A negative feedback loop at the nuclear periphery regulates *GAL* gene expression

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ABSTRACT **The genome is nonrandomly organized within the nucleus, but it remains unclear how gene position affects gene expression. Silenced genes have frequently been found associated with the nuclear periphery, and the environment at the periphery is believed to be refractory to transcriptional activation. However, in budding yeast, several highly regulated classes of genes, including the *GAL7-10-1* gene cluster, are known to translocate to the nuclear periphery concurrent with their activation. To investigate the role of gene positioning on *GAL* gene expression, we monitored the effects of mutations that disrupt the interaction between the *GAL* locus and the periphery or synthetically tethered the locus to the periphery. Localization to the nuclear periphery was found to dampen initial *GAL* gene induction and was required for rapid repression after gene inactivation, revealing a function for the nuclear periphery in repressing endogenous *GAL* gene expression. Our results do not support a gene-gating model in which *GAL* gene interaction with the nuclear pore ensures rapid gene expression, but instead they suggest that a repressive environment at the nuclear periphery establishes a negative feedback loop that enables the *GAL* locus to respond rapidly to changes in environmental conditions.**

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INTRODUCTION

The correct spatial organization of the genome within the nucleus is believed to play a critical role in the regulation of gene expression (Fraser and Bickmore, 2007). Genomes are nonrandomly arranged, and the radial position of a gene within the nucleus can vary, correlating with its functional status (Misteli, 2007). The periphery of the nucleus, comprising the nuclear envelope and associated proteins, was originally implicated in transcriptional silencing. In mammalian cells, cytological observations indicate that heterochromatin is localized near the periphery of the nucleus (Taddei *et al.*, 2004). Chromosomes with low gene density, and therefore low transcriptional activity, are often found near the nuclear periphery, whereas chromosomes with high gene density tend to localize to the nuclear interior (Croft *et al.*, 1999). In addition, a number of genes have been identified that move away from the nuclear periphery upon transcriptional activation, including *IgH* in B lymphocytes (Kosak *et al.*, 2002; Lanctot *et al.*, 2007). Similar to mammalian cells, heterochromatic regions in *Saccharomyces cerevisiae* such as telomeres and silent mating-type loci are found at the nuclear periphery (Akhtar and Gasser, 2007), and tethering to the nuclear envelope can be sufficient to repress a crippled silencer (Andrulis *et al.*, 1998). Together these data supported the hypothesis that the nuclear periphery is predominantly a site of transcriptional repression. However, more recent evidence suggests that regulation of gene expression at the nuclear periphery is not limited to repression (Liang and Hetzer, 2011). An investigation of the murine β-globin locus revealed that it localizes to the nuclear periphery during transcriptional activation and only subsequently relocalizes to the nuclear interior (Ragoczy *et al.*, 2006). Moreover, hypertranscription of the X chromosome in *Drosophila* males occurs at the nuclear periphery and appears to be dependent on components of the nuclear pore complex (NPC; Mendjan *et al.*, 2006; Vaquerizas *et al.*, 2010), although a direct role of NPC components in this process has been controversial (Grimaud and Becker, 2009). Work in *Drosophila* has also demonstrated a role for some NPC components in transcriptional activation of stress-responsive

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Abbreviations used: GFP, green fluorescent protein; NPC, nuclear pore complex; qRT-PCR, quantitative reverse transcription-PCR; SEM, standard error of the mean.

and developmentally regulated genes (Capelson *et al.*, 2010; Kalverda *et al.*, 2010). Of interest, this gene activation does not occur at the NPC, as the chromatin–Nup interaction occurs away from the nuclear periphery in the nucleoplasm (Capelson *et al.*, 2010; Kalverda *et al.*, 2010; Vaquerizas *et al.*, 2010).

In budding yeast, multiple genomic loci, including genes that respond to carbon source shifts, heat shock, and mating pheromones, move to the nuclear periphery upon transcriptional activation (Brickner and Walter, 2004; Casolari *et al.*, 2004, 2005; Dieppois *et al.*, 2006; Taddei *et al.*, 2006; Berger *et al.*, 2008). Chromatin near highly transcribed genes associates with members of the NPC and the mRNA export machinery (Casolari *et al.*, 2004). Furthermore, direct physical links have been identified between the NPC and components of the transcriptional machinery, particularly the SAGA complex (Rodriguez-Navarro *et al.*, 2004). In addition, mutations in components of the NPC, the transcriptional regulator SAGA and other chromatin remodeling factors, and the transcription/RNA export complex TREX-2 have been shown to interfere with the association of active genes and the nuclear periphery (Rodriguez-Navarro *et al.*, 2004; Cabal *et al.*, 2006; Dieppois *et al.*, 2006; Drubin *et al.*, 2006; Kohler *et al.*, 2008; Ahmed *et al.*, 2010). In specific cases, the gene–periphery interaction also appears to be dependent on DNA sequence elements in active gene promoters (Ahmed *et al.*, 2010; Light *et al.*, 2010). In combination, these observations challenged the tenet that the nuclear periphery demarcates an exclusively repressive zone, particularly within the yeast nucleus, and led to the suggestion that a zone of transcriptional activity exists at or near nuclear pore complexes (Akhtar and Gasser, 2007; Taddei, 2007). A number of models have been proposed to ascribe functionality to this active gene–nuclear periphery interaction. For example, localization of the *GAL7-10-1* gene cluster at the nuclear periphery has been suggested to be consistent with the gene gating hypothesis, in which active genes are found near nuclear pore complexes to facilitate efficient export of mRNAs from the nucleus (Blobel, 1985; Cabal *et al.*, 2006). Other models suggest that the nuclear periphery plays a role in chromatin-mediated transcriptional memory at active loci (Brickner *et al.*, 2007), is associated with the formation of gene loops (Laine *et al.*, 2009; Tan-Wong *et al.*, 2009), or recruits genes for activation via localized transcription components (Sarma *et al.*, 2007). Furthermore, there is evidence that genes associate with the nuclear periphery posttranscriptionally (Abruzzi *et al.*, 2006; Vodala *et al.*, 2008) and that the gene–periphery interaction can exist independent of active transcription (Schmid *et al.*, 2006). However, the functional significance of active gene positioning at the nuclear periphery remains unclear.

In this work, we identify the functional role of *GAL* locus movement to the nuclear periphery in budding yeast. We take advantage of mutations that disrupt the gene–periphery interaction to address the significance of the spatial regulation of the *GAL* gene locus. Consistent with a model in which the nuclear periphery constitutes a repressive environment, we find that *GAL* gene expression is negatively regulated at the nuclear periphery both upon activation and after repression. Our results suggest that the nuclear position of a gene is important to modulate gene expression, allowing cells to rapidly respond to changes in environmental conditions.

