC components capable of inducing boundary activity (Ishii et al. 2002; Dilworth et al. 2005). A boundary factor blocks the spread of heterochromatin without inducing transcription. Tethering of the nuclear pore protein Nup2, Exportins Cse1, Mex67 and Los1 and the RAN GEF Prp20 beside a reporter gene prevented the spread of silencing from the *HML* locus without activating an adjacent gene (Ishii et al. 2002; Dilworth et al. 2005). Endogenous Nups may also possess boundary active. Loss of endogenous Nup2 alleviates telomeric repression (Dilworth et al. 2005). Also Nup2 physically interacts with chromatin-modifying proteins and histone variant H2A.Z and binds to intergenic regions near telomeres (Dilworth et al. 2005).

4.6 Mechanisms of Gene Recruitment

The molecular mechanisms underlying gene recruitment to the nuclear periphery and interactions with NPC are not completely understood. Consistent with the gene gating hypothesis, factors involved in early transcription and mRNA export are required for recruitment of genes to the NPC. For example, peripheral localization of *INO1* requires components of both SAGA (Gcn5, Spt7 or Spt20) and TREX-2 (Sac3, Thp1, Sus1; (Ahmed et al. 2010). Likewise, recruitment of *GAL* genes to the NPC is blocked in strains lacking components of SAGA, Mediator (Med31, Cdk8), TREX-2 and the mRNA export receptor Mex67 (Luthra et al. 2007; Schneider et al. 2015; Brickner et al. 2016). SAGA and Mediator complexes mediate two complementary pathways for transcriptional activation (Bhaumik 2011). Mediator stabilizes the transcription factor TFIID, which is involved in general housekeeping genes, whereas SAGA-dependent genes are involved in environmental stress responses. It is conceivable that TREX-2 and Mex67 are recruited to active genes, acting as a bridge that anchors genes to NPC by interacting with components of the SAGA or mediator complexes bound to the genes. However, several observations are not consistent with this model. For example, recruitment of both *INO1* and *GAL1* to the nuclear periphery occur independent of either the transcriptional activator or RNA polymerase II, suggesting that transcription is not required for repositioning to the NPC (Schmid et al. 2006; Brickner et al. 2007, 2016). Thus, although the requirement for these factors is clear, the interpretation of their role is not.

Gene recruitment to the NPC is controlled by *cis*-acting elements in promoters of these genes (Randise-Hinchliff and Brickner 2016). For example, recruitment

of *INO1* to the nuclear periphery is controlled by two DNA Gene Recruitment Sequences called GRSI and GRSII. GRSI and GRSII redundantly control targeting of *INO1* to the NPC and a mutation that disrupts both elements blocks *INO1* recruitment to the nuclear periphery (Ahmed et al. 2010). When inserted at an ectopic locus that is normally nucleoplasmic (*URA3*), each GRS is sufficient to promote recruitment to the nuclear periphery. Thus, these GRS elements function as *DNA zip* codes; being both necessary and sufficient to control interactions with the NPC and contribute to the spatial organization of the genome.

GRS elements are binding sites for transcription factors (TFs; (Brickner et al. 2012; Brickner and Brickner 2012; Randise-Hinchliff et al. 2016). The TFs Put3 and Cbf1 bind to GRSI and GRSII, respectively, and are both necessary for *INO1* gene recruitment (Fig. 4.2a; (Randise-Hinchliff et al. 2016). Interestingly, neither Put3 or Cbf1 control *INO1* transcription. *INO1* expression is regulated by the Ino2

