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Interaction of a DNA Zip Code with the Nuclear Pore Complex Promotes H2A.Z Incorporation and *INO1* Transcriptional Memory

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SUMMARY

DNA "zip codes" in the promoters of yeast genes confer interaction with the NPC and localization at the nuclear periphery upon activation. Some of these genes exhibit transcriptional memory: after being repressed, they remain at the nuclear periphery for several generations, primed for reactivation. Transcriptional memory requires the histone variant H2A.Z. We find that targeting of active *INO1* and recently repressed *INO1* to the nuclear periphery is controlled by two distinct and independent mechanisms involving different zip codes and different interactions with the NPC. An 11 base pair memory recruitment sequence (MRS) in the *INO1* promoter controls both peripheral targeting and H2A.Z incorporation after repression. In cells lacking either the MRS or the NPC protein Nup100, *INO1* transcriptional memory is lost, leading to nucleoplasmic localization after repression and slower reactivation of the gene. Thus, interaction of recently repressed *INO1* with the NPC alters its chromatin structure and rate of reactivation.

INTRODUCTION

Eukaryotic chromosomes fold and localize in stereotypical ways with respect to each other and with respect to nuclear structures (Cremer et al., 2006). The spatial arrangement of chromosomes correlates with the differentiation status of the cell and the localization of individual genes within the nucleus can impact their expression. Many developmentally regulated genes localize near the nuclear periphery when repressed and move to a more internal, nucleoplasmic location after differentiation (Takizawa et al., 2008). Likewise, the tethering of telomeres to the nuclear envelope promotes the repression of subtelomeric genes (Gasser, 2001; Hediger and Gasser, 2002; Taddei et al., 2004; Taddei et al., 2009). These observations have suggested that the nuclear periphery is a transcriptionally repressive environment. Consistent with this notion, lamin-associated parts of the genome tend to be silenced, and artificially tethering DNA to the nuclear lamina is sufficient to cause repression of many neighboring genes (Finlan et al., 2008; Kumaran and Spector, 2008; Reddy et al., 2008). However, localization to the nuclear periphery does not always result in transcriptional repression. A number of genes in yeast relocalize from the nucleoplasm to the nuclear periphery upon activation (Brickner and Walter, 2004; Casolari et al., 2005; Casolari et al., 2004; Sarma et al., 2007; Schmid et al., 2006; Taddei et al., 2006). Therefore, the effects of peripheral localization on transcription are not simple and may be different for different genes or may depend on the targeting mechanism (Akhtar and Gasser, 2007).

We have used the recruitment of active genes to the nuclear periphery in yeast as a model to understand both the mechanisms that control the localization of individual genes and how localization affects gene expression. Genes that localize to the nuclear periphery in yeast physically associate with the nuclear pore complex (NPC) (Casolari et al., 2004). We have found that the yeast *INO1* gene is targeted to the NPC by two gene recruitment sequences (GRSs) in its promoter (Ahmed et al., 2010). These elements act as "zip codes"; they are sufficient to target the normally nucleoplasmic *URA3* locus to the nuclear periphery when integrated nearby (Ahmed et al., 2010). Finally, loss of peripheral targeting leads to defective expression of both *INO1* and another GRS-containing gene, *TSA2* (Ahmed et al., 2010), in the nucleoplasm, suggesting that interaction of these genes with the NPC promotes their full transcriptional activation.

INO1 is activated by inositol starvation (Greenberg et al., 1982). When cells are shifted to medium lacking inositol, *INO1* quickly relocalizes to the nuclear periphery (Brickner et al., 2007; Brickner and Walter, 2004). If inositol is added back, *INO1* transcription is rapidly repressed. However, after being repressed, *INO1* remains at the nuclear periphery within the population for greater than 6 hr, or up to four cell divisions (Brickner et al., 2007). In other words, the localization of recently repressed *INO1* both reflects the previous transcription of the gene and represents a heritable, epigenetic state. While at the nuclear periphery, *INO1* is primed for reactivation (see below). We have termed this phenomenon "transcriptional memory," defined as the persistent localization of a gene at the nuclear periphery after repression in a primed state that promotes reactivation. Transcriptional memory is not unique to *INO1*; galactose-regulated genes that are targeted to the nuclear periphery upon activation (such as *GAL1*) also remain at the nuclear periphery in an H2A.Z-dependent manner for generations after repression (Brickner et al., 2007). In fact, in the case of the *GAL* genes, the rate of reactivation is much faster than the rate of initial activation (Brickner et al., 2007; Brickner, 2009).

Epigenetic transcriptional memory is associated with increased H2A.Z incorporation at the *INO1* promoter (Brickner et al., 2007; Brickner, 2009). H2A.Z is a universally conserved variant of histone H2A that is found in nucleosomes within the promoters of most genes from yeast to plants to humans (Creyghton et al., 2008; Guillemette et al., 2005; Li et al., 2005; Raisner et al., 2005; Zhang et al., 2005; Zilberman et al., 2008). Loss of H2A.Z in yeast leads to defects in the activation of certain genes and to loss of boundary activity between transcriptionally silent and nonsilent parts of the genome (Meneghini et al., 2003; Raisner and Madhani, 2006). This boundary function has been linked to the NPC; H2A.Z physically interacts with NPC components, loss of NPC components leads to loss of boundary activity (Dilworth et al., 2005; Meneghini et al., 2003), and artificially tethering DNA to the NPC is sufficient to create a boundary (Ishii et al., 2002). H2A.Z also plays an essential role in transcriptional memory. Mutants lacking H2A.Z fail to retain recently repressed *INO1* and *GAL1* at the nuclear periphery and exhibit a strong defect in their reactivation (Brickner et al., 2007). This role is specific; loss of H2A.Z does not affect the targeting of active *INO1* to the nuclear periphery or the rate of its initial activation (Brickner et al., 2007). Thus, the mechanisms of activation and reactivation are different for these genes and peripheral localization after repression promotes reactivation.

To better understand the molecular mechanism of transcriptional memory, we determined the molecular requirements for localization of recently repressed *INO1* to the nuclear periphery and H2A.Z incorporation into the *INO1* promoter. We find that the previously identified GRS elements do not contribute to the localization of recently repressed *INO1* to the nuclear periphery. Instead, a different *cis*-acting DNA zip code called the memory recruitment sequence (MRS) controls peripheral targeting of recently repressed *INO1*. The MRS is also necessary and sufficient to induce local H2A.Z incorporation. Furthermore, certain components of the nuclear pore complex are necessary for the peripheral localization of recently repressed *INO1* but are not necessary for the peripheral localization of active *INO1*. One of these proteins, Nup100, plays an essential and specific role in transcriptional memory. Nup100 physically interacts with recently repressed *INO1* but not with active *INO1*. Mutants lacking either the MRS or Nup100 fail to retain *INO1* at the nuclear periphery and fail to incorporate H2A.Z into the repressed promoter. This leads to loss of transcriptional memory in *nup100Δ* mutants; the recently repressed *INO1* gene is unable to associate with a poised RNA polymerase II and shows slower reactivation. Thus, the *INO1* gene utilizes two independent mechanisms to interact with the NPC, one that promotes the transcriptional output of the active gene and another that primes the recently repressed gene for reactivation.

RESULTS

Distinct Mechanisms Control Targeting of Active and Recently Repressed *INO1* to the Nuclear Periphery

INO1 localizes to the nuclear periphery both when active and for ~6 hr after being repressed (Brickner et al., 2007; Brickner and Walter, 2004). Two *cis*-acting DNA zip codes called GRS I and GRS II control the targeting of active *INO1* to the nuclear periphery (Ahmed et al., 2010). To dissect the molecular mechanisms controlling *INO1* gene localization, we have integrated *INO1* at a test site, the *URA3* gene, which normally localizes in the nucleoplasm (Figure 1A) (Ahmed et al., 2010). We used this system to ask whether localization of recently repressed *INO1* to the nuclear periphery was recapitulated when the gene was integrated beside *URA3*. The *INO1* gene (504 base pairs 5′ to 685 base pairs 3′ of the coding sequence) and an array of Lac repressor binding sites were integrated adjacent to *URA3* (*URA3:INO1*; Figure 1A). The GFP-Lac repressor was expressed in this strain, and we used immunofluorescence to visualize GFP and the nuclear envelope (marked with Sec63-myc). To quantify the peripheral localization of *URA3:INO1*, we determined the fraction of the population in which the GFP spot colocalized with the nuclear envelope (Brickner et al., 2010; Brickner and Walter, 2004). The *URA3* gene colocalized with the nuclear envelope in 25%–30% of cells, which represents the baseline level of peripheral localization in this assay (Figure 1B; indicated as a hatched blue line throughout) (Brickner and Walter, 2004). *URA3:INO1* colocalized with the nuclear envelope in ~30% of cells under repressing conditions (Figure 1B; +inositol) and in ~65% of the cells under activating conditions (Figure 1B; −inositol) (Ahmed et al., 2010). After 1 hr of repression, *URA3:INO1* remained at the nuclear periphery (Figure 1B; −ino → +ino). Therefore, like the endogenous *INO1* gene, *URA3:INO1* localized to the nuclear periphery after repression (Brickner et al., 2007; Brickner and Walter, 2004).

We next asked whether the GRS elements contribute to the retention of *INO1* at the nuclear periphery after repression. Because the plasmid used to create *URA3:INO1* lacks GRS II, the targeting of active *URA3:INO1* to the nuclear periphery is dependent exclusively on the GRS I zip code (Ahmed et al., 2010). Transversion mutations in GRS I (*grs I* mutant) block targeting of *URA3:INO1* to the nuclear periphery in activating conditions (Figure 1B, −inositol) (Ahmed et al., 2010). However, after shifting of cells from activating to repressing conditions, *grs I* mutant *URA3:INO1* was localized to the nuclear periphery (Figure 1B). Therefore, localization of recently repressed *INO1* at the nuclear periphery is not dependent on GRS I (or GRS II, which is absent from *URA3:INO1*). Furthermore, this result indicates that localization of active *INO1* to the nuclear periphery is not a prerequisite for the localization of recently repressed *INO1* to the nuclear periphery.

The histone variant H2A.Z is essential for retention of *INO1* at the nuclear periphery after repression (Brickner et al., 2007). To test whether the targeting of recently repressed *URA3:INO1* to the nuclear periphery is also dependent on H2A.Z, we examined the localization of *URA3:INO1* with or without the GRS I element (*Δgrs* in Figure 1C; a 50 base pair deletion within the promoter, called Δ3 in Ahmed et al. [2010]) and with or without H2A.Z (*htz1Δ*). These four strains were shifted from activating to repressing conditions, and the localization of *URA3:INO1* was monitored over time. At the beginning of the time course (i.e., activating conditions), wild-type *URA3:INO1* was localized to the nuclear periphery, whereas *grs IΔ* mutant *URA3:INO1* was localized to the nucleoplasm (Figure 1C). After repression, wild-type *URA3:INO1* remained at the nuclear periphery in strains with H2A.Z, but not in the *htz1Δ* mutant (Figure 1C).