RESULTS

The *GAL* locus is positioned at the nuclear periphery in galactose, raffinose, and glycerol

The interaction of the *GAL* locus with the nuclear periphery concomitant with the induction of gene expression has been shown to depend on the function of diverse protein complexes, including the NPC and the transcriptional activation complex SAGA (Casolari *et al.*, 2004; Cabal *et al.*, 2006; Dieppois *et al.*, 2006; Luthra *et al.*, 2007; Kohler *et al.*, 2008; Ahmed *et al.*, 2010). *GAL* genes exist in at least three distinct functional states (Johnston *et al.*, 1994; Santangelo, 2006). When cells are grown in glucose, *GAL* genes are fully repressed. Growth in the presence of other carbon sources, such as the trisaccharide raffinose or nonfermentable glycerol, relieves the glucose-mediated repression but does not induce transcription of the *GAL* genes. By contrast, growth in galactose transcriptionally induces all *GAL* genes (Johnston *et al.*, 1994; Santangelo, 2006). To analyze the dynamic localization of the *GAL* genes in these distinct physiological states, we performed live-cell imaging on yeast strains containing 256 copies of the LacO repeat integrated 3 kb downstream of the *GAL1* gene (see Figure 3A later in the paper), the LacO-binding protein LacI fused to green fluorescent protein (GFP), and the fluorescent protein dsRED fused to an HDEL peptide to retain it in the nuclear envelope. Consistent with previous reports (Casolari *et al.*, 2004; Cabal *et al.*, 2006; Drubin *et al.*, 2006; Taddei *et al.*, 2006), we verified that the *GAL* locus interacts with the nuclear periphery at a higher frequency during transcriptional activation by galactose than when repressed by glucose. Of interest, we also observed significant interaction between the *GAL* locus and the nuclear periphery when cells were grown in raffinose or glycerol (Figure 1A). This demonstrates a greater degree of interaction between the *GAL* locus and the nuclear periphery than previously described and suggests that peripheral positioning of the *GAL* locus occurs in response to the relief of glucose repression.

These observations prompted us to examine whether the interaction of *GAL1* with the periphery relies on similar molecular mechanisms in both active conditions (galactose) and noninduced conditions (raffinose/glycerol). We first examined the role of SAGA and the NPC in the raffinose- and galactose-induced tethers. The interaction between the *GAL* locus and the nuclear periphery had previously been shown to require components of the SAGA complex, such as Ada2, and components of the NPC, such as Nup1 (Cabal *et al.*, 2006). We verified that deletion of either *ADA2* or *NUP1* prevented the efficient interaction of the *GAL* locus with the periphery in galactose, and we also observed decreased interaction with the periphery in raffinose (Figure 1B). These data indicate that SAGA and the NPC are required for the peripheral localization of the *GAL* locus in both derepressed and induced conditions.

Previous work showed that the gene–periphery interaction can occur independent of transcription (Schmid *et al.*, 2006; Brickner *et al.*, 2007), consistent with our finding that *GAL* gene recruitment to the nuclear periphery occurs in derepressed conditions (i.e., in raffinose or glycerol medium). This observation led us to reinvestigate the role of transcription in the gene–periphery interaction, asking whether the interaction in galactose requires ongoing transcription by using the temperature-sensitive allele of RNA polymerase II, *rpb1-1*. We monitored the position of the *GAL* locus in cells containing the *rpb1-1* allele grown continuously in galactose or in raffinose. When transcription is halted by *RPB1* inactivation at the nonpermissive temperature (37°C), the *GAL* locus is no longer maintained at the nuclear periphery in galactose, whereas there is no significant change in wild-type cells at this temperature (Figure 1C). Moreover, there is no change in the interaction between the *GAL* locus and the periphery in raffinose in the absence of functional RNA pol II. These results demonstrate that the maintenance of the *GAL* locus at the nuclear periphery in galactose is dependent on active transcription, but there is no role for active transcription in the pretranscriptional tether in raffinose. Together these findings demonstrate the existence of two distinct gene–periphery interactions, differentiated