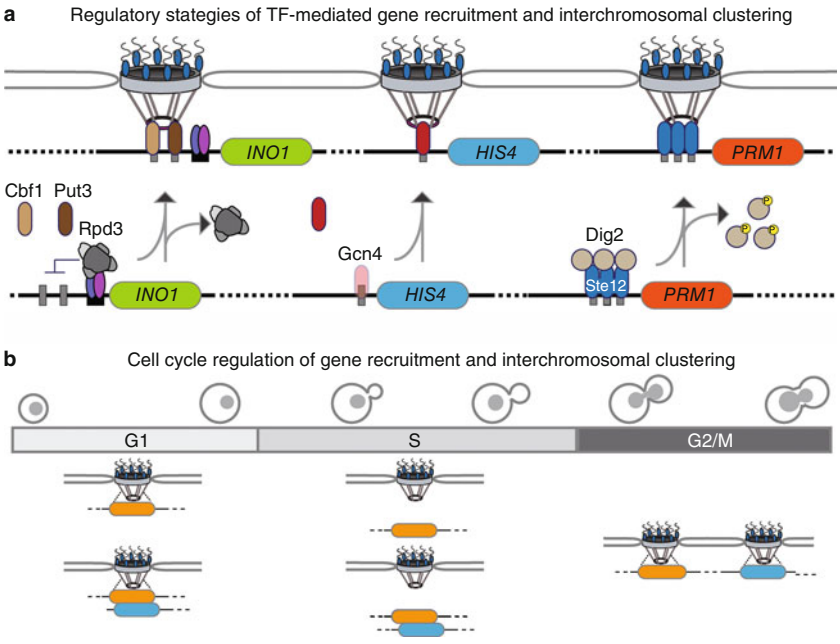

Fig. 4.2 Regulation of gene recruitment and interchromosomal clustering at the NPC. (a) Transcription factor (TF)-mediated recruitment and clustering are regulated by multiple strategies. (Left) Put3 and Cbf1 bind to GRSI and GRSII respectively and mediate *INO1* recruitment to the NPC upon inositol starvation. Recruitment of *INO1* is regulated by the local recruitment of Rpd3(L) histone deacetylase. (Middle) Gcn4-mediated recruitment of *HIS4* to the NPC is controlled by Gcn4 protein abundance. Gcn4 is translationally regulated. (Right) Upon mating pheromone stimulation, Ste12 mediates recruitment of *PRM1* to the NPC. Ste12 is regulated by MAPK phosphorylation of the inhibitor Dig2. (b) Gene recruitment and interchromosomal clustering are regulated through the cell cycle. During G1, gene recruitment and interchromosomal clustering of two loci (orange and blue) are localized at the NPC. During S-phase, phosphorylation of Nup1 blocks recruitment to the periphery but clustering is maintained in the nucleoplasm. During G2/M-phase the two loci are repositioned to the nuclear periphery, unclustered

and Ino4 TFs, neither of which are necessary to promote peripheral localization (Graves and Henry 2000; Randise-Hinchliff and Brickner 2016). Thus, genes such as *INO1* have separate elements and TFs controlling their transcription and positioning. In contrast, recruitment of *HIS4* and *PRM1* to the nuclear periphery is controlled by the same TFs that regulate their expression (Arndt and Fink 1986; Hagen et al. 1991; Randise-Hinchliff et al. 2016). *HIS4* repositions to the nuclear periphery upon activation by amino acid starvation and *PRM1* repositions upon mating pheromone stimulation and this requires Gcn4 and Ste12, respectively (Fig. 4.2a; (Randise-Hinchliff et al. 2016). The binding sites of Gcn4 (Gcn4BS) and Ste12 (pheromone-response element, 3xPRE) also function as DNA zip codes to target *URA3* to the nuclear periphery. This suggests that some, but not all, TFs function in mediating gene recruitment to the NPC. What distinguishes TFs that mediate gene recruitment from ones that do not? Put3, Cbf1, Gcn4 and Ste12 are not obviously similar in structure; representing four different families of TFs (Randise-Hinchliff and Brickner 2016). Furthermore, it is unclear how these factors mediate recruitment to the NPC. Is it through direct interaction with NPC components or through intermediate anchors such as TREX-2 or Mediator?

4.7 Interchromosomal Clustering at the NPC

Zip code-mediated targeting to NPC leads to interchromosomal clustering of genes. This can be observed by comparing the position of two loci that are targeted to the nuclear periphery in either haploid or diploid yeast cells (Brickner and Brickner 2012). Active *INO1* clusters at the NPC with another GRSI-containing gene, *TSA2* and with ectopic GRSI inserted at the *URA3* locus, but does not cluster with these loci in the nucleoplasm when repressed (Brickner et al. 2012). In diploid cells, two active alleles of *INO1* also cluster together. GRSI-mediated clustering requires the Put3 TF, which binds to GRSI. In contrast, *INO1* does not cluster with genes recruited to the nuclear periphery by different zip codes such as the *HSP104* gene (targeted by a different zip code called GRS3). Importantly, GRS3 inserted at *URA3* is sufficient to induce clustering with *HSP104*. Thus, clustering is zip code-specific. Interchromosomal clustering at the NPC has been observed for many genes such as *INO1*, *GAL1*, *HIS4*, *PRM1* and *HSP104* (Brickner et al. 2012, 2016; Randise-Hinchliff et al. 2016). Therefore, zip code-mediated targeting to the NPC leads to interchromosomal interactions and likely impacts the spatial organization of the yeast genome.