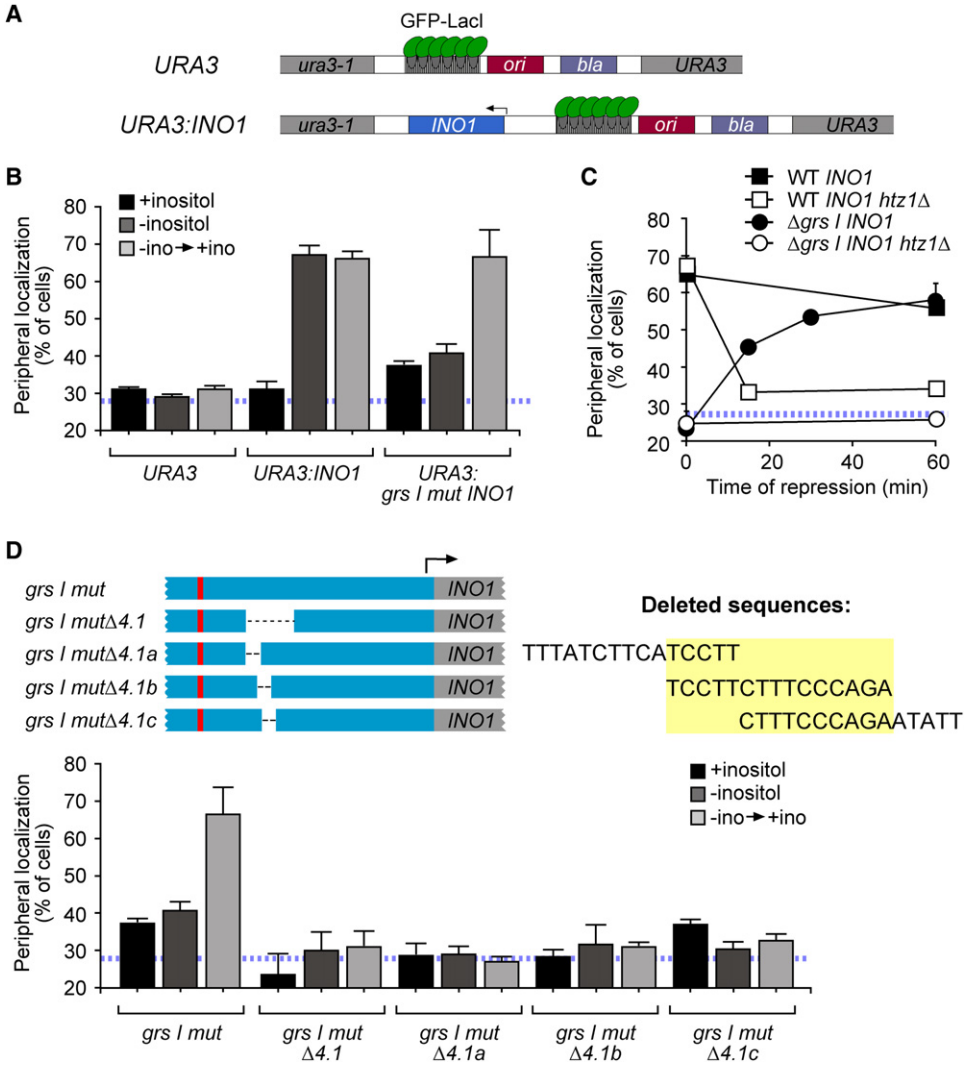

Figure 1. Different *cis*-Acting DNA Elements Control Peripheral Localization of Active and Recently Repressed *INO1*

(A) Schematic of the lac operator array plasmid, with or without *INO1*, integrated at *URA3*.

(B) Quantitative localization assay of *URA3*, *URA3:INO1*, or *grs I* mutant *URA3:INO1*. The hatched blue line indicates the baseline for this assay. Cells were grown in the presence or absence of 100 μM inositol or switched from medium lacking inositol to medium containing inositol for 1 hr (−ino → +ino).

(C) Time course of peripheral localization after repression. The indicated strains were shifted from activating to repressing conditions, and cells were harvested and fixed for immunofluorescence and chromatin localization at the indicated times.

(D) Top: left, schematic of *INO1* promoter mutants; right, deleted sequences. Bottom: peripheral localization from strains having *grs I* mutant *INO1* (data same as in B) or *grs I* mutant *INO1* with each of the deletion mutations at *URA3*.

Error bars represent the SEM from three biological replicates.

Finally, *grs IΔ* mutant *URA3:INO1* was recruited from the nucleoplasm to the nuclear periphery after repression, and this targeting required H2A.Z (Figure 1C). Therefore, like the endogenous *INO1* gene (Brickner et al., 2007), localization of recently repressed *URA3:INO1* to the nuclear periphery required H2A.Z.

A DNA Zip Code Controls Peripheral Localization of Recently Repressed *INO1*

Because targeting of *URA3:INO1* to the nuclear periphery after repression was unaffected by loss of the known GRS elements, we hypothesized that additional *cis*-acting DNA zip codes were responsible for the localization of recently repressed *INO1* to the nuclear periphery. Consistent with this hypothesis, loss of either a 50 base pair region (segment 4.1) or smaller, overlapping segments (Δ4.1a–c) of the *INO1* promoter resulted in nucleoplasmic localization of *grs I* mutant *URA3:INO1* after repression (Figure 1D). This suggested that the 15 base pair region common to these segments is necessary for localization of recently repressed *INO1* to the nuclear periphery (Figure 1D).

DNA zip codes like GRS I and GRS II are both necessary for targeting of active *INO1* to the nuclear periphery and sufficient,

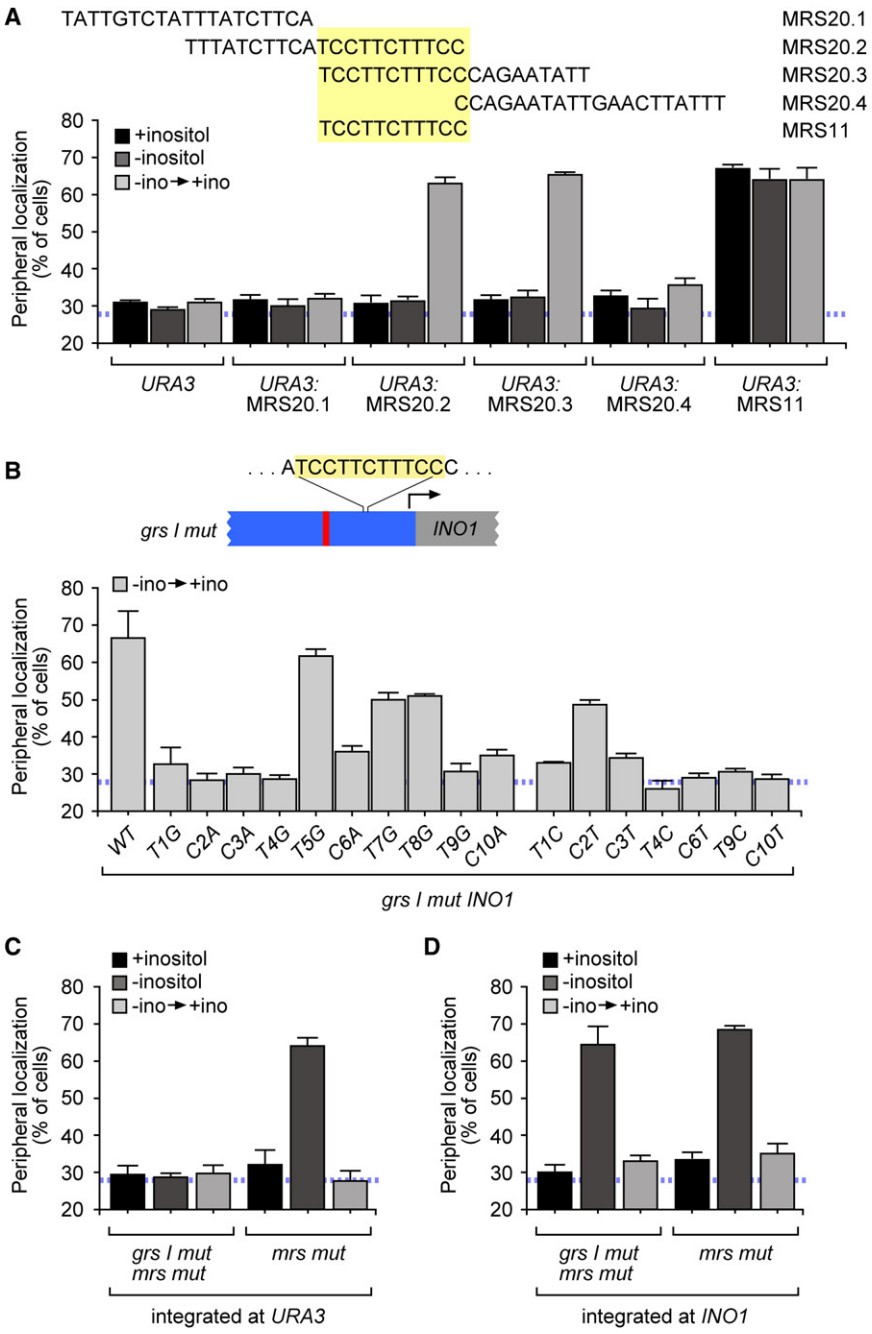

Figure 2. The MRS Is a Sequence-Specific DNA Zip Code

(A) The MRS functions as a DNA zip code. Top: sequences of inserts tested for peripheral localization when integrated at *URA3* using the strategy shown in Figure 3A. Bottom: peripheral localization of *URA3* (data same as in Figure 1B) or *URA3* having each of the indicated DNA fragments integrated nearby.

(B) Top: schematic of *INO1* promoter, indicating relative positions of the *grs I* mutation (red bar) and the MRS. Bottom: peripheral localization of either the *grs I* mutant (labeled WT; same data as shown in Figure 1B) or the indicated single base pair substitutions within the MRS integrated beside *URA3*.

(C and D) Peripheral localization of *mrs* mutant *INO1* or *grs I mrs* mutant *INO1* integrated at *URA3* (C) or at the endogenous *INO1* locus (D).