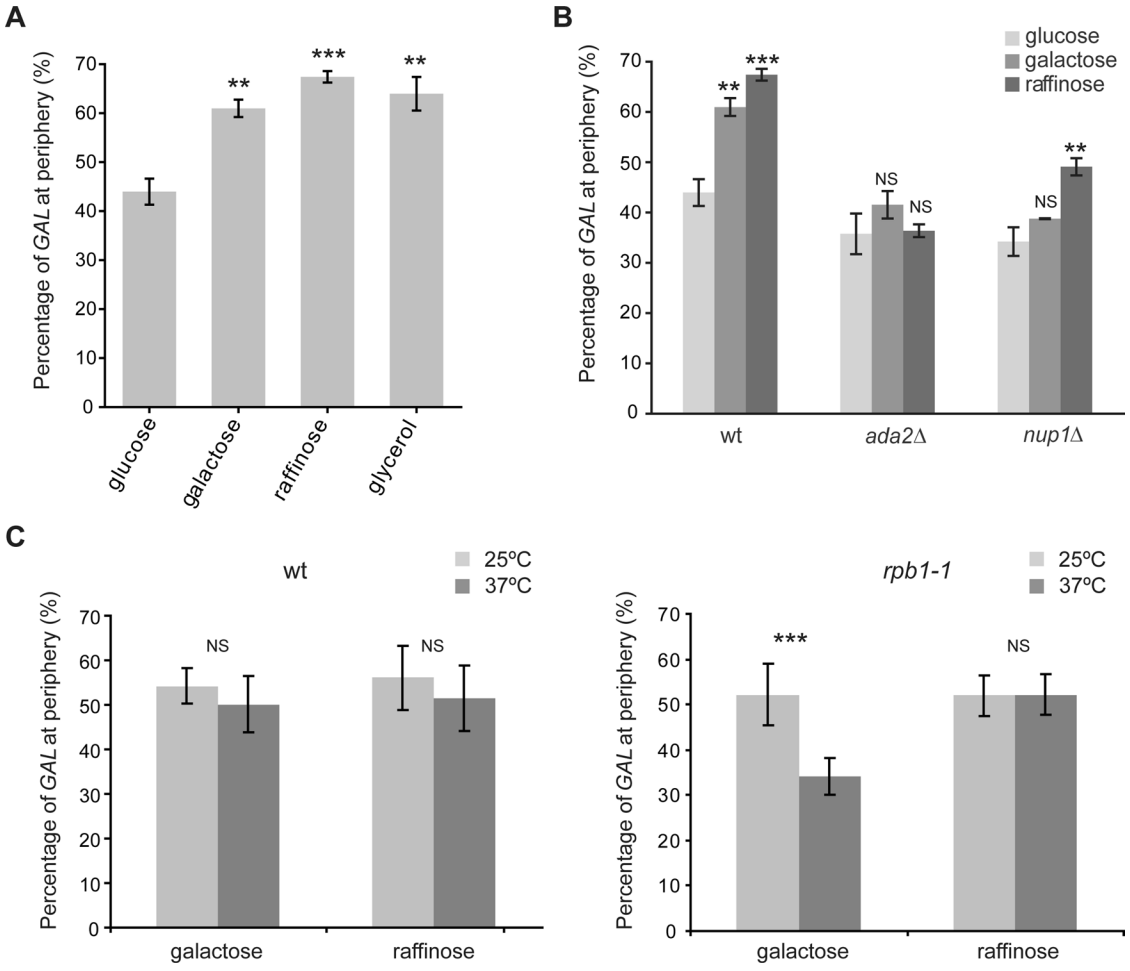

FIGURE 1: Determinants of peripheral positioning of the *GAL* locus. (A) The *GAL* locus is at the nuclear periphery in the absence of glucose. Wild-type cells expressing the LacO/LacI system and dsRED-HDEL were grown in synthetic medium containing 2% raffinose (SRaf) medium to mid-log phase and then maintained in raffinose or shifted to 2% glucose (SGlu), 2% galactose (SGal), or 2% glycerol (SGly) for 2 h prior to imaging. Percentage of cells with peripheral localization was scored as described in *Materials and Methods*. (B) *GAL* locus localization in wild-type and *nup1*Δ or *ada2*Δ mutant cells. The percentage of cells with the *GAL* locus at the nuclear periphery in SGlu, SGal, or SRaf for wild-type, *nup1*Δ, and *ada2*Δ cells was determined by microscopy. Cells were grown and scored as described for A. (C) Localization of the *GAL* locus after transcriptional inhibition. Wild-type and *rpb1-1* mutant cells were grown continuously in SGal or SRaf medium at 25°C and then shifted to 37°C. Cells were imaged and scored as described for A following 2 h at 37°C. Error bars represent the SEM for at least three independent experiments in which at least 100 cells were scored for each condition or time point. An unpaired, two-tailed Student's *t* test was used to determine statistical significance for all microscopy experiments. Statistical significance is as follows: $p > 0.05$ is not significant (NS); *$0.05 > p > 0.01$; **$0.01 > p > 0.001$; ***$p < 0.001$. p values represent a comparison between percentage of cells at the nuclear periphery in glucose to other selected media in wild-type or mutant cells.

by their requirement for transcription, although both the pretranscriptional and transcriptional tethers share a requirement for components of the NPC and SAGA nuclear subcomplexes.

The nuclear periphery negatively regulates *GAL* gene expression

Despite intense investigation, the functional significance of *GAL* gene movement to the nuclear periphery has remained elusive. One prevalent hypothesis is that gene gating at the NPC increases the overall rate of gene expression by facilitating efficient mRNA export (Blobel, 1985; Cabal *et al.*, 2006; Luthra *et al.*, 2007). To test this hypothesis and to analyze the functional role of the gene–periphery interaction, we took advantage of mutations in distinct nuclear subcomplexes—the nucleoporin *NUP1* and the SAGA component *ADA2*—that abrogate the interaction between the *GAL* gene cluster and the nuclear periphery (Figure 1B). We investigated changes in gene expression associated with disrupting the *GAL*–nuclear periphery interaction using quantitative PCR after reverse transcription (qRT-PCR) to monitor *GAL1* mRNA levels in wild-type, *nup1*Δ, and *ada2*Δ cells pregrown in glucose and treated with galactose. *GAL1* mRNA levels were measured relative to the non–galactose-regulated mRNAs *ACT1*, *TFC1*, and *ALG9* at 30 and 60 min following galactose induction (Figure 2A and unpublished data). It is surprising that, in both *nup1*Δ and *ada2*Δ cells, an increase in *GAL1* mRNA could be detected compared with wild-type cells. At later time points, the expression levels in all strains reach similar steady-state levels (Supplemental Figure S1), consistent with prior reports (Cabal *et al.*, 2006). When mutant cells were

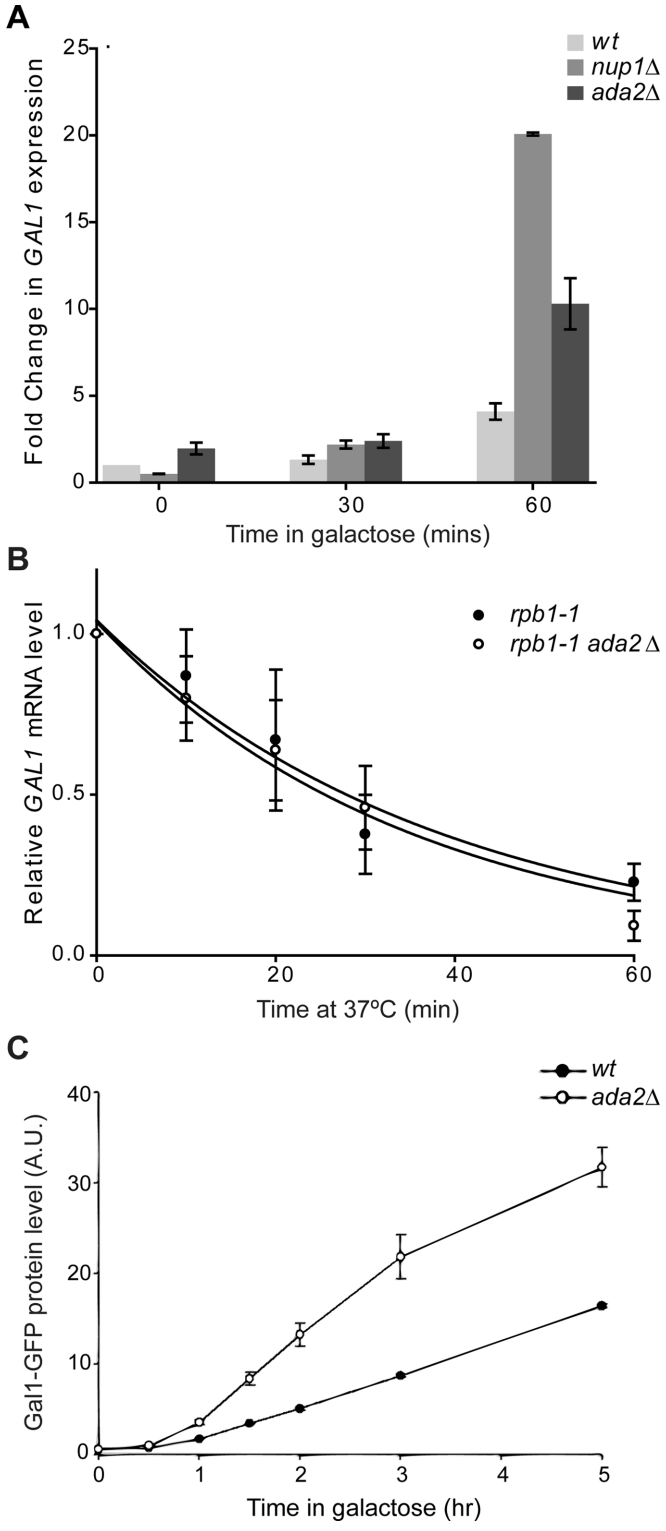

FIGURE 2: The nuclear periphery inhibits *GAL1* mRNA expression. (A) Changes in *GAL1* mRNA expression in yeast with disrupted peripheral localization of the *GAL* locus. Wild-type, *ada2*Δ, or *nup1*Δ mutant cells were grown in YP plus 2% glucose (YPD) to mid-log phase. *GAL1* mRNA expression was induced by galactose. *GAL1* mRNA levels were measured at the indicated times by quantitative RT-PCR. *GAL1* mRNA levels were normalized to levels of a control gene, *TFC1*, and the fold change in expression was calculated relative to the baseline expression at the zero time point for each strain. Error bars represent SEM for three independent experiments. (B) *GAL1* mRNA turnover in galactose in wild-type and *ada2*Δ mutant cells. *rpb1-1* and *rpb1-1 ada2*Δ cells were grown in YPGal medium at 25°C and then shifted to 37°C to inactivate transcription. *GAL1* mRNA levels were measured at the indicated time points by qRT-PCR and plotted as a function of time following the shift to the nonpermissive temperature. *GAL1* mRNA levels were normalized to the RNA pol III transcript *SCR1* and expressed relative to the level of transcript at the zero time point, defined as 1.0. (C) Gal1 protein levels increase in *ada2*Δ cells. Cells were grown in raffinose medium and induced with 2% galactose for the indicated time, and Gal1-GFP protein levels were analyzed using flow cell cytometry. Mean GFP intensity of the population (in arbitrary units) is plotted as a function of time in galactose. Error bars represent SEM for three independent experiments.

pregrown in raffinose and then treated with galactose a similar *GAL1* mRNA hyperinduction phenotype was observed (Supplemental Figure S2).

