Transcriptional memory at the nuclear periphery

Jason H. Brickner
Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL, USA

Jason H. Brickner: j-brickner@northwestern.edu

Summary

A number of inducible yeast genes are targeted to the nuclear periphery upon transcriptional activation. However, when repressed again, the *INO1* and *GAL1* genes remain at the nuclear periphery for multiple generations. Retention at the nuclear periphery represents a novel type of transcriptional memory; the peripherally localized, recently repressed state of *GAL1* is activated more rapidly than the nucleoplasmically localized long-term repressed state of *GAL1*. This rapid reactivation involves localization at the nuclear periphery, the SWI/SNF chromatin remodeling complex, the histone variant H2A.Z and the Gal1 protein itself. Here, I review what we have learned about this type of transcriptional memory in yeast, what remains to be resolved and the challenges associated with understanding such epigenetic phenomena.

Gene recruitment to the nuclear periphery

DNA is spatially organized within the nucleus. It has long been appreciated that certain parts of the genome localize at the nuclear periphery and associate with the nuclear envelope [1–4]. Peripheral localization has been proposed to promote transcriptional repression. Much of the DNA at the nuclear periphery is transcriptionally repressed; heterochromatin, centromeres, telomeres and chromatin insulators (which can block enhancer function) localize at the nuclear periphery [5]. Proximity to the nuclear envelope in *Saccharomyces cerevisiae* [6] and association with the mammalian nuclear lamina [7,8] promotes transcriptional silencing of some genes.

Recent work, however, shows that peripheral localization is not always repressive. Artificially tethering a reporter gene to the nuclear envelope that lacks any silencing elements to the nuclear envelope does not promote repression [6]. Furthermore, certain inducible yeast genes are targeted rapidly to the nuclear periphery upon activation [9–11••]. A number of additional inducible genes have now been shown to undergo recruitment to the nuclear periphery in yeast [12–15] and peripheral localization correlates with a physical association with the NPC [10,12,16]. Peripheral targeting promotes transcription [9,13,17,18] and may be important for post-transcriptional events such as mRNA processing, mRNA export or translation [10,12].

The mechanism of gene recruitment

Physical interaction of genes with the NPC *in vivo* has been observed using different methods [10,12,16,19]. Furthermore, targeting of genes to the nuclear periphery requires NPC components, the SAGA histone acetyltransferase complex, the transcription-mRNA export complex TREX2 and the export receptor Mex67 [14,18–20]. These observations suggest that relocalization of genes to the nuclear periphery represents a "tethering" of genes to the NPC. Consistent with this interpretation, in mutant strains lacking in which NPCs cluster together into plaques, there is a significant increase in the colocalization of the *HXK1* gene with clustered NPCs upon transcriptional activation [13]. However, the fraction of cells

in which *HXK1* colocalized with clustered NPCs (17% of the cells in the population) is still much lower than the fraction of the cells in which *HXK1* is localized at the nuclear periphery (>85% of the cells in the population). Furthermore, it is unclear if the ~150 NPCs in the typical haploid nucleus in yeast would be able to accommodate both a large number of transcriptionally active genes (as suggested by the NPC association data in refs 11, 12 and 16) and nucleo-cytoplasmic transport without interference. The current model for gene recruitment to the NPC, on which I will build for the purposes of this review, is consistent with both the *in vivo* physical interaction of genes with the NPC and the genetic requirements for various NPC and NPC-associated proteins for gene recruitment. However, a direct physical interaction between genes and the NPC has not been demonstrated and it remains possible that the site to which genes are recruited is actually the nuclear envelope, a platform of the filamentous Mlp/Tpr proteins or other, unidentified structures at the nuclear periphery.

Gene recruitment of at least one gene to the nuclear periphery also requires the 3′ UTR [13]. Furthermore, the association of certain genes with NPC components, as measured by chromatin immunoprecipitation, is sensitive to RNase treatment [12]. The connection between the NPC, RNA elements important for mRNA export and the peripheral relocalization of genes suggested that relocalization might represent a <u>consequence</u> of transcription. In other words, relocalization of genes to the nuclear periphery might be mediated by a bridging interaction between chromosomal DNA, the mRNA, export factors and the NPC. If so, then relocalization to the nuclear periphery might simply represent an emergent property of highly expressed genes, rather than a specific targeting event.