Error bars represent the SEM from three biological replicates. See also Figure S1.

in isolation, to target an ectopic locus like *URA3* to the nuclear periphery (Ahmed et al., 2010). When removed from the *INO1* promoter, GRS I and GRS II function as constitutive targeting elements, suggesting that they are normally negatively regulated (Ahmed et al., 2010). To test whether the sequences that were necessary for *URA3:INO1* localization to the nuclear periphery after repression functioned as DNA zip codes, we integrated a series of 20 base pair sequences derived from segment 4.1 beside *URA3* (integration scheme in Figure 3A). Remarkably, two of the four 20 base pair sequences resulted in relocalization of *URA3* to the nuclear periphery under recently repressing conditions but not under activating or long-term repressing conditions (Figure 2A). Thus, the ability of these fragments to function as zip codes was dependent on the previous expression of *INO1*. This suggests that peripheral targeting can be regulated in *trans* and is not necessarily an effect of being part of a promoter that was previously expressed (see the Discussion). Furthermore, the regulation of targeting was affected by sequences from the *INO1* promoter both 5′ and 3′ of the element. However, the 11 base pair element common to these sequences was sufficient to target *URA3* to the nuclear periphery regardless of inositol growth conditions (Figure 2A). This suggests that the targeting function and its regulation are separable and that the protein(s) responsible for targeting are present under all of these growth conditions. Thus, this minimal MRS functions as a DNA zip code to target an ectopic locus to the nuclear periphery.

To ask whether the targeting function of the MRS is determined by its sequence, we introduced single base pair transversion mutations at ten positions of the MRS within the promoter of *grs I* mutant *URA3:INO1* and tested the localization of these mutants after 1 hr of repression (Figure 2B). Mutations at positions 1, 2, 3, 4, 6, 9, and 10 blocked peripheral targeting after repression (Figure 2B). Mutations at positions 2, 7, and 8 resulted in partial defects in peripheral targeting, while the T5G mutant did not affect localization. Transition mutations at positions 1, 3, 4, 6, 9, and 10, but not position 2, also blocked

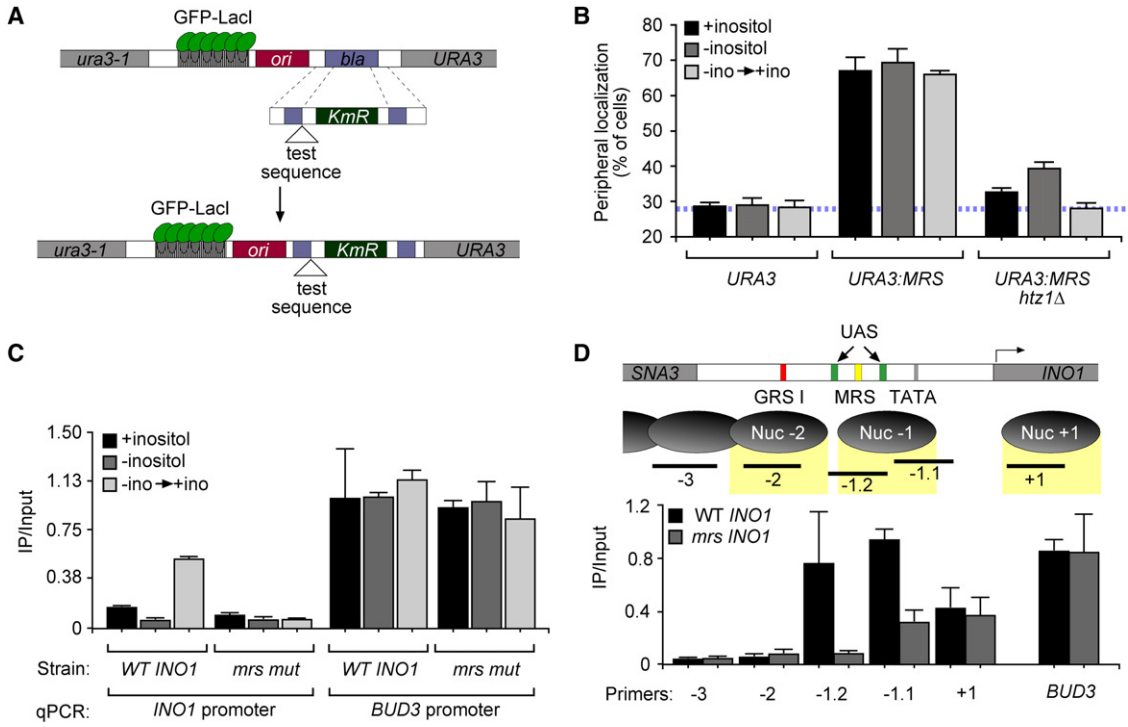

Figure 3. The MRS and the Histone Variant H2A.Z Control Peripheral Targeting of Recently Repressed *INO1*

(A) Scheme for integrating DNA elements for localization experiments (Ahmed et al., 2010).

(B) Peripheral localization of *URA3*, *URA3:MRS*, and *URA3:MRS* in the *htz1Δ* strain.

(C) Chromatin immunoprecipitation of HA-H2A.Z (Meneghini et al., 2003) from wild-type and *mrs* mutant *INO1* strains and quantified using primers to amplify −197 to −284 relative to the *INO1* ORF or primers to amplify the *BUD3* promoter.

(D) Top: map of nucleosomes in the *INO1* promoter. Shown are the positions of GRS I (red box), the MRS (yellow box), the TATA box (gray box), two UAS_{INO} elements (green boxes), and the PCR products associated with each nucleosome. Bottom: ChIP of HA-H2A.Z from either a wild-type or *mrs* mutant *INO1* strain, quantified using primers corresponding to the locations in the top panel or the *BUD3* promoter.

Error bars represent the SEM from three biological replicates.

peripheral targeting (Figure 2B). These results indicate that the sequence of the MRS is essential for its function as a DNA zip code.

GRS I and GRS II function are not necessary to target recently repressed *INO1* to the nuclear periphery. However, it remained possible that these elements might be sufficient and redundant with the MRS element for targeting of recently repressed *INO1*, either at *URA3* or at the endogenous *INO1* locus. To test this possibility, we introduced mutations in the MRS (*mrs* mutant) either alone or in combination with the *grs I* mutation, in the promoter of *INO1* and tested these mutants for peripheral targeting at *URA3* or the endogenous locus. As expected, mutations in GRS I blocked targeting of active *URA3:INO1* but did not block targeting of active endogenous *INO1* to the nuclear periphery because of the presence of GRS II (Figures 2C and 2D) (Ahmed et al., 2010). Mutation of the MRS alone blocked targeting of recently repressed *URA3:INO1* and recently repressed endogenous *INO1* (Figures 2C and 2D). Therefore, the MRS is the only *cis*-acting DNA element governing peripheral localization of recently repressed *INO1*. Furthermore, if cells were switched from recently repressing conditions to activating conditions again, the *URA3:mrs mut INO1* returned to the nuclear periphery, indicating that targeting of active *INO1* to the nuclear periphery is independent of targeting of recently repressed *INO1* to the nuclear periphery (Figure S1A available online).

The MRS Controls H2A.Z Incorporation

H2A.Z is essential for retention of *INO1* and *GAL1* at the nuclear periphery after repression (I. Cajigas and J.H.B., unpublished data) (Brickner et al., 2007). Loss of H2A.Z also results in a strong defect in reactivation of *INO1* and *GAL1* (Brickner et al., 2007). To explore the connection between H2A.Z incorporation and gene localization, we asked whether MRS-mediated targeting of *URA3* to the nuclear periphery also required H2A.Z. In cells lacking H2A.Z (*htz1Δ*), *URA3:MRS* localized in the nucleoplasm (Figure 3B). Therefore, H2A.Z is essential both for peripheral targeting of recently repressed *INO1* and for MRS-mediated peripheral targeting of an ectopic locus.

We next used chromatin immunoprecipitation (ChIP) to determine whether the MRS affects H2A.Z incorporation at *INO1*. As an internal positive control for H2A.Z nucleosomes, we also measured the association of HA-H2A.Z with the *BUD3* promoter (Raisner et al., 2005). We observed weak association of H2A.Z with the long-term repressed *INO1* promoter and an increased association of H2A.Z with the recently repressed *INO1* promoter

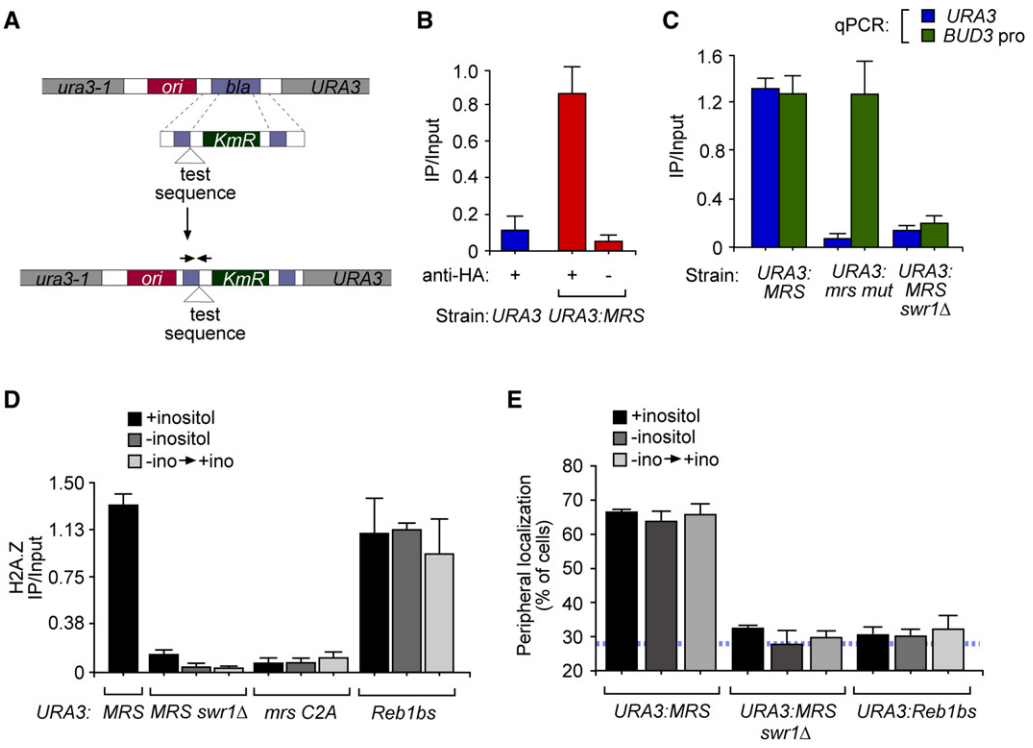

Figure 4. The MRS Is Sufficient to Induce H2A.Z Incorporation

(A) Integration scheme for inserting DNA elements for ChIP experiments (as in Ahmed et al., 2010).