Because a comparable increase in *GAL1* mRNA levels can be observed in *nup1*Δ and *ada2*Δ mutants, our results suggest that association with the nuclear periphery dampens the induction of the *GAL1* gene. To distinguish whether this repressive effect was primarily caused by direct regulation of transcription or by changes in mRNA turnover, we analyzed the rate of *GAL1* mRNA decay in galactose in wild-type and *ada2*Δ strains, which also contained the RNA polymerase II temperature-sensitive allele, *rpb1-1*. Cells were shifted to the nonpermissive temperature to inactivate transcription, and *GAL1* mRNA turnover was monitored by qRT-PCR and normalized to the RNA polymerase III transcript, *SCR1*. Both wild-type and *ada2*Δ cells displayed identical rates of *GAL1* mRNA decay ($t_{1/2} \approx 25$ min; Figure 2B). These data argue that the increased levels of *GAL1* mRNA in strains lacking the gene–periphery tether are due to an increase in transcription and not to a decreased mRNA turnover rate.

In addition to monitoring the kinetics of mRNA expression in the mutants, we also analyzed the affect of disrupting the gene–periphery tether on the kinetics of Gal1 protein production. Using flow cytometry of yeast cells expressing a GFP-tagged Gal1, we followed the protein levels of Gal1-GFP following galactose induction after growth in raffinose. For these experiments we predicted that any delay in mRNA export due to the absence of the gene–periphery tether would be reflected by a slowed rate of protein production. Therefore we monitored the protein expression only in *ada2*Δ and wild-type cells since *nup1*Δ cells were previously shown to be deficient in mRNA export (Schlaich and Hurt, 1995; Fischer *et al.*, 2002). We observed that the increased level of *GAL1* mRNA found in *ada2*Δ cells was reflected by an increase in protein levels of Gal1-GFP after induction with galactose (Figure 2C). This demonstrates that the overexpressed mRNA in *ada2*Δ cells is functional and suggests that the localization of the *GAL1* gene adjacent to the nuclear pore complex is not critical for efficient nuclear export of the *GAL1* mRNA.

The repressive effect of the nuclear periphery on the *GAL* locus

The *GAL* gene cluster contains two additional galactose-induced genes, *GAL7* and *GAL10*, which are flanked by neighboring genes *KAP104* and *FUR4* (Figure 3A). *KAP104* encodes for a nuclear transport factor that is constitutively expressed, whereas *FUR4* encodes for a uracil permease previously shown to be partially regulated by Gal4, the key regulator of genes required for galactose metabolism (Ren *et al.*, 2000). To determine the influence of the nuclear periphery on the expression patterns of the entire locus, we also monitored

