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Dissecting gene repression: not just location, location, location

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Large heterochromatic domains are found tethered to the lamina. But is this nuclear environment repressive *per se*, or just the “ground state” of inactive chromatin? Elegant studies from the van Steensel group recently demonstrated that the lamina is indeed repressive, but that intrinsic promoter properties also dictate gene activity.

Much of the transcriptionally inactive portion of the mammalian genome is located within condensed heterochromatin, predominantly at the nuclear periphery. Around one third of the genome forms kilobase-to-megabase-sized domains that are tethered to the nuclear lamina (LADs, lamina-associated domains). These domains are enriched in heterochromatic histone modifications and comprising gene-poor regions of low expression [1]. A long-standing question is whether the nuclear lamina is in itself a repressive nuclear environment or is merely the passive “stopping point” for inert chromatin. Artificially tethering specific genomic loci to the lamina caused heterogeneous repression of the target genes and their neighbors [2, 3], and around one tenth of genes within LADs are expressed [1], pointing to a complex interplay of lamin association and gene expression regulation. High-throughput experiments providing functional decoupling of nuclear localization and transcriptional output are thus required to dissect active and passive repressive roles of LADs. A new study from the van Steensel group has achieved just that, formally demonstrating that the lamina does indeed form a repressive compartment, but that promoter and local chromatin context determine the extent to which transcription can be modulated [4]. Their study combines two promoter transplantation strategies, SuRE (survey of regulatory elements [5]) and TRIP (thousands of reporters integrated in parallel [6]) to ask two complementary questions (Figure 1):

1. Are LAD promoters active when removed from the lamina?

SuRE uses a barcoding system and RNA-seq to quantitatively measure the autonomous promoter activity of millions of random genomic fragments inserted into episome libraries [5]. In other words, the propensity of all promoters to activate transcription when removed from their endogenous chromatin context can be assessed. By comparing SuRE results to

those from methods measuring (nascent) transcription from the endogenous promoter, the extent to which the local chromatin affects gene expression can be gauged. In this manner, promoters found in LADs are on average ten-fold less expressed than those outside of LADs that have similar autonomous activity (SuRE signal), supporting an active repression role of the lamina. However not all LAD promoters behave identically. Whereas around a third of LAD promoters display more transcriptional activity when moved to episomes, implying that they are repressed by their chromatin environment within LADs, another half are actually silent in both SuRE and nascent transcript measures. These genes thus do not need to be actively repressed, and are more likely to lack other factors required for their activation, such as binding by specific transcription factors or engagement with enhancers. In agreement, these “inactive” genes tend to be tissue-specific, suggesting that they are not readily induced by ubiquitous transcription factors. Another portion of LAD promoters are active whether within LADs or episomes, reminiscent of the previously reported “escaper” LAD genes [1]. In contrast to the inactive gene class, escapers tend to be more ubiquitously expressed across tissues.

2. How is promoter activity altered when transplanted to different LAD regions?

Conversely, TRIP inserts the same reporter construct into many different genomic locations; high-throughput sequencing approaches allow the transcriptional output from each inserted location to be measured simultaneously [6]. Three LAD “escaper” promoters, three LAD-repressed promoters and one housekeeping gene promoter not found within a LAD were compared in this manner [4]. Two major conclusions could be drawn. Firstly, when comparing expression after insertion into LAD or inter-LAD locations, escaper promoters were much less sensitive to repression by LAD environments, suggesting that the “escaper”

or “repressed” property of LAD response is indeed promoter-intrinsic. Notably, the housekeeping reporter appeared completely insensitive to LAD localization, implying that strong enough intrinsic promoter properties can completely override any lamin-mediated repressive mechanisms. Secondly, the variability of reporter expression was very high between LAD inserts, particularly for the repressed promoters, suggesting that the extent of LAD-mediated repression is very heterogeneous according to the local chromatin context. Modeling generally correlated the most repressive parts of LADs as those with the highest enrichment in lamin binding, implying tightest attachment to the lamina. As may be expected, the most transcriptionally permissive regions of LADs are generally enriched for active histone modifications.

In combination, this study confirms and quantifies what has been previously been proposed: that LADs are genuine repressive, but heterogeneous, nuclear environments, and that the interplay of promoter-intrinsic properties (e.g. bound transcription factors) and local chromatin context determines final transcriptional output. However, some fundamental questions remain. Firstly, how do LADs mediate gene repression? At the local scale, tighter lamin binding correlates with greater repression, but it is unclear if this is more a consequence of transcription displacing lamins than the lamina directly inhibiting expression. This study found evidence that LADs impair transcriptional elongation of escaper genes, rather than initiation, but mechanisms are still unclear. A growing body of biochemical evidence suggests that inner nuclear membrane proteins interact with transcriptional repressors and may even activate histone-modifying enzymes [e.g. 7], but such mechanisms have yet to be observed *in vivo*. Secondly, what distinguishes “escaper” promoters from LAD-repressed ones? With the exception of the completely insensitive housekeeping gene promoter, LAD resistance cannot be simply explained by promoter

strength, since the escaper promoters tested by TRIP were not inherently stronger than the LAD-repressed ones. It is intriguing that acetylation of lysine-122 of histone 3, a globular domain modification that has been found to distinguish a specific subset of enhancers in mouse embryonic stem cells [8], correlates with higher activity of escaper promoters but repression of LAD-repressed ones. It remains to be seen if this finding still holds when TRIP is extended to more promoters. Further, extension of the SuRE analysis to compare transcriptional activity of enhancers found within or outside of LADs found a moderate enrichment for binding of pioneer transcription factors. Carefully controlled perturbation studies will be required to see if such correlations translate into mechanisms of LAD escape. Since LADs themselves can vary in different tissues [9], it will also be very interesting to extend the promoter transplantation assays to multiple cell types. In this manner, we can see if “escaper” or “LAD-repressed” properties are completely intrinsic to DNA sequence, or are modulated by the repertoire of available transcription factors. Combined with mechanistic studies, these assays promise to be a Sure TRIP on the path to understanding gene repression via nuclear localization.

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Figure 1. Promoter transplantation assays to understand LAD repression. (a) SuRE assay identifies three classes of LAD promoters: inactive (red), repressed (yellow) and escaper (green). Inactive and repressed genes are transcriptionally shut down at the lamina in their endogenous context, whereas escaper genes are expressed (left). When inserted into episomal reporter constructs (right), inactive genes remain silent and escaper genes remain active, but repressed genes, free from the LAD environment, are activated. Inactive genes tend to be tissue-specific, whereas escaper genes are more ubiquitously expressed. (b) TRIP assay assesses the heterogeneity of lamina-mediated repression. Promoters from the repressed (yellow) or escaper (green) class are randomly integrated into the genome, and the reporter expression from LAD insertions are assessed. Escaper promoters are less affected by LAD insertion than repressed promoters. The most effective lamina-mediated repression occurs where there is tightest lamin binding, and is weakest where there are active histone marks; the histone modification H3K122ac appears to mark silencing and activation, respectively, at repressed and escaper promoters. Figure adapted from [4]

(a)

(b)