(B) ChIP of HA-H2A.Z at *URA3*. The MRS or a control insert were integrated at *URA3*. Immunoprecipitations were performed with or without 12CA5 mAb against the HA tag.

(C) ChIP of HA-H2A.Z from *URA3:MRS*, *mrsmut:URA3*, or *URA3:MRS swr1Δ* strains.

(D and E) ChIP of HA-H2A.Z (D) or peripheral localization (E) from strains having the MRS (with or without Swr1), the mrs C2A, or the Reb1bs integrated at *URA3*. For (B)–(D), immunoprecipitated DNA was quantified relative to input DNA by using real-time PCR with primers for both the *INO1* promoter and *BUD3* promoter. Error bars represent the SEM from three biological replicates.

(Figure 3B) (Brickner et al., 2007). The ChIP signal at *INO1* was slightly lower than that at *BUD3* (likely due to the primers used for this experiment; see below). However, the association of H2A.Z with the *INO1* promoter was MRS dependent; we did not detect H2A.Z associated with the promoter of *mrs* mutant *INO1* (Figure 3C). Therefore, the MRS is necessary both for targeting of recently repressed *INO1* to the nuclear periphery and for H2A.Z incorporation into the *INO1* promoter.

Our previous work and genome-wide studies have mapped several nucleosomes within the *INO1* promoter (Figure 3D) (Brickner et al., 2007; Kaplan et al., 2009; Segal et al., 2006). Relative to the transcriptional start site, one nucleosome is centered ~75 bp downstream (Nuc+1), another is centered ~175 bp upstream (Nuc-1), and another, less well-positioned nucleosome is evident starting ~250 bp upstream (Nuc-2). The MRS element is within sequences protected by Nuc −1 (−201 to −211; yellow box in Figure 3D). Mutations in the MRS element did not alter the position of these nucleosomes (Figure S1B). We performed ChIP against HA-H2A.Z from wild-type or *mrs* mutant *INO1* strains and quantified the recovery using primers corresponding to each of these nucleosomes (Figure 3D, top panel). The peak of DNA recovered with HA-H2A.Z from wild-type strains was within Nuc-1, and this association was lost from *mrs* mutant *INO1* strains (Figure 3D). This is consistent with the analysis in Figure 3B, in which we used primers slightly upstream of Nuc −1 (INO1prom For/Rev; −197 to −284). DNA associated with Nuc+1 was also weakly associated with HA-H2A.Z, but this was not MRS dependent. This suggests that, upon repression, the MRS promotes H2A.Z incorporation into a single nucleosome in the *INO1* promoter that includes the MRS, one of the upstream activating sequences (UAS_{INO}) and the TATA box (Figure 3D).

We next asked whether the MRS is sufficient to promote H2A.Z incorporation. We used ChIP against HA-H2A.Z in a strain having the MRS element integrated beside *URA3* (Figure 4A). H2A.Z immunoprecipitated *URA3:MRS* but not *URA3* (Figure 4B), *URA3:mrs* mutant (Figure 4C), or *URA3:mrsC2A* point mutant that was defective for peripheral targeting (Figure 4D). Thus, the defects we observed in localization correlate with the absence of H2A.Z. Furthermore, loss of the catalytic subunit of the ATPase responsible for incorporation of H2A.Z, the Swr1 protein (Mizuguchi et al., 2004), blocked both H2A.Z incorporation (Figures 4C and 4D) and targeting to the nuclear periphery (Figure 4E). Therefore, the MRS is sufficient to promote Swr1-mediated H2A.Z incorporation at an ectopic location.

Peripheral Targeting Requires Both H2A.Z and the MRS

Because H2A.Z incorporation was necessary for MRS-mediated targeting to the nuclear periphery, we next asked whether H2A.Z incorporation alone is sufficient to promote targeting to the nuclear periphery. To test this idea, we introduced a different DNA sequence adjacent to *URA3* that has previously been shown to promote H2A.Z incorporation (Raisner et al., 2005). The Reb1 binding site along with a poly T sequence (Reb1bs) is sufficient to induce a nucleosome-free region and incorporation of H2A.Z into the flanking nucleosomes (Hartley and Madhani, 2009; Raisner et al., 2005). Indeed, as measured by ChIP, integration of the Reb1bs beside *URA3* was sufficient to promote H2A.Z incorporation (Figure 4D). However, *URA3:Reb1bs* did not localize to the nuclear periphery (Figure 4E). Therefore, incorporation of H2A.Z is not sufficient to control subnuclear localization and targeting of recently repressed *INO1* to the nuclear periphery requires a bipartite signal involving both the MRS and H2A.Z.

The MRS Is Essential for *INO1* Transcriptional Memory

Loss of H2A.Z leads to both a defect in retention of recently repressed *INO1* at the nuclear periphery and a defect in the reactivation of *INO1* (Brickner et al., 2007). Having identified a second component of a bipartite signal for *INO1* targeting to the nuclear periphery, we next asked whether the MRS affects reactivation of *INO1*. We assayed the kinetics of *INO1* activation and reactivation in wild-type, *htz1Δ* and *mrs* mutant strains using RT-qPCR. The rate of activation of *INO1* was very similar in the wild-type, *htz1Δ*, and *mrs* mutant *INO1* strains (Figure 5A). Therefore, H2A.Z and the MRS element are not involved in *INO1* activation (Brickner et al., 2007). Unlike the *GAL* genes, the reactivation of *INO1* is not faster than the initial activation (Brickner et al., 2007). The reactivation occurs after a delay of approximately 90 min (Figure 5B). This is likely due to the effect of persistent Ino1 enzyme and the recent addition of inositol on the concentration of inositol in cells and the time required for cells to perceive its absence (Brickner et al., 2007). However, the rate at which reactivation occurs was strongly affected by loss of H2A.Z and by mutations in the MRS element, suggesting that these components normally promote *INO1* reactivation (Figure 5B). The ultimate steady-state levels of *INO1*, upon activation or reactivation, were indistinguishable between the wild-type and *mrs* mutant *INO1* strains (Figure S1C). This indicates that *INO1* transcriptional memory affects the rate of transcriptional reactivation and requires both the MRS and H2A.Z.

To assess whether MRS-mediated transcriptional memory affects the recruitment of RNA polymerase II to the *INO1* promoter, we performed ChIP using a monoclonal antibody against the carboxy-terminal domain (CTD) of the large subunit of RNA polymerase II during activation and reactivation of wild-type and *mrs mut INO1* (Figures 5C and 5D). During activation, we observed no difference in the rate of recruitment of RNA polymerase II to the *INO1* promoter between wild-type and *mrs* mutant *INO1* (Figure 5C). However, during reactivation, we were surprised to find RNA polymerase II associated with the wild-type *INO1* promoter at the beginning of the time course (Figure 5D). In contrast, we did not observe RNA polymerase II associated with the *mrs* mutant *INO1* promoter until after ~90 min after starving cells for inositol (Figure 5D). This suggested that MRS-mediated transcriptional memory leads to association of RNA polymerase II with the repressed *INO1* promoter.

We also monitored RNA polymerase II association with the *INO1* promoter after repression. The *INO1* gene is repressed very rapidly after addition of inositol (Brickner et al., 2007; Greenberg et al., 1982), and the association of RNA polymerase II with the coding sequence was lost within minutes of addition of inositol (Figure S1D). However, RNA polymerase II remained associated with the wild-type *INO1* promoter for ≥6 hr (three to four generations) after repression (Figure 5E). This is consistent with the duration of transcriptional memory as measured by localization of *INO1* at the nuclear periphery after repression (Brickner et al., 2007). In contrast, RNA polymerase II association with the *mrs* mutant *INO1* promoter was lost after repression (Figure 5E). This effect was specific to the *INO1* gene; RNA polymerase II did not remain associated with the promoters of two other inositol-repressed genes, *OPI3* and *CHO2*, after repression (Figure S1E).

The RNA polymerase II that was associated with the recently repressed *INO1* promoter was not phosphorylated on either Serine 2 or 5 of the CTD (Figure 5F), suggesting that it is not active for transcription. Inactive RNA polymerase II also associates with hundreds of poised promoters during stationary phase in yeast, allowing them to be rapidly induced upon entry into log phase (Radonjic et al., 2005). This suggests that *INO1* transcriptional memory promotes reactivation by permitting association of poised RNA polymerase II with the repressed promoter.

Distinct Nuclear Pore Components Control the Targeting of Active and Recently Repressed *INO1*

In budding yeast and metazoans, localization of active genes to the nuclear periphery requires NPC components (Ahmed et al., 2010; Brickner et al., 2007; Brown et al., 2008; Cabal et al., 2006; Casolari et al., 2004; Kurshakova et al., 2007; Schmid et al., 2006). To determine whether the NPC also plays a role in the localization of recently repressed *INO1*, we quantified the peripheral localization of recently repressed *INO1* in 30 mutant strains lacking different NPC components or associated proteins (Figures 6A and 6B; summarized in Figure S2). These proteins could be grouped into three classes: those that were not required for targeting of either active or recently repressed *INO1* (red bars in Figure 6), those that were required for targeting of both active and recently repressed *INO1* (blue bars in Figure 6), and those that were specifically required for targeting of recently repressed *INO1* (purple bars in Figure 6). Thus, the NPC proteins required for targeting of recently repressed *INO1* represent a superset of the proteins required for targeting of active *INO1*. Proteins required for peripheral targeting of both active and recently repressed *INO1* included proteins in the nuclear basket and basket-associated proteins (Figure S2). The five proteins specifically required for peripheral targeting of recently repressed *INO1* included all of the members of the Nup84 subcomplex that were tested (Nup84, Nup120, Nup133, and the C terminus of Nup145) as well as Nup100 (Figure S2). This further confirms that GRS-dependent and MRS-dependent targeting to the NPC represent two distinct mechanisms that control localization of *INO1* during two distinct phases of its regulation.