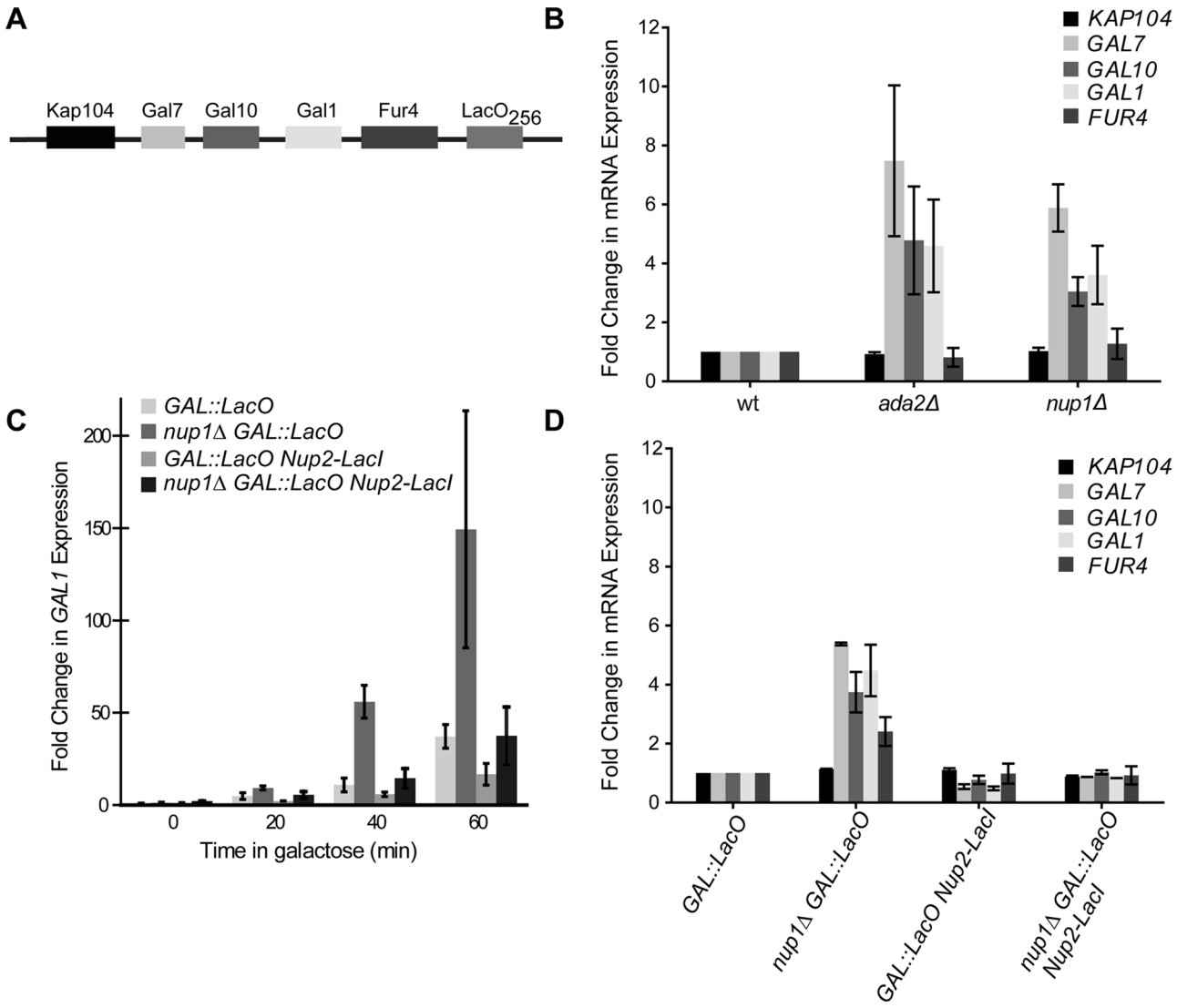

FIGURE 3: The nuclear periphery specifically regulates expression of galactose-induced genes. (A) Schematic of the *GAL* locus and adjacent genes on chromosome II, including the integration site of 256 copies of the LacO repeats. (B) mRNA expression levels of *GAL* locus genes (*GAL1*, *GAL7*, *GAL10*) and neighboring genes (*FUR4* and *KAP104*) in yeast lacking the gene–periphery tether. Wild-type, *ada2*Δ, or *nup1*Δ mutant cells were grown in YPD medium to mid-log phase and shifted to YPGal medium for 1 h. mRNA levels were measured by qRT-PCR, and the expression of each gene was normalized to the control gene, *TFC1*. Fold change of mRNA levels for all genes in *ada2*Δ or *nup1*Δ strains was calculated relative to expression in the wild-type strain, defined as 1.0. (C) Kinetics of *GAL1* expression with constitutive peripheral tethering. *GAL1* gene expression, measured by qRT-PCR in wild-type and *nup1*Δ strains containing the LacO repeats integrated near the *GAL* locus with and without the Nup2-LacI gene tether. Cells were grown in YPD medium, and *GAL1* expression was induced with 2% galactose for the indicated times. *GAL1* mRNA levels were normalized to levels of the control gene *TFC1*, and the fold change in expression was calculated relative to the baseline expression at the zero time point for each strain. (D) mRNA expression levels of *GAL* locus genes and neighboring genes with the Nup2-LacI gene tether. mRNA levels were detected by qRT-PCR following 1 h induction with galactose. The expression of each gene was normalized to the control gene *TFC1*, and the fold change of mRNA levels for all genes was calculated relative to expression in the *GAL::LacO* strain, defined as 1.0. Error bars represent SEM for three independent experiments.

the expression levels of *FUR4*, *GAL7*, *GAL10*, and *KAP104* in addition to *GAL1* in wild-type, *nup1*Δ, and *ada2*Δ cells by qRT-PCR. Similar to *GAL1*, we observed greatly increased levels of the galactose-regulated genes *GAL7* and *GAL10* in the *nup1*Δ and *ada2*Δ mutants (Figure 3B). However, we observed no changes for *KAP104* and only a very modest increase in the mRNA levels of *FUR4*.

If hyperactivation of the *GAL* genes in both *nup1*Δ and *ada2*Δ cells is indeed caused by the altered position of the gene within the nucleus, then constitutive tethering of the *GAL* locus to the nuclear periphery should rescue this defect in transcriptional regulation. To test this hypothesis, we constructed a gene-tethering system using the nucleoporin Nup2. The lac repressor (LacI) was fused to the C-terminus of Nup2 and expressed in yeast cells with LacO repeats integrated downstream of the *GAL* locus. The interaction between Nup2-LacI and the LacO repeats was sufficient to tether the *GAL* locus constitutively to the nuclear periphery in both glucose and

galactose at a frequency higher than observed for wild-type cells in galactose (Supplemental Figure S3). We monitored the induction of *GAL1* over time in the presence and absence of the Nup2-LacI/LacO tether in cells shifted from glucose to galactose (Figure 3C). Expression of only Nup2-LacI did not alter transcript levels (Supplemental Figure S4), and *GAL1* hyperactivation was still observed in the presence of the LacO repeats alone in *nup1*Δ cells. Of importance, the kinetics of induction was slower in the presence of the full Nup2-LacI/LacO tether in *nup1*Δ, correlating with the restored peripheral localization of the gene in this strain background. These data show that constitutive tethering of the *GAL* locus to the nuclear periphery is sufficient to rescue the aberrant *GAL1* expression observed in *nup1*Δ cells.

To investigate whether the Nup2-LacI/LacO gene tether caused generalized transcriptional repression around the *GAL* locus or whether it was specific for the *GAL* genes, we also monitored expression of the galactose-induced genes *GAL7* and *GAL10* and the two surrounding genes *KAP104* and *FUR4*. mRNA levels of all genes were detected by qRT-PCR following treatment with galactose in both wild-type and *nup1*Δ strains with and without the Nup2-LacI/LacO gene tether (Figure 3D). As observed for *GAL1*, the Nup2-LacI/LacO gene tether rescued the hyperinduction phenotype of *GAL7* and *GAL10* detected in *nup1*Δ cells. However, the Nup2-LacI/LacO gene tether did not repress *KAP104* or *FUR4* levels below the levels observed in untethered wild-type control cells. Overall, these data suggest that the negative transcriptional control at the nuclear periphery is specific for galactose-regulated genes and is not due to generalized repression of the locus or gene silencing at the nuclear periphery.

Efficient repression of *GAL1* requires interaction with the nuclear periphery

Our analysis of *GAL1* expression kinetics paradoxically shows that relocalization to the nuclear periphery, which is observed upon gene activation, leads to a repression of *GAL* gene induction. However, for yeast cells it is not only critical to rapidly induce appropriate amounts of galactose-responsive genes upon exposure to galactose, but it is also very important to swiftly repress *GAL* genes in the presence of glucose, the preferred carbon source. We therefore analyzed whether the gene–periphery interaction also stimulates repression of *GAL1* upon glucose addition. Using qRT-PCR (Figure 4), we measured the amount of *GAL1* mRNA in wild-type, *nup1*Δ, and *ada2*Δ cells that were grown in galactose and subsequently shifted to glucose. As expected (Johnston *et al.*, 1994), repression of galactose-induced genes occurs rapidly in the presence of glucose in wild-type cells. In contrast, the kinetics of repression following the shift to glucose was significantly delayed in both *nup1*Δ and *ada2*Δ mutants. These data show that disrupting the *GAL1*–nuclear periphery interaction delays the rate of repression of *GAL1* upon glucose addition, thereby limiting the cellular response to changes in carbon source.

Taken together, our experiments investigating the effects of gene position on *GAL1* activation and repression demonstrate that the nuclear periphery constitutes a repressive environment for *GAL* gene expression despite the fact that peripheral localization occurs concurrently with transcriptional activation. Furthermore, our results argue that disrupting the interaction between the periphery and the *GAL* locus prevents cells from rapidly repressing *GAL* gene expression upon return to the more favorable glucose conditions.

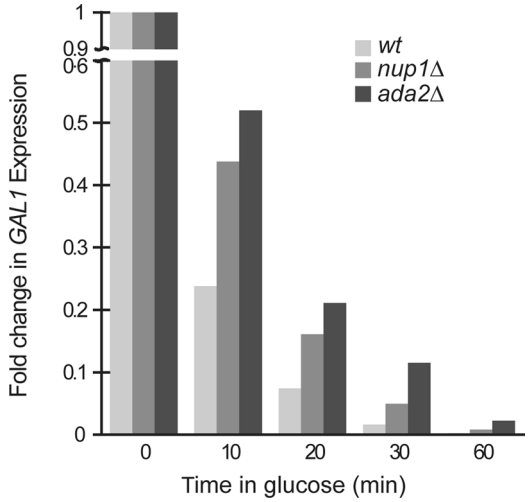

FIGURE 4: Repression of *GAL1* is delayed with a disrupted gene–periphery tether. Wild-type, *nup1*Δ, and *ada2*Δ strains were grown at room temperature in YP plus 2% raffinose to mid-log phase. *GAL1* mRNA expression was induced by galactose addition for 2 h and then inhibited by the addition of glucose for the indicated time. *GAL1* mRNA levels were monitored by qRT-PCR and normalized to the control gene, *ACT1*. The fold change is calculated relative to the transcript levels for each strain at the zero time point, defined as 1.0.