Several results argue against this interpretation. From experiments using a temperature-sensitive allele of RNA polymerase to block transcription, it became clear that transcription is not essential for either the interaction of *GAL1* with the NPC [16] or for relocalization of *INO1* to the nuclear periphery [11••]. Furthermore, association of the Mex67 mRNA export receptor with the *GAL2* gene was independent of RNA, suggesting that the export receptor can interact with DNA or chromatin directly [14]. Also, both *INO1* and *GAL1* remain at the nuclear periphery for generations after transcription is repressed (see below; ref. [11••]). Finally, we have recently identified an 8 basepair element in the *INO1* promoter that functions as a "DNA zip code" to target an ectopic locus to the nuclear periphery [18]. Therefore, although the mRNA may play important roles in the localization of genes with the NPC after transcription is initiated (see below), transcription-independent mechanisms of gene relocalization occur and relocalization to the nuclear periphery represents an active and specific targeting mechanism.

Transcriptional memory at the nuclear periphery

In addition to a role in transcriptional activation, we have identified a role for peripheral targeting in the rapid reactivation of recently repressed genes. When cells are shifted from activating to repressing conditions, both the *INO1* and *GAL1* genes remain at the nuclear periphery through multiple cell divisions [11••]. Retention of *GAL1* at the nuclear periphery is very stable, lasting greater than seven generations. This suggested that peripheral localization might represent a novel epigenetic state that reflects previous transcription. We hypothesized that this recently repressed "memory" state might be functionally different from the long-term repressed state. Indeed, we found that the reactivation of the *GAL1* gene, even after seven generations of repression, was much faster than the initial activation of the *GAL1* gene [11••]. This suggested that cells have cellular and molecular mechanisms to mark previously expressed genes and to promote their reactivation, a phenomenon I call adaptive transcriptional memory.

Concurrent work independently demonstrated that previous transcription of *GAL1*, *GAL7* and *GAL10* promoted their rapid reactivation [21••]. Rapid reactivation of *GAL1* required as little as one hour of activation and persisted through cell division. The rapid reactivation of the *GAL1* gene was not due to the lingering association of RNA polymerase II, the TATA binding protein (which binds upstream of preinitiation complex formation) or coactivators such as Mediator (which interacts with RNA Polymerase II to promote transcription), the SWI/SNF chromatin remodeling complex or the SAGA histone acetyltransferase [21••]. Therefore, recently repressed *GAL1* is not primed through a lingering association with a stalled RNA polymerase II, but through faster recruitment of RNA polymerase II upon reactivation.

Defects in mRNA processing can also lead to post-transcriptional targeting of genes to the nuclear periphery. *GAL1* promoter-driven reporter genes that produce mRNAs that are improperly polyadenylated remain associated with post-transcriptional RNA "dots" at the nuclear periphery [22]. Localization at the nuclear periphery is maintained for approximately 1 hour after transcription is repressed [22,24•], increases with defects in mRNA processing [22] and requires both an mRNA export factor (TREX 2; ref. [23•]) and the nuclear exosome, an ribonuclease that degrades aberrant mRNAs in the nucleus [24•]. In mutants lacking the THO complex (which coordinates mRNA processing with export), an intermediate in the mRNA processing pathway accumulates associated chromatin [25•]. This intermediate also appears as RNA dots, requires the nuclear exosome for its formation and leads to the relocalization of genes to the nuclear pore complex [25•]. It is unclear how this mechanism of peripheral retention relates to the epigenetic peripheral retention observed for the endogenous, wild type *GAL1* gene.

The mechanism of transcriptional memory

Although both *INO1* and *GAL1* are retained at the nuclear periphery after transcriptional repression, the mechanism of *GAL1* transcriptional memory is easier to study because it lasts longer [11••]. Recently repressed *GAL1* is both rapidly reactivated and localized at the nuclear periphery. The mechanism of reactivation of recently repressed *GAL1* is different from the mechanism of activation of long-term repressed *GAL1* (see below; refs. [11••, 21••]). Therefore, I propose that the cell marks recently repressed genes and that this alters their mechanism of transcriptional activation.