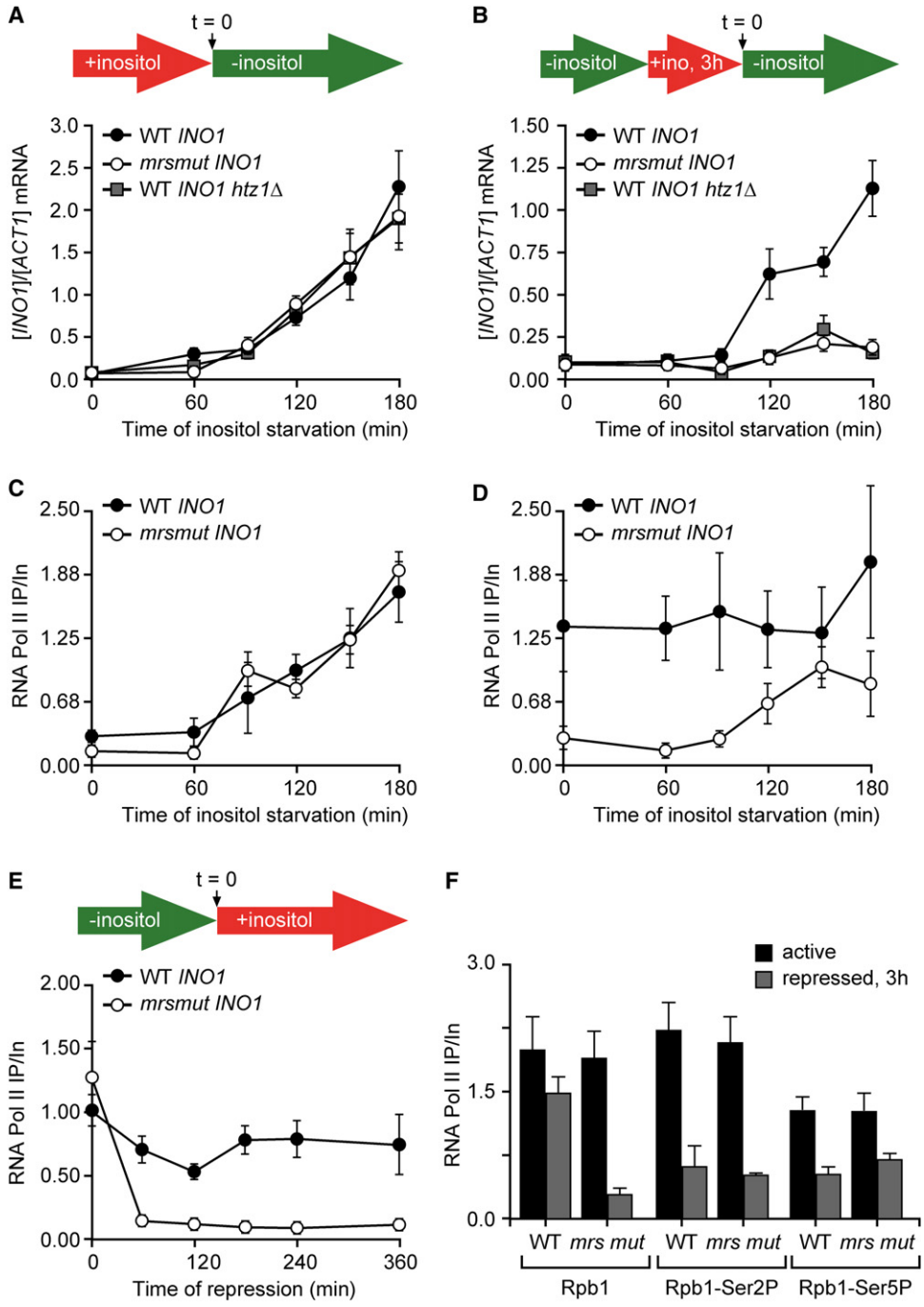

Figure 5. The MRS Is Required for Transcriptional Memory

(A) *INO1* activation. At time = 0, cells were shifted from repressing medium containing 100 μM inositol (red arrow in schematic) to medium without inositol (green arrow in schematic). Cells were harvested at the indicated time points, and *INO1* mRNA levels were quantified relative to *ACT1* mRNA levels by RT-qPCR.

(B) *INO1* reactivation. Cells were shifted from activating medium to repressing medium containing 100 μM inositol for 3 hr. At time = 0, cells were harvested and returned to medium without inositol. Cells were harvested at the indicated time points, and *INO1* mRNA levels were quantified relative to *ACT1* mRNA levels by RT-qPCR.

(C) ChIP with anti-Rbp1 antibody at the indicated time points during activation (same scheme as in A).

(D) ChIP with anti-Rbp1 antibody at the indicated time points during reactivation (same scheme as in B).

(E) ChIP with anti-Rbp1 antibody (8WG16) from wild-type or *mrs mut INO1* strains after repression.

(F) ChIP using anti-Rpb1, anti-phospho Ser2 CTD, or anti-phospho Ser5 CTD antibodies from wild-type or *mrs mut INO1* strains under activating conditions or after 3 hr of repression.

Error bars represent the SEM from three biological replicates. See also Figure S1.

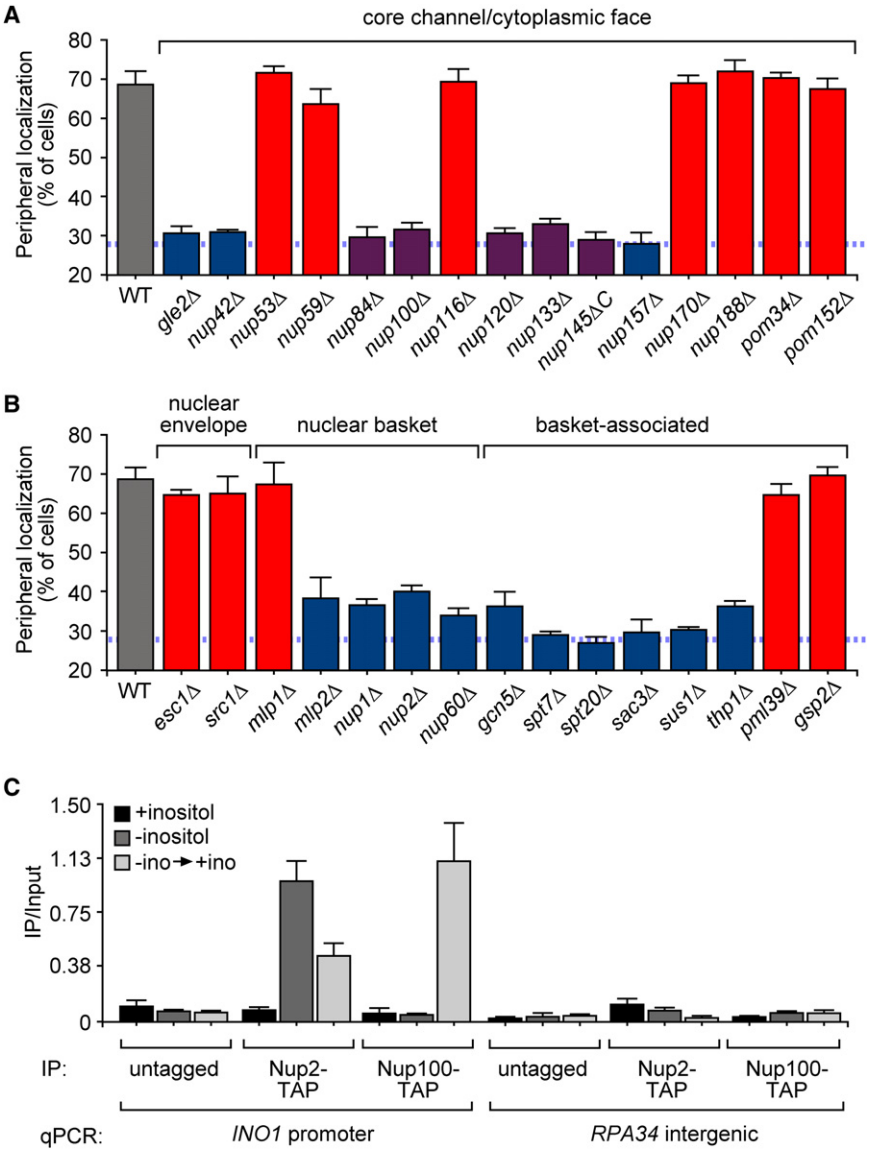

Figure 6. NPC Proteins required for Targeting of Active and Recently Repressed *INO1*

(A and B) Peripheral localization of recently repressed *INO1* in NPC mutant strains. The wild-type and mutant strains were grown overnight in medium lacking inositol, inositol was added for 1 hr, and cells were fixed for immunofluorescence. Red bars highlight strains that targeted both active and recently repressed *INO1* to the nuclear periphery. Blue bars highlight strains that failed to target both active and recently repressed *INO1* to the nuclear periphery. Purple bars highlight strains that targeted active *INO1* to the nuclear periphery but failed to target recently repressed *INO1* to the nuclear periphery.

(C) ChIP of TAP-tagged Nup2 or Nup100. Immunoprecipitated DNA was quantified relative to input DNA using real-time PCR and primers specific for the *INO1* promoter and *RPA34* intergenic region (negative control).

Error bars represent the SEM from three biological replicates. See also Figure S2.

These observations suggest that active and recently repressed *INO1* interact differently with the NPC.

We also tested whether Nup100 was required for transcriptional memory. In mutants lacking Nup100, we did not observe H2A.Z incorporation into the *INO1* promoter after repression (Figure 7A) or at *URA3:MRS* (data not shown). Incorporation of H2A.Z into the *BUD3* promoter was unaffected by loss of Nup100 (Figure 7B). Strains lacking Nup100 activated *INO1* at the same rate as wild-type strains but showed significantly slower reactivation of *INO1* (Figures 7C and 7D). Loss of Nup100 did not affect the ultimate steady state expression of *INO1* (Figure S1F). Finally, loss of either Nup100 or H2A.Z led to loss of poised RNA polymerase II from the recently repressed *INO1* promoter (Figures 7E and 7F). Therefore, the MRS element and Nup100 play essential and specific roles in *INO1* transcriptional memory: they promote (1) localization of recently repressed *INO1* to the nuclear periphery, (2) H2A.Z incorporation after repression, (3) association of poised RNA polymerase II with the *INO1* promoter, and (4) rapid reactivation of recently repressed *INO1*.

Nup100 Interacts Specifically with Recently Repressed *INO1* and Is Required for Transcriptional Memory

A number of NPC components physically interact with active genes by ChIP (Ahmed et al., 2010; Brickner et al., 2007; Casolari et al., 2005; Casolari et al., 2004; Dieppois et al., 2006; Luthra et al., 2007). We used ChIP to monitor the interaction of two NPC components, Nup2 and Nup100, with active and recently repressed *INO1*. The nucleoplasmic basket protein Nup2, which is required for peripheral targeting of both active and recently repressed *INO1*, interacted with active *INO1* and recently repressed *INO1*, but not long-term repressed *INO1* (Figure 6C) (Ahmed et al., 2010). Therefore, both active *INO1* and recently repressed *INO1* physically interact with nuclear pore components. Nup100, a protein that was specifically required for peripheral localization of recently repressed *INO1*, gave a robust ChIP interaction *only* with recently repressed *INO1* (Figure 6C).