DISCUSSION

The nuclear periphery represses *GAL* gene expression

The correlation between peripheral *GAL* locus position and transcriptional activation has encouraged the development of numerous models that suggest that the nuclear periphery is directly involved in transcriptional activation or promotes more efficient mRNA export from the nucleus (Blobel, 1985; Cabal *et al.*, 2006; Brickner *et al.*, 2007; Sarma *et al.*, 2007). Here we monitor the position of the *GAL* locus in live yeast and first demonstrate that the *GAL* locus is positioned at the nuclear periphery both prior to and following transcriptional activation. Both of the pretranscriptional and transcriptional gene–periphery tethers depend on components of the nuclear pore complex (Nup1) and the SAGA complex (Ada2), previously identified as required for association of *GAL1* with the nuclear periphery concomitant with transcriptional activation (Cabal *et al.*, 2006). The persistence of the *GAL* locus–nuclear periphery interaction in multiple environmental conditions suggests a complex interplay between active genes and the nuclear periphery, the function of which has yet to be elucidated.

To investigate the functional significance of the *GAL* locus–nuclear periphery interaction, we took advantage of yeast mutants in the nucleoporin *NUP1* or the SAGA component *ADA2* to disrupt the interaction between the *GAL* locus and the nuclear periphery. It is surprising that in these mutants we observed a significant increase in *GAL* gene expression kinetics compared with wild-type cells (Figure 2A). This hyperinduction seems to be primarily mediated by an increase in transcription (Figure 2B) and could be observed in all *GAL* genes tested (*GAL1*, *GAL7*, and *GAL10*). By contrast, the peripheral localization had little or no effect on two neighboring genes, *KAP104* and *FUR4* (Figure 3B). It is of note that our results do not contradict previous measurements of steady-state mRNA levels (Cabal *et al.*, 2006), in that we see little difference between wild-type, *nup1*Δ, and *ada2*Δ mutants once expression levels have reached saturation (Supplemental Figure S1). These results also parallel recent work showing that disruption of peripheral positioning of actively transcribing ribosomal protein genes results in their increased expression (Yoshida *et al.*, 2010).

Our observations demonstrate three important aspects of the gene–nuclear periphery interaction. First, although association of

the *GAL* locus with the nuclear periphery *correlates* with gene activation, localization at the nuclear periphery itself is not required for transcriptional activation or gene expression, and in fact peripheral localization negatively regulates *GAL* gene expression. Second, our findings do not support the original gene gating hypothesis (Blobel, 1985) because mRNA processing and/or export of *GAL1* are not significantly delayed when the gene is not peripheral (Figure 2C). This also suggests that, at least in yeast, the diffusion of mRNA from the site of transcription to the nuclear pore complex is not a rate-limiting step in gene expression. Third, our observations indicate that the spatial position of the *GAL* genes within the nucleus is important for regulating their activity. Similar increases in *GAL1* expression are observed in strains with deletions of either *ADA2* or *NUP1*, whose gene products function in separate nuclear complexes. This is most likely explained by their shared phenotype: the disruption of the gene–periphery interaction and the mislocalization of the gene to the nuclear interior.

We further show that the nuclear periphery is not only necessary but also sufficient for establishing repressive conditions for modulating *GAL* gene expression levels, as we observed a decrease in *GAL1*, *GAL7*, and *GAL10* expression in *nup1*Δ cells when the *GAL* locus is constitutively tethered to the nuclear pore complex (Figure 3, C and D). Because the aberrant *GAL* gene expression pattern in *nup1*Δ cells can be rescued by tethering the genes to the nuclear periphery, this indicates that the misregulation in these mutants can be attributed specifically to the disrupted gene–periphery interaction. Thus, the nuclear periphery likely plays a role in dampening the activation of the *GAL* genes to maintain the appropriate transcript levels for the cell's response to galactose, consistent with several previous studies linking the nuclear periphery with transcriptional repression and gene silencing (Andrulis *et al.*, 1998; Feuerbach *et al.*, 2002; Akhtar and Gasser, 2007).

Whether this repressive effect is specific to the *GAL* genes or whether the nuclear periphery and the nuclear pore also inhibit other dynamic gene loci is unclear. Here we analyzed only the effects of Nup1, but the nuclear pore complex provides a versatile platform that recruits components of multiple activities, and it is very likely that the regulation of gene expression at the nuclear periphery is not limited to repression (reviewed in Arib and Akhtar, 2011; Liang and Hetzer, 2011). Consistent with this, wide-ranging variability in results has been observed in several other gene-tethering experiments that have been performed in both yeast (Andrulis *et al.*, 1998; Brickner and Walter, 2004; Brickner *et al.*, 2007; Taddei *et al.*, 2006) and metazoans (Finlan *et al.*, 2008; Kumaran and Spector, 2008; Reddy *et al.*, 2008) using either reporter or endogenous genes. This suggests that there are gene-specific means of transcriptional control occurring at the nuclear periphery, in different peripheral subdomains, or at the nuclear pore. This would be in agreement with the differential effects on expression that we observe in the presence of the gene–periphery tether for the SAGA-dependent *GAL1*, *GAL7*, and *GAL10* genes and SAGA-independent genes such as *KAP104*. In this context, it is also intriguing that the *INO1* gene appears to rely on a distinct peripheral tethering mechanism since it requires *cis*-acting DNA elements that promote either the initial localization to the periphery or a peripheral interaction that serves as a form of transcriptional memory. Of interest, these DNA elements are not present at the *GAL* locus (Ahmed *et al.*, 2010; Light *et al.*, 2010).

A negative feedback loop governs gene expression at the nuclear periphery

In addition to modulating levels of *GAL* gene expression upon induction, we also identified a role for peripheral positioning of the *GAL* locus in facilitating rapid repression of *GAL1* following the shift from galactose to glucose. When *GAL1* is positioned in the nuclear interior, repression by the addition of glucose is slowed significantly when compared with peripherally positioned *GAL1* (Figure 4). The molecular mechanism of *GAL* gene repression at the periphery remains unclear. One plausible hypothesis is that repositioning of the *GAL* locus toward the nuclear periphery upon activation may increase access to either a general or gene-specific repressive factor that modulates transcription. Of interest, the glucose repressor Mig1 is enriched at the nuclear periphery (Sarma *et al.*, 2007) and is required for the movement of the *GAL* locus toward the nuclear envelope upon activation (Vodala *et al.*, 2008). While this manuscript was in revision, it was further shown that components of the nuclear pore are important for Mig1 recruitment to target promoters (Sarma *et al.*, 2011). It will now be interesting to investigate the role of Mig1 in *GAL* gene repression at the nuclear periphery described here.

What is the physiological relevance of *GAL* gene movement to the repressive environment of the nuclear periphery upon transcriptional activation? Our results suggest that *GAL* gene localization to the nuclear periphery is an important mechanism for cells to quickly respond to changing environmental conditions. This supports a model in which the nuclear periphery is part of a negative feedback loop that regulates *GAL* gene expression and provides yeast cells with a mechanism for adaptive plasticity in changing environmental conditions. A very rapid repression of *GAL* gene expression may be particularly important for yeast cells when they return to their preferred carbon source, glucose. Further investigations into the molecular mechanism of this novel feedback loop in *GAL* gene regulation will provide important insight into how position-dependent gene expression patterns emerge in response to environmental stimuli.