Many epigenetic phenomena require post-translational histone modifications or alterations in nucleosome composition [26–29]. Adaptive transcriptional memory requires two chromatin-based mechanisms: the histone variant H2A.Z [11••] and the SWI/SNF chromatin remodeling complex [21••] play essential roles in either the establishment of transcriptional memory or the rapid reactivation of the memory state.

H2A.Z nucleosomes are found in two regions of the genome. They are found in subtelomeric regions, where they are important for insulating euchromatin from the spread of heterochromatin [30]. They are also found in the promoters of many eukaryotic genes, where they play a role in promoting transcriptional activation [31–36]. We identified a third role for H2A.Z nucleosomes: H2A.Z is essential both for the retention of *INO1* and *GAL1* at the nuclear periphery after repression and for the rapid reactivation of these genes [11••]. Importantly, the initial targeting of *INO1* or *GAL1* to the nuclear periphery and the initial activation of these genes was not dependent on H2A.Z. Thus, H2A.Z plays a specific, essential role in the peripheral retention and reactivation of certain recently repressed genes.

The reactivation of *GAL1* does not require histone modifications that are associated with transcriptional activation [21••]. This is important because previous work had shown that

immediately after *GAL1* transcription is repressed, the trimethylation of histone H3 lysine 4 that is associated with transcription persists [37]. However, the reactivation of *GAL1* does require the SWI/SNF chromatin-remodeling complex. In the absence of the Swi2 protein, the activation and reactivation of *GAL1* are indistinguishable and slow. The initial activation of *GAL1* was normal in the *swi2Δ* mutant, indicating that, like H2A.Z, the role of SWI/SNF was specific to the reactivation of the memory state.

The role of SWI/SNF appears to be to counteract the repressive effects of either Isw1 or Isw2 remodeling complexes. The requirement for SWI/SNF in *GAL1* reactivation was bypassed in strains lacking either Isw1 of Isw2 [21••]. Isw1 and Isw2 are the catalytic subunits of two related chromatin-remodeling complexes implicated in transcriptional repression. When the *swi2Δ* mutation was combined with either *isw1Δ* or *isw2Δ*, the double mutant exhibited normal transcriptional memory, reactivating *GAL1* more rapidly.

Both the SWI/SNF chromatin remodeling complex and the histone variant H2A.Z are required for the rapid reactivation of *GAL1*. The major difference between the studies that implicated H2A.Z and that implicated SWI/SNF in transcriptional memory is the particular regime of repression before reactivation. Kundu *et al.* (2007) analyzed the effect of SWI/SNF on *GAL1* reactivation after one hour of repression, whereas our work has focused on the effect of H2A.Z on *GAL1* reactivation after 12 hours of repression. It remains to be determined how these regimes are related.

The role of DNA localization in transcriptional memory

The correlation of rapid reactivation with peripheral localization suggests that localization may be important for transcriptional memory. Loss of H2A.Z causes both a dramatic decrease in the reactivation rate and loss of peripheral localization of *INO1* and *GAL1* after repression. Therefore, peripheral localization is coupled to reactivation and both are dependent on H2A.Z. Intriguingly, H2A.Z physically associates with the NPC-associated protein Nup2 [38]. Tethering silenced genes to the NPC creates a boundary that prevents the spreading of silencing [39]. Furthermore, loss of Nup2 causes a spread of silencing from telomeres, similar to the effect of loss of H2A.Z [30,38]. Therefore, perhaps the memory state and boundary activity represent mechanistically related chromatin states that both require H2A.Z nucleosomes and interaction with the NPC.

Artificially tethering the *INO1* gene to the nuclear periphery leads to more rapid transcriptional activation [9,11••]. This effect may represent a type of "artificial memory". Like genuine transcriptional memory, tethering does not affect the ultimate steady-state activation of *INO1* [11••]. However, unlike genuine memory, tethering to the nuclear envelope induces rapid activation in the absence of previous transcription.