Targeting to the NPC is a prerequisite for zip-code mediated clustering. However, the molecular mechanisms controlling targeting to the NPC and interchromosomal clustering are distinct. For example, the recruitment of *GAL1* to the NPC, like *INO1*, is controlled by two redundant zip codes GRS4 and GRS5 (Brickner et al. 2016). Although both GRS4 and GRS5 are sufficient to target *URA3* locus to the nuclear periphery, GRS4 alone controls *GAL1* clustering (Brickner et al. 2016). Likewise, GRSI is both necessary and sufficient for *INO1* clustering whereas GRSII is not

(Brickner et al. 2012). Therefore, not all zip codes that are sufficient to target *URA3* to the nuclear periphery are sufficient to induce interchromosomal clustering. Clustering, unlike gene targeting, requires both transcription and transcriptional activators such as Gal4 (Brickner et al. 2016). Finally, the set of NPC components required for clustering are overlapping, but distinct, from the set required for targeting. Loss of Nup1, Nup60 and Mlp2 block both targeting to the nuclear periphery and clustering of *GAL1*, whereas loss of Mlp1 specifically blocks *GAL1* clustering without affecting peripheral targeting (Brickner et al. 2016).

4.8 Regulation of Gene Recruitment and Clustering

Gene recruitment to the NPC and interchromosomal clustering of many genes are conditional and occur under specific environmental stimuli (Randise-Hinchliff et al. 2016). This reflects how each zip code and the TF that binds to them are regulated. Put3-, Cbf1-, Ste12-, and Gcn4- mediated recruitment are regulated through different strategies (Randise-Hinchliff and Brickner 2016). Put3 and Cbf1 are regulated by a context-dependent mechanism. While Put3 and Cbf1 conditionally recruit *INO1* upon inositol starvation, when the GRSI and GRSII are inserted at an ectopic site, recruitment to the nuclear periphery is constitutive. This suggests that Put3 and Cbf1 have the capacity to recruit chromatin under repressing conditions, but are negatively regulated in the context of the *INO1* promoter. Indeed, Put3 and Cbf1 are regulated at *INO1* by local recruitment of Rpd3(L) histone deacetylase complex by transcriptional repressors, Ume6 and Opi1 (Fig. 4.2a; (Randise-Hinchliff et al. 2016). Loss of Rpd(L) leads to constitutive binding of Put3 and constitutive recruitment and interchromosomal clustering of *INO1* at the NPC. Many transcriptional repressors are sufficient to block GRSI- and GRSII-mediated recruitment to the NPC as well as GRSI-mediated clustering (Randise-Hinchliff et al. 2016). Sixteen of twenty one transcriptional repressors tested were able to block GRSI zip code activity (Randise-Hinchliff et al. 2016). This suggests that this is a general function of transcriptional repressors, which may provide multiple, alternative strategies to regulate the recruitment to the NPC mediated by a particular TF. For example, the *TSA2* gene is recruited to the NPC by Put3. *TSA2* is induced by protein folding stress, is not regulated by Ume6 or Opi1, and is recruited by different environmental stimuli (Ahmed et al. 2010; Brickner and Brickner 2012). Therefore, the context contributes to zip code regulation.

Gcn4- and Ste12-mediated gene recruitment are regulated through context-independent mechanisms (Fig. 4.2a; (Randise-Hinchliff and Brickner 2016). The zip code activity of the Gcn4 and Ste12 binding sites inserted at an ectopic site in the genome are still regulated (Randise-Hinchliff et al. 2016). Ste12-mediated recruitment is regulated downstream of DNA binding by MAPK phosphorylation of the inhibitor Dig2. Loss of Dig2 or a phosphomimetic mutation in Dig2 led to constitutive Ste12-mediated recruitment of both *PRM1* and the 3xPRE at *URA3*. Gcn4-mediated targeting is regulated by its abundance. Upregulating Gcn4 protein

levels led to an increased level of peripheral localization of *HIS4* and ectopic GCN4BS at *URA3*. Thus, in addition to regulation by local recruitment of transcriptional repressors, targeting to the NPC can be regulated by other mechanisms.

