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Chromatin: A Connection Between Loops and Barriers?

Dispatch

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A genetic screen for proteins that can block the spread of silenced heterochromatin has identified components of the nuclear pores with potential barrier activity. These results suggest that formation of loops of chromatin anchored to the pore could be one mechanism of barrier function.

The eukaryotic nucleus is organized into distinct territories and a gene's location within these chromosomal domains has consequences for its expression potential [1]. While a preponderance of potentially active genes reside in euchromatic domains, heterochromatic domains are gene deficient and usually associated with transcriptionally silent genes. These domains are thought to be composed of chromatin loops associated with a proteinaceous scaffold, and several DNA elements that preferentially associate with this scaffold to structurally delineate the loops have been identified.

Heterochromatic and euchromatic domains are established and maintained by specific regulatory elements, such as silencers, locus control regions and enhancers. Despite the close proximity in which antagonistic elements such as enhancers and silencers may reside along a chromosomal fiber, they only affect the expression of relevant associated genes. Functional elements, termed insulators, have been defined and characterized which separate distinct transcriptional domains and block the spread of silenced heterochromatin into neighboring euchromatin [2]. Currently it is unclear if the functionally defined active and inactive domains are coincident with the loop domains.

The budding yeast *Saccharomyces cerevisiae* has several loci — including the silent mating-type loci *HML* and *HMR*, as well as telomeric loci — with all of the molecular and biochemical characteristics of heterochromatin. Silencing at these loci is mediated by nearby regulatory elements and uses the Sir proteins Sir2p (a deacetylase), Sir3p and Sir4p. The genes at the cryptic mating type loci are flanked by silencer elements and proteins bound to these elements are thought to recruit the Sir complex through direct and indirect interactions. Following the recruitment of the Sir proteins, these proteins are thought to spread along the DNA fiber to form a specialized chromatin state that is inaccessible to various enzymatic probes [3]. 'Barrier' elements that block the spread of this domain have been identified, and now Laemmli and colleagues [4] have reported evidence that the formation of such barriers that delimit the extent of such silenced regions may involve interactions between chromatin and nuclear pores.

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Native Yeast Barriers

The silent domain at *HMR* and *HML* has been shown to extend beyond the silencers for a limited distance [5–8] and DNA elements were found that flank the repressed loci and map to the boundaries of the inaccessible chromatin domain [6,7]. Deletion of these barrier elements at the native *HMR* locus led to an increased spread of silenced chromatin and concomitant repression of neighboring euchromatic genes, while the ectopic insertion of this barrier between a silencer and a promoter — either on a plasmid or at its native chromosomal locus — blocked the repressive effects of the silencer [7,9]. These properties precisely fit the definition of barrier elements.

The telomeric ends of *S. cerevisiae* chromosomes contain reiterated binding sites for Rap1p, and it is believed that the Sir complex is recruited via interactions with the telomere-bound Rap1p. Following recruitment, the complex is believed to spread along the sub-telomeric chromatin, resulting in formation of an inaccessible chromatin domain. Barrier elements called STARs were found in middle-repetitive, subtlomeric sequences which can block this spread [10–12]. Thus, two linked reporter genes, *URA3* and *TRP1*, located at telomeres are silenced in wild-type cells, but insertion of a STAR element between the telomere-proximal *URA3* and *TRP1* allowed cells to grow on medium containing 5-FOA but lacking tryptophan, indicating that *URA3* gene was repressed while the downstream *TRP1* reporter was insulated from silencing. Furthermore, bracketing *TRP1* with STAR elements insulated this gene from telomere-driven silencing. This indicated that barrier elements did not function merely by de-repressing the entire chromosomal region or altering the potency of silencing elements.

Analysis of these and other barrier elements [6,13] revealed that transcription factors bound to specific sites are involved in blocking the spread of silencing. Binding sites for the transcription factors Reb1p and Tbf1p found at the telomeric barrier were found to be necessary and sufficient for barrier activity and insulation from silencing [10]. But insulator activity clearly does not involve transcriptional activation of reporter genes. Two other general regulatory factors that are involved in silencing, Rap1p and Abf1p, also harbor potent insulating domains [14].

At the *HMR* locus, a specific tRNA gene was shown to act as a barrier. Mutations in the promoter of this gene, or in the RNA polymerase III transcription factors TFIIIC and TFIIIB, weakened the barrier activity of this tRNA gene, and further analysis showed that the acetyltransferase Sas2p is also required for barrier activity [9]. These results failed, however, to resolve the issue of whether these functionally defined domains are coincident with chromatin loop domains,

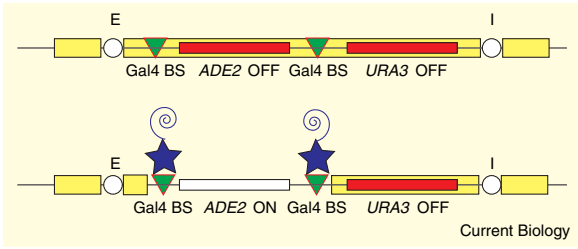

Figure 1. A schematic representation of the boundary trap screen of Ishii *et al.* [4].