The mechanism of epigenetic inheritance

SWI/SNF is required for rapid reactivation of *GAL1*. H2A.Z is required for rapid reactivation of both *GAL1* and *INO1* and for their retention at the nuclear periphery after repression. These results raised the possibility that transcriptional memory might represent a self-perpetuating chromatin state and that this might explain the epigenetic inheritance of transcriptional memory. In other words, a change in the chromatin state that reflects the history of the gene might "bookmark" a gene and, if it persists until DNA replication, this bookmark might somehow promote its own inheritance. The concept that chromatin might promote its own inheritance to produce stable epigenetic changes in gene expression has been broadly endorsed and has even been proposed as a modern definition of epigenetics [40]. However, the perpetuation of epigenetic states through chromatin alone, without input from *trans*-acting factors, is still controversial and has been greeted with skepticism [41].

To directly test the hypothesis that chromatin alone is the source of epigenetic inheritance of *GAL* transcriptional memory, a recent study used heterokaryon analysis, in which cells fuse, but nuclei do not [42••]. They showed that rapid reactivation of *GAL1* could be transferred from one cell to another via cytoplasmic mixing. Therefore, *GAL* transcriptional memory is a <u>cytoplasmically</u> inherited phenomenon. The cytoplasmic factor was identified as the Gal1 protein itself; a *gal1*Δ mutant is unable to rapidly reactivate the *GAL7* gene. Furthermore, constitutive expression of *GAL1* was sufficient to confer rapid reactivation of *GAL7* [42••]. *GAL1* encodes the galactokinase enzyme. Galactokinase is 70% identical to the galactose sensor Gal3, which senses galactose and inactivates the Gal80 transcriptional repressor [43]. Therefore, Gal1 may directly inhibit Gal80, priming the *GAL* genes for reactivation. The ~100 fold activation of the *GAL1* gene, coupled with an extremely long half-life, provides a plausible mechanism for the inheritance of transcriptional memory for > 7 generations [11••, 42••]. Thus, although *GAL* transcriptional memory requires chromatin-based mechanisms, the inheritance of memory is mediated by a cytoplasmic factor.

Based on what we have learned from the regulation of the *GAL* genes, I propose a model for adaptive transcriptional memory (Figure 1). For genes that utilize this type of transcriptional memory, three qualitatively distinct states can exist: a long-term repressed state, an active state and an epigenetic memory state. Each state is distinguished from the other two by its subnuclear localization, its transcriptional activity and its mechanism of regulation (Figures 1 & 2). Furthermore, I propose that it is possible to convert between any state and any other state. Conversion from the active state to the memory state requires a cytoplasmic effector (Gal1 in the case of the *GAL* genes). I propose that the cytoplasmic effector functions upstream of peripheral localization, SWI/SNF and H2A.Z (Figure 2). The long-term repressed state can also be converted directly into the memory state (*i.e.* artificial memory) by expression of the cytoplasmic regulator (Figure 2). I propose that, like genuine memory, this pathway also requires peripheral targeting, SWI/SNF or H2A.Z (Figure 2).

Two observations indicate that the memory state is qualitatively different from the long-term repressed state and that reactivation occurs by a novel mechanism that is not simply due to titration of a negative regulator. First, the long-term repressed state and the memory state localize to different parts of the nucleus (Figure 1). Second, the requirement for H2A.Z and SWI/SNF in rapid reactivation of recently repressed *INO1* and *GAL1* is specific; loss of these proteins has no significant effect on the activation of long-term repressed *INO1* and *GAL1* [11••,21••]. This, too, is not simply due to a change in the rate-limiting step because SWI/SNF and H2A.Z are not required for the rapid activation of *GAL1* when cells are shifted from non-repressing raffinose medium to activating galactose medium (our unpublished data; ref. [21••]). Finally, if the tethering of *INO1* truly generates artificial memory and if cytoplasmic regulators are a general feature of transcriptional memory, this suggests that their role can be bypassed by tethering *INO1* to the nuclear envelope. If so, then the transient expression of a cytoplasmic regulator (like Gal1) generates a transcriptional memory through downstream chromatin modifications mediated by peripheral localization, SWI/SNF and H2A.Z (Figures 1 & 2).