TF-mediated gene positioning and interchromosomal clustering is regulated by at least three different mechanisms that operate on different time scales (Randise-Hinchliff et al. 2016). MAPK signaling is rapid and leads to repositioning and clustering within 15–30 minutes. Changes in Gcn4 protein levels lead to slower repositioning and clustering of Gcn4 targets over 30–60 minutes. *INO1* recruitment and clustering occurs even more slowly over 60–120 minutes, consistent with the slow depression of *INO1* transcription. Thus, cells employ different strategies to regulate TF-mediated gene positioning over different time scales.

4.9 Gene Recruitment and Clustering Through the Cell Cycle

The recruitment of inducible genes to the NPC is regulated through the cell cycle. For active *INO1*, *GAL1* and *HSP104* genes, recruitment to the nuclear periphery occurs during G1 and G2/M, but not in S-phase when the genes localize in the nucleoplasm (Fig. 4.2b; (Brickner and Brickner 2010). Importantly, the loss of peripheral localization is not a nonspecific effect of DNA replication, but rather due to phosphorylation of Nup1 by the cyclin-dependent kinase Cdk1 (Brickner and Brickner 2010). Phosphorylation of Nup1 is required for normal targeting to the nuclear periphery; inactivation of Cdk or mutations that block phosphorylation of Nup1 also block targeting of *INO1* and *GAL1* to the periphery. Conversely, mutations in Nup1 that mimic phosphorylation at either of two sites or loss of the Cdk1 inhibitor, Sic1, led to *INO1* and *GAL1* remaining at the nuclear periphery during S-phase. The phosphomimetic mutations bypass the requirement of Cdk1, suggesting that Nup1 is the only protein whose phosphorylation affects peripheral targeting of these genes.

Interchromosomal clustering is also regulated through the cell cycle, but is out of phase with gene recruitment. *GAL1* clustering is maintained in the nucleoplasm through S-phase, but is lost upon repositioning to the periphery during G2/M (Fig. 4.2b; (Brickner et al. 2016). Interestingly, the regulation of peripheral targeting and clustering are interdependent. Loss of phosphorylation of Nup1 leads to loss of interchromosomal clustering and phosphomimetic Nup1 both maintains *GAL1* at the NPC during S-phase and leads to clustering during G2/M. Therefore, Cdk phosphorylation of the NPC coordinates the positioning of individual genes and the organization of chromosomes with respect to each other through the cell cycle.

4.10 Transcription Memory

Several inducible genes such as *INO1* and *GAL1* that are recruited to the NPC upon activation remain anchored to the pore for several generations after

repression (D'Urso and Brickner 2014). Such epigenetic retention leads to an altered chromatin structure and primes genes for rapid transcriptional reactivation. This phenomenon is called transcriptional memory and represents a mitotically heritable state. Furthermore, transcriptional memory leads to a faster or stronger response when cells are confronted with an environmental challenge previously experienced, presumably impacting cellular fitness and survival (D'Urso and Brickner 2014). Nuclear pore components play important roles in transcriptional memory, but not all genes that interact with the NPC when active exhibit memory. Understanding the mechanisms and specific NPC components involved in transcriptional memory can further elucidate the functions of the NPC.

A well-established model for transcriptional memory is *GAL1* (Brickner et al. 2007; Kundu et al. 2007; Brickner 2009). After being repressed, *GAL1* is retained at the nuclear periphery, primed for faster reactivation for up to seven generations (Brickner et al. 2007). During the first few hours, *GAL1* is anchored to the NPC as an intragenic loop between its promoter and 3' end; called a memory gene loop (MGL; (Tan-Wong et al. 2009). MGLs are stabilized at the NPC by Mlp1 and are thought to prime genes for reactivation by retaining transcription initiation factors, such as TBP. Indeed, destabilizing *GAL1* MGL, through loss of Mlp1, significantly reduces both TBP binding and the rate of reactivation (Tan-Wong et al. 2009). However, this is not the sole mechanism of *GAL1* transcriptional memory, since the *GAL1* MGL does not persist as long as memory (Brickner et al. 2007; Tan-Wong et al. 2009). It is possible that MGLs initiate memory and downstream mechanisms maintain transcriptional memory. Consistent with this notion, the chromatin remodeling complex, SWI/SNF1, is required for *GAL1* memory, but not for loop formation (Kundu et al. 2007). Interestingly, the inheritance of *GAL1* memory is not perpetuated by chromatin alone, but through *trans*-acting Gal1 protein itself, which is necessary for epigenetic memory (Zacharioudakis et al. 2007). Ectopic expression of *GAL1* is sufficient to induce faster induction of the other *GAL* genes (Zacharioudakis et al. 2007). Thus, the rapid reactivation of *GAL* genes involves multiple mechanisms including the formation of gene loops, chromatin-based mechanisms and *GAL1* protein itself.

