

# Dissecting Gene Repression: Not Just Location, Location, Location

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1	Dissecting gene repression: not just location, location, location
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12	Large heterochromatic domains are found tethered to the lamina. But is this nuclear
13	environment repressive per se, or just the "ground state" of inactive chromatin? Elegant
14	studies from the van Steensel group recently demonstrated that the lamina is indeed
15	repressive, but that intrinsic promoter properties also dictate gene activity.

17 Much of the transcriptionally inactive portion of the mammalian genome is located within condensed heterochromatin, predominantly at the nuclear periphery. Around one third of 18 the genome forms kilobase-to-megabase-sized domains that are tethered to the nuclear 19 lamina (LADs, lamina-associated domains). These domains are enriched in heterochromatic 20 histone modifications and comprising gene-poor regions of low expression [1]. A long-21 22 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 23 24 genomic loci to the lamina caused heterogeneous repression of the target genes and their 25 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 26 27 experiments providing functional decoupling of nuclear localization and transcriptional 28 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 29 does indeed form a repressive compartment, but that promoter and local chromatin context 30 31 determine the extent to which transcription can be modulated [4]. Their study combines two 32 promoter transplantation strategies, SuRE (survey of regulatory elements [5]) and TRIP 33 (thousands of reporters integrated in parallel [6]) to ask two complementary questions (Figure 1): 34

#### 35 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

40 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, 41 promoters found in LADs are on average ten-fold less expressed than those outside of LADs 42 that have similar autonomous activity (SuRE signal), supporting an active repression role of 43 the lamina. However not all LAD promoters behave identically. Whereas around a third of 44 45 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 46 47 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, 48 such as binding by specific transcription factors or engagement with enhancers. In 49 agreement, these "inactive" genes tend to be tissue-specific, suggesting that they are not 50 51 readily induced by ubiquitous transcription factors. Another portion of LAD promoters are active whether within LADs or episomes, reminiscent of the previously reported "escaper" 52 LAD genes [1]. In contrast to the inactive gene class, escapers tend to be more ubiquitously 53 54 expressed across tissues.

### 55 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 LADrepressed 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"

63 or "repressed" property of LAD response is indeed promoter-intrinsic. Notably, the housekeeping reporter appeared completely insensitive to LAD localization, implying that 64 strong enough intrinsic promoter properties can completely override any lamin-mediated 65 repressive mechanisms. Secondly, the variability of reporter expression was very high 66 between LAD inserts, particularly for the repressed promoters, suggesting that the extent of 67 68 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 69 70 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 71 active histone modifications. 72

73 In combination, this study confirms and quantifies what has been previously been proposed: 74 that LADs are genuine repressive, but heterogeneous, nuclear environments, and that the 75 interplay of promoter-intrinsic properties (e.g. bound transcription factors) and local chromatin context determines final transcriptional output. However, some fundamental 76 77 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 78 consequence of transcription displacing lamins than the lamina directly inhibiting 79 expression. This study found evidence that LADs impair transcriptional elongation of escaper 80 genes, rather than initiation, but mechanisms are still unclear. A growing body of 81 biochemical evidence suggests that inner nuclear membrane proteins interact with 82 transcriptional repressors and may even activate histone-modifying enzymes [e.g. 7], but 83 84 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 85 housekeeping gene promoter, LAD resistance cannot be simply explained by promoter 86

87 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 88 domain modification that has been found to distinguish a specific subset of enhancers in 89 mouse embryonic stem cells [8], correlates with higher activity of escaper promoters but 90 repression of LAD-repressed ones. It remains to be seen if this finding still holds when TRIP is 91 92 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 93 enrichment for binding of pioneer transcription factors. Carefully controlled perturbation 94 studies will be required to see if such correlations translate into mechanisms of LAD escape. 95 Since LADs themselves can vary in different tissues [9], it will also be very interesting to 96 extend the promoter transplantation assays to multiple cell types. In this manner, we can 97 see if "escaper" or "LAD-repressed" properties are completely intrinsic to DNA sequence, or 98 are modulated by the repertoire of available transcription factors. Combined with 99 mechanistic studies, these assays promise to be a Sure TRIP on the path to understanding 100 101 gene repression via nuclear localization.

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123 Figure 1. Promoter transplantation assays to understand LAD repression. (a) SuRE assay 124 identifies three classes of LAD promoters: inactive (red), repressed (yellow) and escaper 125 (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 126 episomal reporter constructs (right), inactive genes remain silent and escaper genes remain 127 128 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 129 130 assay assesses the heterogeneity of lamina-mediated repression. Promoters from the repressed (yellow) or escaper (green) class are randomly integrated into the genome, and 131 the reporter expression from LAD insertions are assessed. Escaper promoters are less 132 133 affected by LAD insertion than repressed promoters. The most effective lamina-mediated 134 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 135 activation, respectively, at repressed and escaper promoters. Figure adapted from [4] 136