Two reporter genes, *ADE2* and *URA3*, inserted between the *HML-E* and *HML-I* silencers (E and I, respectively) are repressed in wild-type cells. In the screen, the *ADE2* gene is flanked with Gal4p-binding sites (Gal4 BS) to identify proteins that, when recruited to the Gal4-binding sites, cause derepression specifically of the *ADE2* gene; such proteins are represented here by a star, for the Gal4 DNA-binding domain, and spiral, for the fused domain with boundary activity.

though mutations in proteins believed to be involved in the formation of loop domains were shown to negatively affect barrier function [7].

The Boundary Trap Screen

A recent genetic screen [4] for proteins with barrier activity has uncovered a possible connection between structural loop domains and functional barrier elements. This screen also used two sensitive reporter genes, *ADE2* and *URA3*, inserted between the *HML-E* and *HML-I* silencers such that both reporter genes are silenced in wild-type cells. Ishii *et al.* [4] then flanked *ADE2* with binding sites for Gal4 and screened a Gal4 DNA-binding domain fusion library for proteins that, when recruited to the Gal4 binding sites, would insulate *ADE2*, but not *URA3*, from silencing. The screen identified proteins that have previously been shown to be involved in nuclear transport: Cse1p, Los1p, Mex67p, Sxm1p and Gsp2p. Los1p and Mex67p are involved in RNA export; Cse1p and Sxm1p are required for protein export and import, respectively; and Gsp2p is one of two yeast Ran homologs.

These results raise several questions on the mechanism by which these proteins might function in barrier activity. As the barrier effect mediated by these proteins was dependent on the presence of binding sites for Gal4, it is unlikely that the observed phenotype is due to interference with transport of the *ADE2* RNA. It is also unlikely that the barrier effect is due to the recruitment of any large complex, as other known large protein complexes were not identified in this screen. What is not clear is whether the transcriptional states of the insulated *ADE2* gene are clonally inheritable — a key defining characteristic of position effect variegation, the phenomenon in which a gene's state of transcriptional activity is determined by proximity to a region of heterochromatin [15].

The connection between loop domains and barrier function came from the observation that, in wild-type cells, Cse1p was found to be present at the nuclear periphery, and this localization required the nuclear pore protein Nup2p. The barrier activity mediated by Cse1 was also abolished in *nup2Δ* mutants, and tethered Nup2p could also function as a barrier, suggesting that tethering of *ADE2* to the nuclear pore is a requirement for barrier function. Consonant with this was the demonstration that targeting these proteins to an array of *lac* operators caused the relocalization of the sequence from the nuclear lumen to the rim. There was also a tight correlation between association with the nuclear rim and barrier activity, suggesting that tethering to the nuclear rim is important for barrier activity.

From these observations, Ishii *et al.* [4] suggest that the formation of small loops tethered to the nuclear periphery may be required to insulate genes from surrounding heterochromatin. Unfortunately, they [4] do not present evidence that the nuclear pore proteins function as barriers at other silenced loci, such as *HMR* and telomeres, and neither do they demonstrate that nuclear pore protein complexes are involved in barrier activity at the native *HML* [6], *HMR* [7] or telomeric barriers [10].

Interestingly, silenced loci such as *HML* and those near telomeres are normally found at the nuclear periphery [16], and it has been shown that tethering sequences to the nuclear periphery via nuclear membrane protein hybrids results in significant Sir-dependent silencing of a reporter gene [17]. The results with Nup2 [4] suggest that the localization of sequences specifically to the nuclear pore, rather than the periphery in general, may be important for barrier activity. This model is elegant in its simplicity, but is also at variance with previous results on nuclear pore proteins which showed that mutations of Mlp1p, Mlp2p and Nup145p and Nup60p cause reduced silencing at yeast telomeres, accompanied by a concomitant loss of perinuclear clustering of telomeric loci [18,19].

The new results [4] raise questions about the mechanism by which the Nup2p pore proteins might function to block the spread of silencing. Earlier work on native yeast barriers suggested that the ability of a protein complex to form a stable interaction with DNA in competition with the spreading silenced chromatin is what constitutes a barrier [20]. The barrier presumably acts by creating localized regions of open chromatin to impede the propagation of silenced chromatin. The new data raise the possibility that anchoring DNA to a nuclear substructure — the pore complex — might generate a topologically independent domain, and this might be another mechanism for barrier function. The results also indicate that different pore complexes behave differently, and that the nuclear periphery can be differentiated into at least two compartments — the Nup145p-dependent silencing domain [18] and the active domain organized by Nup2p [4]. It is also possible that the Nup2p complex identified has the potential to recruit chromatin remodeling activities involved in transcription activation, though this has not yet been demonstrated. Further experiments should help determine the exact mechanism by which these elements function in restricting the spread of silencing.

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