Future directions

Future work will integrate what we have learned to create a more coherent model of transcriptional memory and to answer a number of important questions. How do cytoplasmic factors, chromatin-based changes and gene localization work together to create a marked promoter that can be activated more rapidly? What cytoplasmic factors, if any, are involved in *INO1* transcriptional memory? How many genes utilize this type of short-term memory? What are the selective advantages of this system? Finally, do metazoan cells utilize this type of transcriptional memory? SWI/SNF and H2A.Z are conserved among eukaryotes and

peripheral targeting of genes has been observed in flies [44•] and mammalian cells [45•]. If metazoan cells utilize adaptive transcriptional memory, it might provide a mechanism by which environmental or physiological inputs can produce short-term to long-term changes in gene expression.

Acknowledgments

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suggests that peripheral targeting of active genes can also occur in mammalian cells. [PubMed: 18316479]
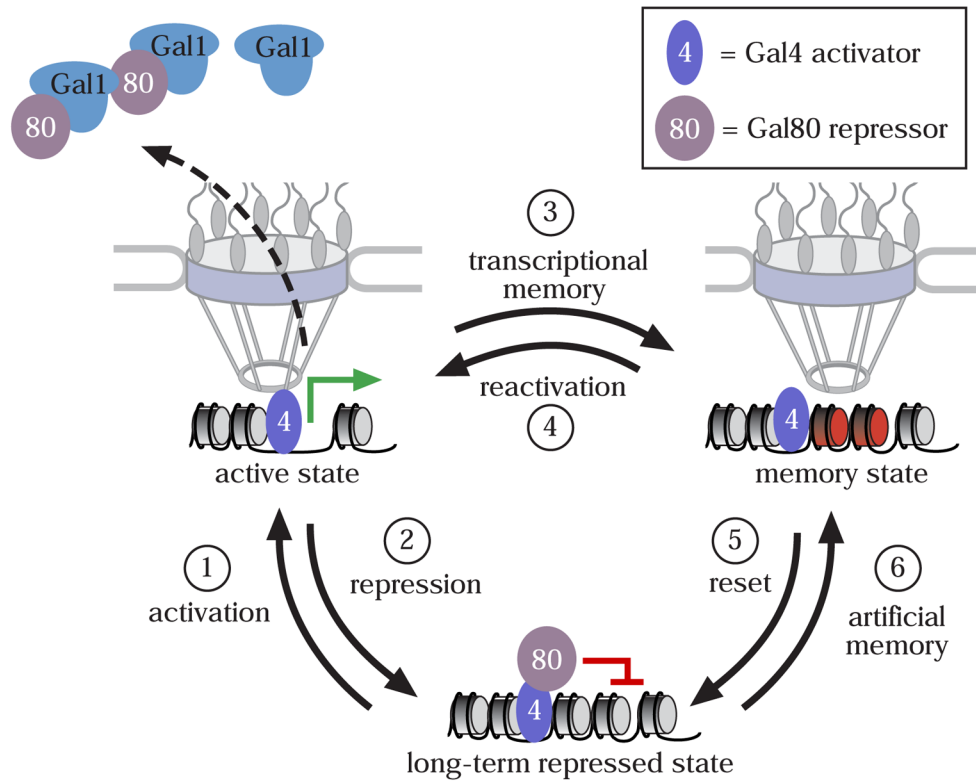
Figure 1. Three distinct states of the *GAL* genes
Initial activation of the *GAL1*, *GAL2*, *GAL7* and *GAL10* genes (step 1) through the Gal3 galactose sensor, leads to relocalization to the nuclear periphery through association with the NPC and the production of the Gal1 protein. Gal1 binds the repressor Gal80 and inhibits its function. After