Loss of the histone variant H2A.Z both blocks periphery localization of *INO1* and *GAL1* and causes a dramatic decrease in the reactivation after repression (Brickner et al. 2007) (our unpublished results). This suggests that peripheral localization is coupled to reactivation. Indeed, H2A.Z incorporation after repression depends on the nuclear pore protein Nup100. H2A.Z also physically associates with Nup2 (Dilworth et al. 2005). However, it is unclear how H2A.Z perpetuates memory. Loss of H2A.Z and Nup100 leads to a strong and specific defect in the rate of reactivation of *INO1* (Light et al. 2010), but loss of H2A.Z affects both the rate of activation and reactivation of *GAL1* (Halley et al. 2010). Similar to Nup2, H2A.Z functions to insulate euchromatin from the spread of heterochromatin (Meneghini et al. 2003). It is found in most inducible promoters and facilitates faster induction (Zhang et al. 2005; Albert et al. 2007; Wan et al. 2009). H2A.Z-containing nucleosomes are also less stable and flank nucleosome-free regions in promoters (Albert et al. 2007). Therefore, perhaps chromatin changes like H2A.Z

incorporation generally enhance the rate of transcriptional induction and such changes can be influenced by interactions with the NPC during memory.

INO1 gene remains associated with the nuclear periphery for up to four generations after repression, dependent on H2A.Z incorporation and Nup100 (Brickner et al. 2007; Light et al. 2010). After repression, the *INO1* promoter is marked with another chromatin mark, dimethylated histone H3 lysine 4 (H3K4me2). Memory leads to binding of poised RNA polymerase II (RNAPII) preinitiation complex (PIC), which enhances the rate of future reactivation (Fig. 4.3a; (Light et al. 2010; Light et al. 2013; D'Urso et al. 2016). Many of the NPC components required for active recruitment were also required in memory such as Nup1, Nup2 and Nup60. However, five Nups are specifically required for retention at the nuclear periphery