MATERIALS AND METHODS

Yeast strains

All *S. cerevisiae* strains used in this study are listed in Supplemental Table S1. The KWY strain background is W303. Standard growth conditions and manipulations were used. All gene deletions and epitope tag integrations were performed using standard techniques (Longtine *et al.*, 1998). Plasmids containing *LacI-GFP::HIS3* and *LacO$_{256}$::LEU2* repeats for integration into yeast were gifts from A. Straight (Straight *et al.*, 1996), but the LacO-containing plasmid was modified to integrate at *YBR022w*. The integration plasmid allowing for expression of dsRED-HDEL was described previously (Madrid *et al.*, 2006). Yeast strains expressing the Nup2-LacI fusion protein were generated by integration of a PCR product amplified from plasmid pKW2139 (pFA6a*LacI::KanMX*) with flanking sequence to target the 3′ end of the *NUP2* coding region to generate a C-terminal fusion. KWY1302 (*MATa rpb1*-1) was generated by backcrossing the *rpb1-1* allele (Morrissey *et al.*, 1999) to KWY165. KWY2619 was constructed using the same strategy as described for KWY1622 but using KWY1302 as the initial strain for construct integration.

Microscopy

All microscopy experiments were performed using synthetic complete (SC) medium. Cells were grown to mid-log phase (OD_{600} ≈ 0.4–0.6) in SC plus either 2% glucose, 2% raffinose, or 2% galactose. To score large numbers of yeast cells efficiently, live yeast were imaged and recorded using an epifluorescence microscope equipped with a Hamamatsu interline CCD camera (Hamamatsu, Hamamatsu, Japan). When overlap between the LacI-GFP signal in the green

channel and the nuclear envelope signal in the red channel was observed, these cells were scored as having the gene locus at the nuclear periphery. To validate this method of scoring, we compared this approach to image analysis on a deconvolution microscope. In these experiments, position in the nucleus was determined by manually identifying the focal plane for the LacI-GFP spot and measuring the shortest distance from the center of the spot to the center of the nuclear rim using the softWoRx program (Applied Precision, Issaquah, WA). If the distance was <200 nm (the approximate resolution of this technique), the *GAL* locus was scored as peripheral. Both techniques yielded comparable changes in peripheral localization upon galactose induction.

RNA isolation

For analysis of mRNA levels of *GAL* locus genes (*GAL1*, *GAL7*, and *GAL10*) and neighboring genes (*FUR4* and *KAP104*) following galactose induction, yeast were grown in yeast extract/peptone (YP) media plus 2% glucose at 25°C until mid-log phase. Cells were harvested, washed in YP two times, and resuspended in YP plus 2% galactose. For analysis of *GAL1* mRNA following glucose repression, cells were grown to mid-log phase in YP plus 2% raffinose at 25°C, then shifted to YP plus 2% galactose for an additional 2 h. Cells were harvested and resuspended in YP plus 2% glucose. For each time point analyzed, an aliquot of cells was harvested by centrifugation and frozen in liquid nitrogen. Total RNA was isolated using the RiboPure-Yeast Kit (Applied Biosystems/Ambion, Austin, TX). RNA samples were treated with DNase I (Applied Biosystems/Ambion) to remove contaminating genomic DNA.

qRT-PCR

An oligo dT_{20} was used for first-strand synthesis for all mRNA expression experiments except for the *GAL1* mRNA decay experiment (Figure 2B), in which random primers (Invitrogen, Carlsbad, CA) were used. Superscript SS III reverse transcriptase (Invitrogen) was used to make cDNA from 2 µg of total RNA for each sample. qRT-PCR was performed in an Applied Biosystems 7300 Real Time PCR machine using Taqman Universal PCR Mastermix (Applied Biosystems, Foster City, CA) with 5′FAM/3′-BHQ-1 Taqman probes or in an Applied Biosystems StepOne Plus Real Time PCR using Absolute Blue QRT-PCR SYBR Green ROX Mix (Thermo Fisher Scientific, Waltham, MA) supplemented with gene-specific primers (described in Supplemental Table S2).

Flow cell cytometry

To monitor the expression levels of Gal1-GFP after induction, yeast were grown to early mid-log phase ($OD_{600} \approx 0.4$) in SC medium containing additional adenine and 2% raffinose. Cells were then diluted to $OD_{600} \approx 0.2$ and simultaneously induced in SC medium containing 2% galactose and adenine. Time points 0, 30, 60, 90, 120, and 180 min were treated with 10 µg/ml cycloheximide and held on ice for 2–4 h to allow all translated Gal1-GFP protein to properly fold before sorting. The 300-min time point was immediately sorted without cycloheximide treatment. Analysis was performed on an EPICS XL-MCL flow cytometer (Beckman Coulter, Brea, CA). Measurements from flow cytometry were plotted as a histogram (*y*-axis, number of cells; *x*-axis, log GFP intensity). Threshold GFP intensities were gated such that only 0–2% of a glucose-grown, wild-type culture would sort as GFP positive. Gal1-GFP expression levels were determined by taking the geometric mean of GFP intensity for the population of cells.

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REFERENCES

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

Ahmed S, Brickner DG, Light WH, Cajigas I, McDonough M, Froyshteter AB, Volpe T, Brickner JH (2010). DNA zip codes control an ancient mechanism for gene targeting to the nuclear periphery. Nat Cell Biol 12, 306.

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

Andrulis ED, Neiman AM, Zappulla DC, Sternglanz R (1998). Perinuclear localization of chromatin facilitates transcriptional silencing. Nature 394, 592–595.

Arib G, Akhtar A (2011). Multiple facets of nuclear periphery in gene expression control. Curr Opin Cell Biol 23, 346–353.

Berger AB, Cabal GG, Fabre E, Duong T, Buc H, Nehrbass U, Olivo-Marin JC, Gadal O, Zimmer C (2008). High-resolution statistical mapping reveals gene territories in live yeast. Nat Methods 5, 1031–1037.

Blobel G (1985). Gene gating: a hypothesis. Proc Natl Acad Sci USA 82, 8527–8529.

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

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

Cabal GG *et al.* (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, Hetzer MW (2010). Chromatin-bound nuclear pore components regulate gene expression in higher eukaryotes. Cell 140, 372–383.

Casolari JM, Brown CR, Drubin DA, Rando OJ, Silver PA (2005). Developmentally induced changes in transcriptional program alter spatial organization across chromosomes. Genes Dev 19, 1188–1198.

Casolari JM, Brown CR, Komili S, West J, Hieronymus H, Silver PA (2004). Genome-wide localization of the nuclear transport machinery couples transcriptional status and nuclear organization. Cell 117, 427–439.

Croft JA, Bridger JM, Boyle S, Perry P, Teague P, Bickmore WA (1999). Differences in the localization and morphology of chromosomes in the human nucleus. J Cell Biol 145, 1119–1131.

Dieppois G, Iglesias N, 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.

Drubin D, Garakani A, Silver P (2006). Motion as a phenotype: the use of live-cell imaging and machine visual screening to characterize transcription-dependent chromosome dynamics. BMC Cell Biol 7, 19.