DISCUSSION

Here, we show that the mechanisms controlling the localization and initial induction of a gene can be different from the mechanisms controlling its localization and reactivation after repression. Both active *INO1* and recently repressed *INO1* localize to the nuclear periphery (Brickner et al., 2007). However, while the targeting of active *INO1* to the NPC requires two *cis*-acting GRS

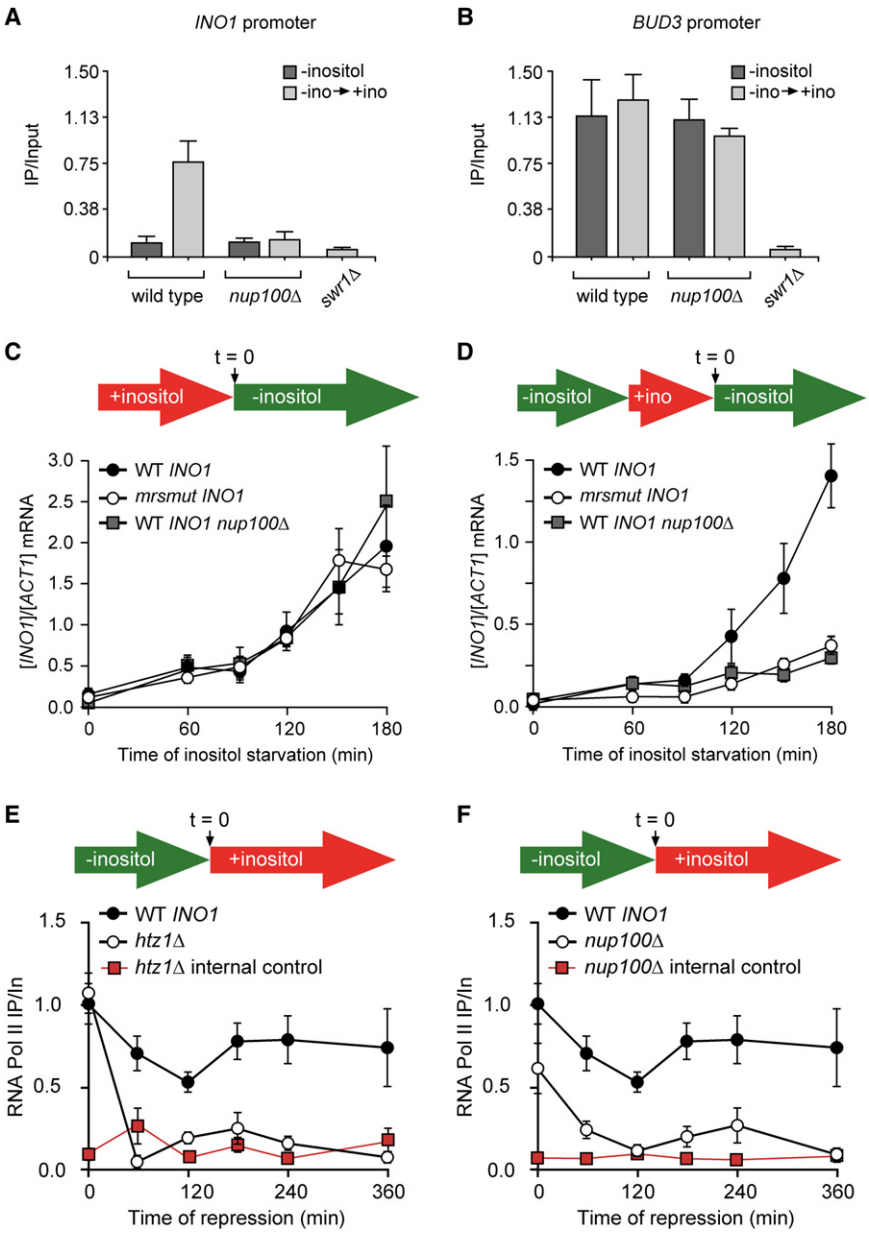

Figure 7. Nup100 Is Essential for *INO1* Transcriptional Memory

(A and B) ChIP of HA-H2A.Z in wild-type, *nup100Δ*, and *swr1Δ* strains grown without inositol or shifted from −inositol to +inositol for 1 hr before crosslinking and processing for ChIP. Immunoprecipitated DNA was quantified relative to input DNA using primers against the *INO1* promoter and the *BUD3* promoter.

(C) Wild-type, *nup100Δ* or *mrs* mutant *INO1* cells were harvested at the indicated times after shifting from repressing to activating conditions. *INO1* mRNA levels were quantified relative to *ACT1* mRNA levels by RT-qPCR.

(D) After 3 hr of repression, at time = 0 the strains were shifted back to activating medium and harvested at the indicated times. *INO1* mRNA levels were quantified relative to *ACT1* mRNA levels by RT-qPCR.

(E) ChIP with anti-Rbp1 after repression from wild-type and *htz1Δ* strains.

(F) ChIP with anti-Rbp1 after repression from wild-type and *nup100Δ* strains.

In (E) and (F), the recovery of the *INO1* promoter or the repressed *GAL1* promoter (internal control), relative to input DNA, was quantified by q-PCR; the wild-type data are the same as in Figure 5E. Error bars represent the SEM from three biological replicates. See also Figure S1.

elements (Ahmed et al., 2010), the targeting of recently repressed *INO1* to the NPC requires a distinct *cis*-acting element, the MRS. The targeting mediated by the GRS elements and the MRS elements involves distinct interactions with the NPC. These two targeting mechanisms are independent of each other; peripheral localization of recently repressed *INO1* does not depend on prior targeting of active *INO1*, and vice versa. The MRS element is necessary and sufficient to promote H2A.Z incorporation and requires H2A.Z to function as a DNA zip code. Retention of *INO1* at the nuclear periphery, incorporation of H2A.Z after repression, and rapid reactivation of *INO1* also require the NPC component Nup100. Therefore, these two targeting mechanisms produce different outcomes. Whereas GRS-mediated targeting of active *INO1* to the NPC promotes robust transcription (Ahmed et al., 2010), MRS-mediated targeting to the NPC promotes H2A.Z incorporation and RNA polymerase II association, promoting faster reactivation.

The Nuclear Pore Complex and Transcription

Recent work in *Drosophila* suggests that active genes physically interact with NPC proteins like the Nup100 homolog Nup98 (Capelson et al., 2010; Kalverda et al., 2010; Kurshakova et al., 2007; Sunthara-lingam and Wente, 2003; Vaquerizas et al., 2010). Furthermore, the expression of many genes requires NPC proteins (Capelson et al., 2010; Vaquerizas et al., 2010). The *hsp70* locus and the hyperactive X chromosome in males both localize at the nuclear periphery (Kurshakova et al., 2007; Vaquerizas et al., 2010). However, contrary to what has been observed in yeast, some of the interactions of genes with NPC components in *Drosophila* occur in the nucleoplasm (Capelson et al., 2010; Kalverda et al., 2010; Vaquerizas et al., 2010). This suggests that NPC proteins may have conserved roles in promoting transcription and that this need not always occur at the nuclear periphery. Furthermore, our data indicate that genes can interact with nuclear pore proteins by multiple pathways that influence expression level, chromatin structure, and transcriptional kinetics. Therefore, interpretation of the correlation between

such interactions and gene expression might be more complex than anticipated. Also, because the two pathways we have identified are independent of each other, this raises the possibility that some genes may use either pathway alone. In other words, certain genes may be targeted to the NPC upon activation only, other genes (like *INO1*) are targeted to the NPC both when active and when recently repressed, and yet other genes might be targeted to the NPC only when repressed. Consistent with this idea, we find that many of the genes that are targeted to the nuclear periphery when active return to the nucleoplasm after repression (D.G.B. and J.H.B., unpublished data).

The zip codes that target active and recently repressed *INO1* to the NPC confer distinct physical interactions with the NPC. We observed this difference by ChIP: active *INO1* interacted robustly with Nup2 but not with Nup100 and recently repressed *INO1* interacted with both proteins. Furthermore, a subset of the proteins of the NPC were specifically required for targeting recently repressed *INO1* to the nuclear periphery. This raises an important point: although interaction with the NPC correlates with localization to the nuclear periphery, it is possible for a gene (i.e., active *INO1*) to localize to the nuclear periphery without interacting with certain NPC proteins by ChIP (i.e., Nup100). This also suggests that active *INO1* and recently repressed *INO1* interact in biochemically distinct complexes with the NPC. We propose that sequence-specific DNA binding proteins interact with these elements in the *INO1* promoter and with different parts of the NPC to produce different effects. Alternatively, it is conceivable that active and recently repressed *INO1* interact with NPCs of distinct molecular composition.

The Mechanism of Transcriptional Memory

Several genes in yeast exhibit transcriptional memory. In addition to *INO1*, the *GAL* genes (*GAL1*, *GAL2*, *GAL7*, and *GAL10*) remain at the nuclear periphery for up to seven generations after repression, primed for reactivation (Brickner et al., 2007; Kundu et al., 2007). Several mechanistic explanations have been offered for *GAL* gene memory. As with *INO1*, loss of H2A.Z causes the *GAL* genes to lose peripheral localization after repression and leads to a strong defect in reactivation of *GAL1* (I. Cajigas and J.H.B., unpublished data) (Brickner et al., 2007). The ATP-dependent chromatin remodeler SWI/SNF is also required for rapid reactivation (Kundu et al., 2007). Subsequent work showed that the Gal1 protein itself played an important role in transcriptional memory (Zacharioudakis et al., 2007). Recently, *GAL* gene transcriptional memory has been linked to the formation of persistent loops between the 5′ and 3′ end of the genes, associated with the NPC (Lainé et al., 2009; Tan-Wong et al., 2009). The experimental regimes used in these studies were different, and this has raised the possibility that *GAL* genes utilize more than one type of transcriptional memory (Brickner, 2009, 2010; Kundu and Peterson, 2010). Furthermore, because SWI/SNF (Bryant et al., 2008), H2A.Z (Gligoris et al., 2007), and Gal1 (Zacharioudakis et al., 2007) also promote the activation of the *GAL* genes, it has been unclear whether they have specific roles in memory. This has led some authors to conclude that SWI/SNF and H2A.Z play roles in activation but are not involved in memory (Bryant et al., 2008; Halley et al., 2010). Although this is not the place to explain all of these results, it is clear from our work that *INO1* memory is simpler. Loss of a DNA element or a nuclear pore protein that are required for H2A.Z incorporation into the *INO1* promoter, like loss of H2A.Z, has a strong and specific effect on reactivation and the binding of RNA polymerase II to the repressed promoter. Thus, H2A.Z plays an essential and direct role in promoting *INO1* transcriptional memory. If the *GAL* genes utilize multiple, independent forms of transcriptional memory that have some overlap in their duration, then it would be difficult to interpret the effects of mutations using transcription rates alone. Examining mutants for their effects on *GAL* gene reactivation at various times after repression, *GAL* gene localization at the nuclear periphery and association of poised RNA polymerase II after repression might clarify the roles of these and other factors in *GAL* gene memory.