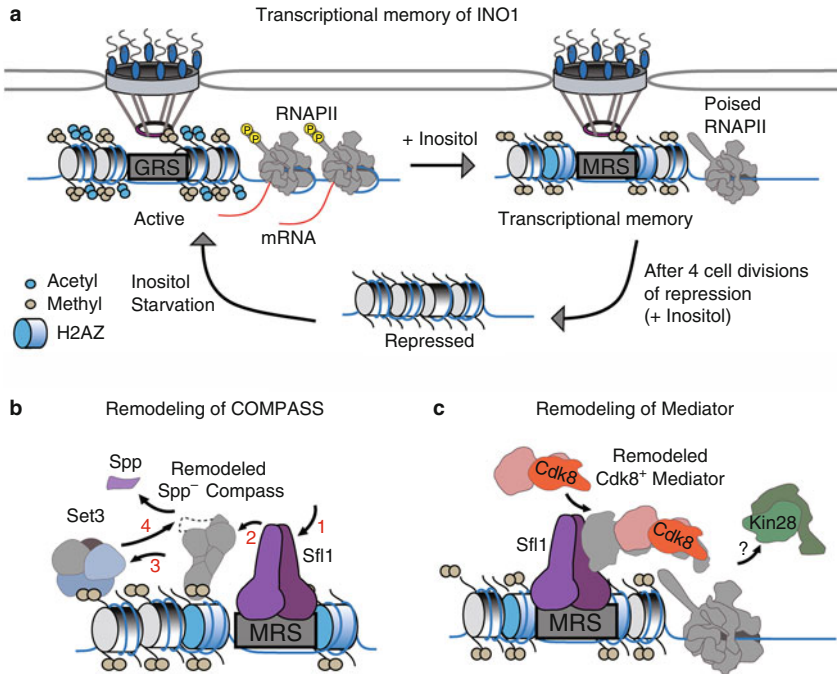

Fig. 4.3 Model of INO1 transcriptional memory at the NPC. (a) *INO1* is recruited to the NPC under inositol starvation, which leads to transcription by RNA polymerase II (RNAPII) as well as hyperacetylation and both di- and trimethylation of histone H3, lysine 4 (H3K4). Upon repression, *INO1* remains associated to the NPC and the preinitiation RNAPII is poised to the promoter for up to four generations. *INO1* transcriptional memory leads to an altered chromatin state involving the incorporation of H2A.Z and dimethylation of H3K4. (b) Transcription factor Sfl1 binds to the MRS upon repression and is required for recruitment and remodeling of Set1/COMPASS (1). The remodeled form of COMPASS lacking the Spp1 subunit is necessary to establish H3K3 dimethylation (2). H3K4me2 recruits Set3C (3). Set3C promotes the persistence of H3K4me2 and potentially the recruitment or remodeling of COMPASS (4). (c) $Cdk8^{+}$ Mediator promotes transcriptional poising. During activation, Mediator lacks Cdk8 and TFIIK (Kin28/Cdk7) phosphorylates Serine 5 of RNAPII to initiate transcription. Upon repression, Kin28 is lost and $Cdk8^{+}$ Mediator is recruited

during transcriptional memory: Nup100 and Nup84 subcomplex components Nup84, Nup120, Nup133, and Nup145C (Light et al. 2010). In contrast to *GAL1*, *INO1* does not require Mlp1 and MGLs do neither form nor are required for *INO1* memory (Tan-Wong et al. 2009; Light et al. 2010). By ChIP, Nup2 binds to the *INO1* promoter both in active and recently repressed conditions, whereas, Nup100 binds specifically during memory (Light et al. 2010). In strains lacking Nup100, the *INO1* promoter loses H2A.Z incorporation, H3K4me2 and poised RNA polymerase II PIC, leading to slower reactivation (Light et al. 2010, 2013).

Targeting of active and recently repressed *INO1* to the NPC is mediated by distinct mechanisms and different zip codes (Light et al. 2010). Recruitment of recently repressed *INO1* to the nuclear periphery does not require GRSI and GRSII. Instead, after repression a zip code called the Memory Recruitment Sequence (MRS) is both necessary and sufficient to recruit *INO1* to the NPC. A mutation in the MRS sequence specifically blocks *INO1* peripheral positioning after repression, but not in active conditions (Light et al. 2010). Finally unlike the GRS, MRS-mediated recruitment is not regulated throughout the cell cycle (Brickner and Brickner 2010).

Transcriptional memory also leads to interchromosomal clustering of *INO1* (Brickner et al. 2015). During memory, two alleles *INO1* remain clustered in diploid cells, which requires the MRS and Nup100. Unlike recruitment during memory, *INO1* clustering during memory also requires GRSI and GRSII zip codes (Brickner et al. 2015). Furthermore, neither GRSI or MRS inserted at *URA3* is sufficient to cause clustering with *INO1* during memory. In contrast, the ectopic GRSI clusters with *INO1* in active conditions (Brickner and Brickner 2012). This suggest clustering during memory requires previous clustering of *INO1* during activation. Therefore, the MRS zip code is necessary, but not sufficient, to induce clustering. Clustering of *INO1* during transcriptional memory is regulated through the cell cycle. In G2/M phase, *INO1* clustering is lost (Brickner et al. 2015). Therefore, MRS- and GRS- mediated recruitment and clustering of *INO1* share some similarities, but function by distinct mechanisms.

4.11 Molecular Mechanism of INO1 Transcriptional Memory

INO1 transcriptional memory is initiated by binding of a TF to the MRS zip code. The TF Sfl1 binds to the MRS upon shifting cells from activating to repressing conditions (Fig. 4.3; (D'Urso et al. 2016). Sfl1 has a genetic interaction with the Nup84 subcomplex component, Nup120, and is both necessary and sufficient to recruit chromatin to the nuclear periphery (Robertson and Fink 1998, D'Urso et al. 2016). Sfl1 and the MRS, like Nup100, are essential for all aspects of transcriptional memory (D'Urso et al. 2016). This suggests that binding of Sfl1 to the MRS initiates *INO1* transcriptional memory and may determine the duration of memory.