Feuerbach F, Galy V, Trelles-Sticken E, Fromont-Racine M, Jacquier A, Gilson E, Olivo-Marin J-C, Scherthan H, Nehrbass U (2002). Nuclear architecture and spatial positioning help establish transcriptional states of telomeres in yeast. Nat Cell Biol 4, 214–221.

Finlan LE, Sproul D, Thomson I, Boyle S, Kerr E, Perry P, Ylstra B, Chubb JR, Bickmore WA (2008). Recruitment to the nuclear periphery can alter expression of genes in human cells. PLoS Genet 4, e1000039.

Fischer T, Straber K, Racz A, Roriguez-Navarro S, Oppizzi M, Ihrig P, Lechner J, Hurt E (2002). The mRNA export machinery requires the novel Sac3p-Thp1p complex to dock at the nucleoplasmic entrance of the nuclear pores. EMBO J 21, 5843–5852.

Fraser P, Bickmore W (2007). Nuclear organization of the genome and the potential for gene regulation. Nature 447, 413–417.

Grimaud C, Becker PB (2009). The dosage compensation complex shapes the conformation of the X chromosome in *Drosophila*. Genes Dev 23, 2490–2495.

Johnston M, Flick JS, Pexton T (1994). Multiple mechanisms provide rapid and stringent glucose repression of GAL gene expression in *Saccharomyces cerevisiae*. Mol Cell Biol 14, 3834–3841.
Kalverda B, Pickersgill H, Shloma VV, Fornerod M (2010). Nucleoporins directly stimulate expression of developmental and cell-cycle genes inside the nucleoplasm. Cell 140, 360–371.
Kohler A, Schneider M, Cabal GG, Nehrbass U, Hurt E (2008). Yeast ataxin-7 links histone deubiquitination with gene gating and mRNA export. Nat Cell Biol 10, 707–715.
Kosak ST, Skok JA, Medina KL, Riblet R, Le Beau MM, Fisher AG, Singh H (2002). Subnuclear compartmentalization of immunoglobulin loci during lymphocyte development. Science 296, 158–162.
Kumaran RI, Spector DL (2008). A genetic locus targeted to the nuclear periphery in living cells maintains its transcriptional competence. J Cell Biol 180, 51–65.
Laine JP, Singh BN, Krishnamurthy S, Hampsey M (2009). A physiological role for gene loops in yeast. Genes Dev 23, 2604–2609.
Lanctot C, Cheutin T, Cremer M, Cavalli G, Cremer T (2007). Dynamic genome architecture in the nuclear space: regulation of gene expression in three dimensions. Nat Rev Genet 8, 104–115.
Liang Y, Hetzer MW (2011). Functional interactions between nucleoporins and chromatin. Curr Opin Cell Biol 23, 65–70.
Light WH, Brickner DG, Brand VR, Brickner JH (2010). Interaction of a DNA zip code with the nuclear pore complex promotes H2A.Z incorporation and INO1 transcriptional memory. Mol Cell 40, 112–125.
Longtine MS, McKenzie A 3rd, Demarini DJ, Shah NG, Wach A, Brachat A, Philippsen P, Pringle JR (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. Yeast 14, 953–961.
Luthra R, Kerr SC, Harreman MT, Apponi LH, Fasken MB, Ramineni S, Chaurasia S, Valentini SR, Corbett AH (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.
Madrid AS, Mancuso J, Cande WZ, Weis K (2006). The role of the integral membrane nucleoporins Ndc1p and Pom152p in nuclear pore complex assembly and function. J Cell Biol 173, 361–371.
Mendjan S *et al.* (2006). Nuclear pore components are involved in the transcriptional regulation of dosage compensation in *Drosophila*. Mol Cell 21, 811–823.
Misteli T (2007). Beyond the sequence: cellular organization of genome function. Cell 128, 787–800.
Morrissey JP, Deardorff JA, Hebron C, Sachs AB (1999). Decapping of stabilized, polyadenylated mRNA in yeast pab1 mutants. Yeast 15, 687–702.
Ragoczy T, Bender MA, Telling A, Byron R, Groudine M (2006). The locus control region is required for association of the murine beta-globin locus with engaged transcription factories during erythroid maturation. Genes Dev 20, 1447–1457.
Reddy KL, Zullo JM, Bertolino E, Singh H (2008). Transcriptional repression mediated by repositioning of genes to the nuclear lamina. Nature 452, 243–247.
Ren B *et al.* (2000). Genome-wide location and function of DNA binding proteins. Science 290, 2306–2309.
Rodriguez-Navarro S, Fischer T, Luo M-J, Antunez O, Brettschneider S, Lechner J, Perez-Ortin JE, Reed R, Hurt E (2004). Sus1, a functional component of the SAGA histone acetylase complex and the nuclear pore-associated mRNA export machinery. Cell 116, 75–86.
Santangelo GM (2006). Glucose signaling in *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev 70, 253–282.
Sarma NJ, Buford TD, Haley T, Barbara-Haley K, Santangelo GM, Willis KA (2011). The nuclear pore complex mediates binding of the Mig1 repressor to target promoters. PLoS ONE 6, e27117.
Sarma NJ, Haley TM, Barbara KE, Buford TD, Willis KA, Santangelo GM (2007). Glucose-responsive regulators of gene expression in *Saccharomyces cerevisiae* function at the nuclear periphery via a reverse recruitment mechanism. Genetics 175, 1127–1135.
Schlaich NL, Hurt EC (1995). Analysis of nucleocytoplasmic transport and nuclear envelope structure in yeast disrupted for the gene encoding the nuclear pore protein Nup1p. Eur J Cell Biol 67, 8–14.
Schmid M, Arib G, Laemmli C, Nishikawa J, Durussel T, Laemmli UK (2006). Nup-PI: the nucleopore-promoter interaction of genes in yeast. Mol Cell 21, 379–391.
Straight AF, Belmont AS, Robinett CC, Murray AW (1996). GFP tagging of budding yeast chromosomes reveals that protein-protein interactions can mediate sister chromatid cohesion. Curr Biol 6, 1599–1608.
Taddei A (2007). Active genes at the nuclear pore complex. Curr Opin Cell Biol 19, 305–310.
Taddei A, Hediger F, Neumann FR, Gasser SM (2004). The function of nuclear architecture: a genetic approach. Annu Rev Genet 38, 305–345.
Taddei A, Van Houwe G, Hediger F, Kalck V, Cubizolles F, Schober H, Gasser SM (2006). Nuclear pore association confers optimal expression levels for an inducible yeast gene. Nature 441, 774–778.
Tan-Wong SM, Wijayatilake HD, Proudfoot NJ (2009). Gene loops function to maintain transcriptional memory through interaction with the nuclear pore complex. Genes Dev 23, 2610–2624.
Vaquerizas JM, Suyama R, Kind J, Miura K, Luscombe NM, Akhtar A (2010). Nuclear pore proteins nup153 and megator define transcriptionally active regions in the *Drosophila* genome. PLoS Genet 6, e1000846.
Vodala S, Abruzzi KC, Rosbash M (2008). The nuclear exosome and adenylation regulate posttranscriptional tethering of yeast GAL genes to the nuclear periphery. Mol Cell 31, 104–113.
Yoshida T *et al.* (2010). Actin-related protein Arp6 influences H2A.Z-dependent and -independent gene expression and links ribosomal protein genes to nuclear pores. PLoS Genet 6, e1000910.