The MRS and Nup100 are required for H2A.Z incorporation into the *INO1* promoter after repression, suggesting that targeting of the gene to the NPC promotes incorporation of the histone variant. How does interaction of recently repressed *INO1* with the NPC affect H2A.Z incorporation? The functional relationship between the MRS, the NPC, and H2A.Z is not a simple, linear genetic pathway. The MRS is necessary and sufficient to promote H2A.Z incorporation. Loss of Nup100 blocks H2A.Z incorporation. However, MRS-mediated targeting to the nuclear periphery also requires H2A.Z, and H2A.Z incorporation by itself is not sufficient to induce peripheral localization. We propose that transcriptional memory utilizes a positive feedback system involving a bipartite NPC targeting mechanism requiring both H2A.Z and the MRS. The interaction of the *INO1* promoter with the NPC might allow the SWR1 complex to exchange H2A.Z/H2B for H2A/H2B in Nuc-1. H2A.Z nucleosomes and NPC-associated nucleosomes are among the most dynamic nucleosomes in the yeast genome (Dion et al., 2007). Indeed, the nuclease protection conferred by the MRS-dependent H2A.Z nucleosome in the *INO1* promoter is less robust in the recently repressed *INO1* promoter than in the long-term repressed *INO1* promoter (Brickner et al., 2007). Therefore, this nucleosome might be unstable or more rapidly turned over. If so, the disassembly of this nucleosome could allow both NPC interaction and RNA polymerase II association. In this way, NPC targeting could promote H2A.Z incorporation, and H2A.Z incorporation could promote retention at the NPC and prime the promoter for reactivation.

One aspect of the transcriptional memory phenomenon that is particularly striking is that the localization of the *INO1* and *GAL1* genes to the nuclear periphery is maintained within the population for generations after repression (Brickner et al., 2007). Thus, both the gene that was physically transcribed and its descendants are localized to the nuclear periphery and primed for reactivation. How is this transcriptional memory inherited? We envision that targeting of recently repressed *INO1* and incorporation of H2A.Z could be controlled by a sequence-specific DNA binding protein whose ability to function at the *INO1* promoter is affected by recent growth conditions. Consistent with this idea, 20 base pair fragments from the *INO1* promoter, when introduced beside *URA3*, were targeted to the nuclear periphery only when *INO1* has been recently repressed. Incorporation of H2A.Z at *URA3* in these strains also occurs only after shifting of cells from activating to repressing conditions

(Figure S3). This suggests that diffusible factors control targeting and MRS-mediated H2A.Z incorporation in *trans*. We hypothesize that the localization and chromatin structure of genes that exhibit transcriptional memory are regulated by proteins that are produced during activation but function only on the repressed form of the gene. If so, such proteins might interfere with the normal repression of these genes for a period of time that can extend through multiple generations, depending on their rate of turnover and the critical concentration required for their function. Such a system would then allow cells to more rapidly produce enzymes required to grow under conditions that they have previously encountered.

H2A.Z Incorporation

The association of H2A.Z with MRS-containing promoters is significantly higher than the association of H2A.Z with all promoters, based on genome-wide ChIP experiments ($p = 0.02$ with a two-tailed t test; Figure S4) (Zhang et al., 2005). The MRS element is the second example of a DNA sequence that is sufficient to induce H2A.Z incorporation. The Reb1 binding site and a polyT sequence confer H2A.Z incorporation, when inserted into the coding sequence of the *PRM1* gene, through the recruitment of the RSC chromatin remodeler (Hartley and Madhani, 2009; Raisner et al., 2005). We think that the MRS represents a different mechanism for several reasons. First, MRS-mediated incorporation of H2A.Z, both within the *INO1* promoter and when the MRS is integrated near *URA3* (data not shown), requires Nup100. This is not a generally true of H2A.Z nucleosomes; loss of Nup100 had no effect on the incorporation of H2A.Z in the promoter of the *BUD3* gene. Second, unlike the Reb1bs, the MRS behaves as a DNA zip code, targeting *URA3* to the nuclear periphery. Third, insertion of the MRS element and the Reb1bs result in distinct patterns of H2A.Z incorporation within the *PRM1* coding sequence (Figure S5). Finally, in the context of the *INO1* promoter, the MRS promotes incorporation of H2A.Z into a single nucleosome, and, unlike the Reb1bs, it does not create an obvious nucleosome-free region (Hartley and Madhani, 2009). Therefore, the interaction of the MRS with the NPC may represent a pathway by which H2A.Z incorporation can be controlled to create an altered chromatin state that primes genes for reactivation after repression.

EXPERIMENTAL PROCEDURES

Chemicals and Reagents

Unless noted otherwise, chemicals were from Sigma Aldrich, media components were from Q-BIOgene, oligonucleotides were from Operon or Integrated DNA Technologies, and restriction enzymes were from New England Biolabs. Fluorescent secondary antibodies, antibodies against GFP, Protein G dynabeads, and Pan mouse IgG dynabeads were from Invitrogen. Other antibodies were from Santa Cruz Biotechnology (anti-myc 9E10), Encore Biotechnology (anti-Nsp1), Covance (anti-Rpb1, 8WG16), and Abcam (anti-CTD Ser2P and anti-CTD Ser5P). The 12CA5 anti-HA antibody was a generous gift from Robert Lamb.

Chromatin Localization Assay

Chromatin localization was performed as described previously (Brickner and Walter, 2004) and detailed in Brickner et al. (2010), in the Northwestern University Biological Imaging Facility. Error bars represent the standard error of the mean for three biological replicates of 30–50 cells each. The hatched blue line in Figures 1, 2, 3, and 6 represents the mean peripheral localization of the *URA3* gene.

Plasmid Construction

Plasmids pRS306, pRS306-INO1, p6LacO128, and p6LacO128INO1 have been described (Brickner and Walter, 2004). All oligonucleotides used in this study are listed in Table S1. Site-directed mutagenesis was performed with high-fidelity PCR and mutagenic primers, followed by digestion with *Dpn*I. Transformants were then screened and confirmed by sequencing. Mutant sequences were then cloned into p6LacO128.

Yeast Strains

Yeast strains used in this study are listed in Table S2. The integration of test sequences at *URA3* was performed as shown in Figure 3A and as described previously (Ahmed et al., 2010). The *mrs* mutation (or, as a control, wild-type *INO1*) was introduced into the endogenous *INO1* promoter via homologous recombination (Ahmed et al., 2010). The MRS, *mrs* mutant, *mrsC2A* mutant, and the Reb1bs were introduced within *PRM1* with the *Delitto Perfetto* system (Storici et al., 2003).

Chromatin Immunoprecipitation

ChIP was performed as described (Ahmed et al., 2010). Recovery of the *INO1* promoter by ChIP was analyzed with INO1prom For and Rev, except in Figure 3D (Table S1). TAP tagged Nup2 and Nup100 were recovered with Pan Mouse IgG Dynabeads. HA-H2A.Z was recovered with 12CA5 anti-HA antibody and Protein G Dynabeads. Error bars represent the SEM from three biological replicates.

Reverse Transcriptase Real-Time Quantitative PCR

For experiments in which mRNA levels were quantified, RT-qPCR was performed as described (Brickner et al., 2007). Error bars represent the SEM of three biological replicates.

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures and two tables and can be found with this article online at doi:10.1016/j.molcel.2010.09.007.

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REFERENCES

Ahmed, S., Brickner, D.G., Light, W.H., Cajigas, I., McDonough, M., Froyshteter, A.B., Volpe, T., and Brickner, J.H. (2010). DNA zip codes control an ancient mechanism for gene targeting to the nuclear periphery. Nat. Cell Biol. *12*, 111–118.

Akhtar, A., and Gasser, S.M. (2007). The nuclear envelope and transcriptional control. Nat. Rev. Genet. *8*, 507–517.

Brickner, J.H. (2009). Transcriptional memory at the nuclear periphery. Curr. Opin. Cell Biol. *21*, 127–133.

Brickner, J.H. (2010). Transcriptional memory: staying in the loop. Curr. Biol. *20*, R20–R21.

Brickner, J.H., and Walter, P. (2004). Gene recruitment of the activated INO1 locus to the nuclear membrane. PLoS Biol. *2*, e342.

Brickner, D.G., Cajigas, I.C., Fondufe-Mittendorf, Y., Ahmed, S., Lee, P.-C., Widom, J., and Brickner, J.H. (2007). H2A.Z-mediated localization of genes at the nuclear periphery confers epigenetic memory of previous transcriptional state. PLoS Biol. *5*, e81.

Brickner, D.G., Light, W.H., and Brickner, J.H. (2010). Quantitative localization of chromosomal loci by immunofluorescence. In Methods in Enzymology, J. Weissman, C. Guthrie, and G.R. Fink, eds. (Burlington: Academic Press), pp. 569–580.

Brown, C.R., Kennedy, C.J., Delmar, V.A., Forbes, D.J., and Silver, P.A. (2008). Global histone acetylation induces functional genomic reorganization at mammalian nuclear pore complexes. Genes Dev. *22*, 627–639.

Bryant, G.O., Prabhu, V., Floer, M., Wang, X., Spagna, D., Schreiber, D., and Ptashne, M. (2008). Activator control of nucleosome occupancy in activation and repression of transcription. PLoS Biol. *6*, 2928–2939.

Cabal, G.G., Genovesio, A., Rodriguez-Navarro, S., Zimmer, C., Gadal, O., Lesne, A., Buc, H., Feuerbach-Fournier, F., Olivo-Marin, J.C., Hurt, E.C., and Nehrbass, U. (2006). SAGA interacting factors confine sub-diffusion of transcribed genes to the nuclear envelope. Nature *441*, 770–773.

Capelson, M., Liang, Y., Schulte, R., Mair, W., Wagner, U., and Hetzer, M.W. (2010). Chromatin-bound nuclear pore components regulate gene expression in higher eukaryotes. Cell *140*, 372–383.