INO1 transcriptional memory is associated with histone modifications. When *INO1* is repressed, H3K4 is hypoacetylated and unmethylated whereas during

activation, H3K4 is hyperacetylated and both di- and trimethylated (Fig. 4.3a; (D'Urso et al. 2016). However, upon repression, *INO1* loses histone acetylation and trimethylation, but remains dimethylated (H3K4me2; (D'Urso et al. 2016). H3K4me2 is necessary for memory and is established by remodeling of the Set1/COMPASS methyltransferase complex, ejecting the Spp1 subunit (Fig. 4.3b; (D'Urso et al. 2016). The Spp1$^-$ complex is capable of dimethylation, but not trimethylation of H3K4 (Schneider et al. 2005; Takahashi et al. 2009). H3K4me2 recruits the SET3C histone deacetylase, which is also required for memory (D'Urso et al. 2016). Set3 is the eponymous member of SET3C and binds to H3K4me2 through its PHD domain (Kim and Buratowski 2009). SET3C binding to H3K4me2 is required both to recruit RNAPII and to maintain H3K4me2 during memory (D'Urso et al. 2016). Conditional inactivation of SET3C leads to rapid loss of both H3K4me2 and poised RNAPII from the *INO1* promoter (D'Urso et al. 2016). Thus, SET3C has a direct and continuous role in memory. The maintenance of H3K4me2 may provide a chromatin state that allows recruitment of RNAPII and rapid reactivation.

Changes in chromatin composition (H2A.Z) and histone modifications (H3K4me2) are necessary for transcriptional memory. These changes presumably allow RNAPII PIC to remain bound; poising genes for transcriptional reactivation (D'Urso and Brickner 2014). PIC assembly during memory also requires Cdk8$^+$ form of Mediator (Fig. 4.3c). Mediator binds to the *INO1* promoter both under activating and memory condition (D'Urso and Brickner 2014). However, the Cdk8 module only binds during memory. Inactivation of Cdk8 specifically disrupts RNAPII binding during memory and slows reactivation without affecting *INO1* activation (D'Urso and Brickner 2014). Interestingly, Cdk8$^+$ Mediator physically interacts with both Sfl1 and the NPC-associates TREX-2 complex, both of which are required for memory (Song and Carlson 1998; Schneider et al. 2015). The poised PIC complex during memory is partially assembled, missing both Ctk1 and Kin28, which phosphorylate serine 2 and 5 on the caboxy terminal domain, respectively (Light et al. 2010). Unlike Cdk8, Kin28 is also not required for memory and the poised RNAPII is unphosphorylated on Ser2 and 5. It's conceivable that Cdk8 and Kin28 are mutually exclusive and that Cdk8$^+$ Mediator promotes transcriptional poising by blocking Kin28 association with the PIC (Fig. 4.3c). Further experiments will discern this mechanism.

The mechanism of *INO1* memory is related to the mechanism of stress-induced memory in yeast and IFNγ-induced memory in human cells (D'Urso et al. 2016). In both systems, genes that display memory are marked with H3K4me2, bound by RNAPII and Cdk8. In yeast, 77 of the genes induced by oxidative stress are primed for activation in response to previously experienced salt stress (Guan et al. 2012). This effect persists for four generations. However, unlike *INO1*, salt stress-induced memory does not require Sfl1 or Nup100 and requires a different NPC component, Nup42, for faster reactivation (Guan et al. 2012). In human cells, genes that exhibit IFNγ-induced memory physically interact with Nup98, a homologue of Nup100, and require Nup98 for memory (Light et al. 2013). Unlike in yeast, IFNγ-induced genes interact with Nup98 in the nucleoplasm. Despite these

differences, the core mechanism revealed by studies of *INO1* transcriptional memory is both general and conserved (D'Urso et al. 2016).

4.12 Concluding Remarks

From yeast to humans, the NPC plays essential roles in promoting transcription, regulating chromatin structure, spatially organizing eukaryotic genomes. Research in yeast has guided our understanding of these mechanisms, many of which are evolutionarily conserved. These observations have broadened our understanding of the NPC's role as a regulatory hub for genome organization and function.

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