repressing transcription (via the glucose repression system), the genes remain at the nuclear periphery in a memory state that can be rapidly reactivated. The establishment of the memory state requires chromatin changes (indicated as red nucleosomes). Eventually, transcriptional memory is lost (reset, step 5). The memory state differs from the long-term repressed state in its localization, its chromatin requirements for activation and its rate of reactivation.
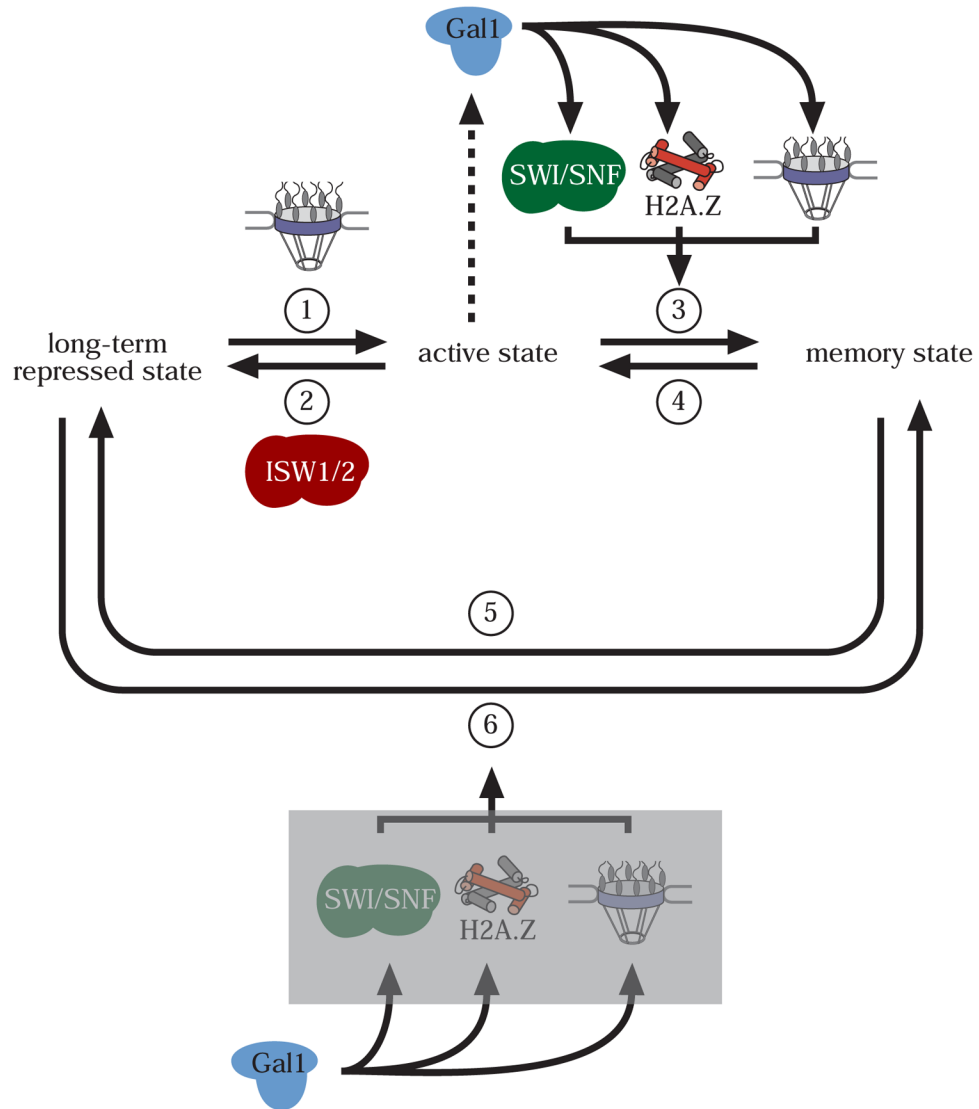
Figure 2. Regulation of transcriptional memory

The states and the interconversion between them are the same as in Figure 1. Conversion between the three states is regulated by the production of a cytoplasmic regulator (the Gal1 protein in the case of the *GAL* genes). The cytoplasmic regulator functions upstream of the SWI/SNF chromatin remodeling complex, the H2A.Z histone variant and peripheral localization (indicated as the NPC) to promote transcriptional memory (step 3). Because we cannot order these events relative to each other currently, I represent them as parallel inputs. SWI/SNF is necessary to counteract the conversion of the active state into the long-term repressed state by the ISW1 and ISW2 complexes. The memory state can be generated directly from the long-term repressed state by expression of the cytoplasmic regulator (artificial memory; step 6). Grey box: possible role for SWI/SNF, H2A.Z and peripheral localization in the generation of artificial memory.