Casolari, J.M., Brown, C.R., Komili, S., West, J., Hieronymus, H., and Silver, P.A. (2004). Genome-wide localization of the nuclear transport machinery couples transcriptional status and nuclear organization. Cell *117*, 427–439.

Casolari, J.M., Brown, C.R., Drubin, D.A., Rando, O.J., and Silver, P.A. (2005). Developmentally induced changes in transcriptional program alter spatial organization across chromosomes. Genes Dev. *19*, 1188–1198.

Cremer, T., Cremer, M., Dietzel, S., Müller, S., Solovei, I., and Fakan, S. (2006). Chromosome territories—a functional nuclear landscape. Curr. Opin. Cell Biol. *18*, 307–316.

Creyghton, M.P., Markoulaki, S., Levine, S.S., Hanna, J., Lodato, M.A., Sha, K., Young, R.A., Jaenisch, R., and Boyer, L.A. (2008). H2AZ is enriched at polycomb complex target genes in ES cells and is necessary for lineage commitment. Cell *135*, 649–661.

Dieppois, G., Iglesias, N., and Stutz, F. (2006). Cotranscriptional recruitment to the mRNA export receptor Mex67p contributes to nuclear pore anchoring of activated genes. Mol. Cell. Biol. *26*, 7858–7870.

Dilworth, D.J., Tackett, A.J., Rogers, R.S., Yi, E.C., Christmas, R.H., Smith, J.J., Siegel, A.F., Chait, B.T., Wozniak, R.W., and Aitchison, J.D. (2005). The mobile nucleoporin Nup2p and chromatin-bound Prp20p function in endogenous NPC-mediated transcriptional control. J. Cell Biol. *171*, 955–965.

Dion, M.F., Kaplan, T., Kim, M., Buratowski, S., Friedman, N., and Rando, O.J. (2007). Dynamics of replication-independent histone turnover in budding yeast. Science *315*, 1405–1408.

Finlan, L.E., Sproul, D., Thomson, I., Boyle, S., Kerr, E., Perry, P., Ylstra, B., Chubb, J.R., and Bickmore, W.A. (2008). Recruitment to the nuclear periphery can alter expression of genes in human cells. PLoS Genet. *4*, e1000039.

Gasser, S.M. (2001). Positions of potential: nuclear organization and gene expression. Cell *104*, 639–642.

Gligoris, T., Thireos, G., and Tzamarias, D. (2007). The Tup1 corepressor directs Htz1 deposition at a specific promoter nucleosome marking the GAL1 gene for rapid activation. Mol. Cell. Biol. *27*, 4198–4205.

Greenberg, M.L., Reiner, B., and Henry, S.A. (1982). Regulatory mutations of inositol biosynthesis in yeast: isolation of inositol-excreting mutants. Genetics *100*, 19–33.

Guillemette, B., Bataille, A.R., Gévry, N., Adam, M., Blanchette, M., Robert, F., and Gaudreau, L. (2005). Variant histone H2A.Z is globally localized to the promoters of inactive yeast genes and regulates nucleosome positioning. PLoS Biol. *3*, e384.

Halley, J.E., Kaplan, T., Wang, A.Y., Kobor, M.S., and Rine, J. (2010). Roles for H2A.Z and Its Acetylation in GAL1 Transcription and Gene Induction, but Not GAL1-Transcriptional Memory. PLoS Biol. *8*, e1000401.

Hartley, P.D., and Madhani, H.D. (2009). Mechanisms that specify promoter nucleosome location and identity. Cell *137*, 445–458.

Hediger, F., and Gasser, S.M. (2002). Nuclear organization and silencing: putting things in their place. Nat. Cell Biol. *4*, E53–E55.

Ishii, K., Arib, G., Lin, C., Van Houwe, G., and Laemmli, U.K. (2002). Chromatin boundaries in budding yeast: the nuclear pore connection. Cell *109*, 551–562.

Kalverda, B., Pickersgill, H., Shloma, V.V., and Fornerod, M. (2010). Nucleoporins directly stimulate expression of developmental and cell-cycle genes inside the nucleoplasm. Cell *140*, 360–371.

Kaplan, N., Moore, I.K., Fondufe-Mittendorf, Y., Gossett, A.J., Tillo, D., Field, Y., LeProust, E.M., Hughes, T.R., Lieb, J.D., Widom, J., and Segal, E. (2009). The DNA-encoded nucleosome organization of a eukaryotic genome. Nature *458*, 362–366.

Kumaran, R.I., and Spector, D.L. (2008). A genetic locus targeted to the nuclear periphery in living cells maintains its transcriptional competence. J. Cell Biol. *180*, 51–65.

Kundu, S., and Peterson, C.L. (2010). Dominant role for signal transduction in the transcriptional memory of yeast GAL genes. Mol. Cell. Biol. *30*, 2330–2340.

Kundu, S., Horn, P.J., and Peterson, C.L. (2007). SWI/SNF is required for transcriptional memory at the yeast GAL gene cluster. Genes Dev. *21*, 997–1004.

Kurshakova, M.M., Krasnov, A.N., Kopytova, D.V., Shidlovskii, Y.V., Nikolenko, J.V., Nabirochkina, E.N., Spehner, D., Schultz, P., Tora, L., and Georgieva, S.G. (2007). SAGA and a novel Drosophila export complex anchor efficient transcription and mRNA export to NPC. EMBO J. *26*, 4956–4965.

Lainé, J.P., Singh, B.N., Krishnamurthy, S., and Hampsey, M. (2009). A physiological role for gene loops in yeast. Genes Dev. *23*, 2604–2609.

Li, B., Pattenden, S.G., Lee, D., Gutiérrez, J., Chen, J., Seidel, C., Gerton, J., and Workman, J.L. (2005). Preferential occupancy of histone variant H2AZ at inactive promoters influences local histone modifications and chromatin remodeling. Proc. Natl. Acad. Sci. USA *102*, 18385–18390.

Luthra, R., Kerr, S.C., Harreman, M.T., Apponi, L.H., Fasken, M.B., Ramineni, S., Chaurasia, S., Valentini, S.R., and Corbett, A.H. (2007). Actively transcribed GAL genes can be physically linked to the nuclear pore by the SAGA chromatin modifying complex. J. Biol. Chem. *282*, 3042–3049.

Meneghini, M.D., Wu, M., and Madhani, H.D. (2003). Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. Cell *112*, 725–736.

Mizuguchi, G., Shen, X., Landry, J., Wu, W.H., Sen, S., and Wu, C. (2004). ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. Science *303*, 343–348.

Radonjic, M., Andrau, J.C., Lijnzaad, P., Kemmeren, P., Kockelkorn, T.T., van Leenen, D., van Berkum, N.L., and Holstege, F.C. (2005). Genome-wide analyses reveal RNA polymerase II located upstream of genes poised for rapid response upon S. cerevisiae stationary phase exit. Mol. Cell *18*, 171–183.

Raisner, R.M., and Madhani, H.D. (2006). Patterning chromatin: form and function for H2A.Z variant nucleosomes. Curr. Opin. Genet. Dev. *16*, 119–124.

Raisner, R.M., Hartley, P.D., Meneghini, M.D., Bao, M.Z., Liu, C.L., Schreiber, S.L., Rando, O.J., and Madhani, H.D. (2005). Histone variant H2A.Z marks the 5′ ends of both active and inactive genes in euchromatin. Cell *123*, 233–248.

Reddy, K.L., Zullo, J.M., Bertolino, E., and Singh, H. (2008). Transcriptional repression mediated by repositioning of genes to the nuclear lamina. Nature *452*, 243–247.

Sarma, N.J., Haley, T.M., Barbara, K.E., Buford, T.D., Willis, K.A., and Santangelo, G.M. (2007). Glucose-responsive regulators of gene expression in Saccharomyces cerevisiae function at the nuclear periphery via a reverse recruitment mechanism. Genetics *175*, 1127–1135.

Schmid, M., Arib, G., Laemmli, C., Nishikawa, J., Durussel, T., and Laemmli, U.K. (2006). Nup-PI: the nucleopore-promoter interaction of genes in yeast. Mol. Cell *21*, 379–391.

Segal, E., Fondufe-Mittendorf, Y., Chen, L., Thåström, A., Field, Y., Moore, I.K., Wang, J.P., and Widom, J. (2006). A genomic code for nucleosome positioning. Nature *442*, 772–778.

Storici, F., Durham, C.L., Gordenin, D.A., and Resnick, M.A. (2003). Chromosomal site-specific double-strand breaks are efficiently targeted for repair by oligonucleotides in yeast. Proc. Natl. Acad. Sci. USA *100*, 14994–14999.

Suntharalingam, M., and Wente, S.R. (2003). Peering through the pore: nuclear pore complex structure, assembly, and function. Dev. Cell *4*, 775–789.

Taddei, A., Hediger, F., Neumann, F.R., Bauer, C., and Gasser, S.M. (2004). Separation of silencing from perinuclear anchoring functions in yeast Ku80, Sir4 and Esc1 proteins. EMBO J. *23*, 1301–1312.

Taddei, A., Van Houwe, G., Hediger, F., Kalck, V., Cubizolles, F., Schober, H., and Gasser, S.M. (2006). Nuclear pore association confers optimal expression levels for an inducible yeast gene. Nature *441*, 774–778.

Taddei, A., Van Houwe, G., Nagai, S., Erb, I., van Nimwegen, E., and Gasser, S.M. (2009). The functional importance of telomere clustering: global changes in gene expression result from SIR factor dispersion. Genome Res. *19*, 611–625.

Takizawa, T., Meaburn, K.J., and Misteli, T. (2008). The meaning of gene positioning. Cell *135*, 9–13.

Tan-Wong, S.M., Wijayatilake, H.D., and Proudfoot, N.J. (2009). Gene loops function to maintain transcriptional memory through interaction with the nuclear pore complex. Genes Dev. *23*, 2610–2624.

Vaquerizas, J.M., Suyama, R., Kind, J., Miura, K., Luscombe, N.M., and Akhtar, A. (2010). Nuclear pore proteins nup153 and megator define transcriptionally active regions in the Drosophila genome. PLoS Genet. *6*, e1000846.

Zacharioudakis, I., Gligoris, T., and Tzamarias, D. (2007). A yeast catabolic enzyme controls transcriptional memory. Curr. Biol. *17*, 2041–2046.

Zhang, H., Roberts, D.N., and Cairns, B.R. (2005). Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. Cell *123*, 219–231.

Zilberman, D., Coleman-Derr, D., Ballinger, T., and Henikoff, S. (2008). Histone H2A.Z and DNA methylation are mutually antagonistic chromatin marks. Nature *456*, 125–129.