



# Xist at the edge: silencing at the nuclear periphery extends to X inactivation

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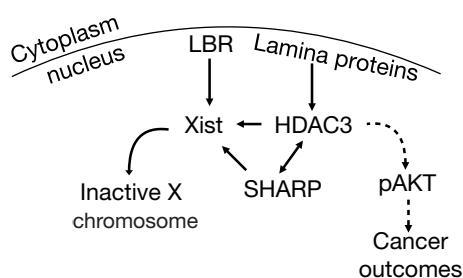
The physical organization of chromatin in the nucleus plays a fundamental role in directing developmental cell fate decisions. The nuclear lamina in particular is a critical regulator of silencing specific genomic regions and establishing facultative heterochromatin (1). Chen *et al.* now demonstrate the connection between Xist-mediated silencing of the inactive X chromosome (XCI) and the repressive properties of the nuclear lamina (2). The absence of the inactive X chromosome has long been associated with tumors (3), and lately Xist, along with other long noncoding RNAs (lncRNAs), has been implicated in cancer etiology in addition to its role in dosage compensation (4,5). These new findings emphasize the links between the Xist lncRNA, repressive epigenetic modifiers such as the polycomb repressive complexes (PRC1, PRC2), histone deacetylase 3 (HDAC3)-containing complexes, and the nuclear lamina, pointing towards new directions in the role of epigenetic silencing in translational cancer research.

The identity of the XCI as a lamina-associated domain is often visible to the eye, as labeled Barr bodies frequently occur at the nuclear periphery, and with reduced frequency at the nucleolus. However, a mechanistic description of this phenomenon has been lacking, with experiments focused on the biochemical establishment of repressive marks via PRC complexes in the absence of information about nuclear organization. Recent studies of the Xist proteome identified among other repressive epigenetic modifiers (PRC and SMRT complexes), architectural factors (SAF-A), RNA-binding proteins (SHARP and RBM15), and several nuclear lamina components including lamin B receptor (LBR) (6-8). LBR, an integral protein of the inner nuclear membrane, plays an essential role in developmentally regulated silencing through establishment and maintenance of facultative heterochromatin

formation at the nuclear lamina by multiple epigenetic modifying complexes (9).

The authors analyzed Xist positioning and gene silencing in mouse ES cells treated with LBR siRNAs, and observed a concomitant loss of lamina-associated Xist signal and gene silencing activity. This behavior was also observed in LBR knockout ES cells. Using a cross-linking immunoprecipitation (CLIP) readout, they pinpointed LBR association to three main regions of the Xist transcript, including a SHARP-bound region previously identified as necessary for silencing. The use of the BoxB/ $\lambda$ N tethering system to force association of Xist transcripts to the nuclear periphery provided clear evidence that the activity was reversible in the siLBR cells, as well as in mutants of the Xist-interacting LBR region and the LBR-binding region of Xist. The absence of Xist spreading to other regions of the inactive X chromosome was confirmed by RAP-DNA experiments showing decreased Xist RNA occupancy across the genomes of cells lacking the LBR-binding region of Xist ( $\Delta$ LBS). However, knockdown of SHARP reduced silencing of the XCI despite maintenance of proximity to the lamina, indicating a multi-step mechanism, not simply lamina association, is necessary for silencing. Together, these findings led the authors to propose a model whereby Xist, silencing mechanisms, and the nuclear lamina are linked together for proper XCI silencing, but not independently sufficient for Xist spreading and silencing.

Several mechanistic questions are now raised by these studies. Super-resolution microscopy identifies on the order of 50–100 Xist foci per Barr body (10,11) and many of these foci are not within a physical range (100s of nm) of the nuclear lamina to interact with LBR or other lamina-associated proteins (12). Thus, only a fraction of Xist molecules at any given time will



**Figure 1** Interactions between Xist, the nuclear lamina, silencing factors, and cancer outcomes. Solid lines represent direct interactions, dashed lines represent indirect interactions. LBR, lamin B receptor.

be mediating lamina-directed silencing. It is unknown whether each of these molecules will interact with the peripheral silencing compartment over the lifetime of an individual cell, or whether silencing activity, promoted by the lamina, will spread after nucleation at a subset of Xist foci. A finer time course of Xist association with the lamina, at super-resolution level, may resolve this question. Additionally, a method of determining what proportion of Xist molecules interact biochemically with LBR in particular or the lamina in general, such as a DAM-ID/CLIP approach, could resolve the necessary questions about stoichiometry.

Another source of uncertainty is the timing and order of establishment of Xist interactions with the lamina. Repositioning experiments indicate that Xist- and LBR-mediated silencing spread to loci such as *Gpc4* over a 16-hour timespan following Xist induction (in the SHARP knockdown, and presumably in WT cells as well), and that knockdowns of LBR or the  $\Delta$ LBS and  $\Delta$ A mutants maintain a larger distance between the *Gpc4* locus and Xist signal within this timeframe. The authors hypothesize that interactions between Xist and LBR are initiated by a dynamic sampling of the nuclear space by Xist-coated chromatin over a timespan of hours. However, lamina-associated domains are generally thought to be established across mitotic divisions, as the nuclear lamina reforms in the proximity of regions containing sequence-specific factors that engage with lamina components, such as in the interaction between GAGA dinucleotide repeats, the GAGA-binding factor cKrox, the nuclear lamina protein Lap2 $\beta$ , and HDAC3 in the establishment of lamina-associated domains (13). Controlling for cell division, and extending the time-resolved analysis of Xist positioning in normal and mutant cells, perhaps with a live-cell approach using MS2-tagged Xist transcripts, would help answer this question.

The previous identification of a SHARP-HDAC3 complex as critical for Xist-mediated silencing (6) by the same group

also reinforces the potential role of the nuclear lamina, as HDAC3 catalytic activity is enhanced at the nuclear lamina (14). Previous studies looking at the effect of various nuclear membrane components such as LMNB1, LBR, or emerin in developmental contexts make this discovery a logical progression of known lamina-mediated silencing phenomena. It would be informative to examine the role of nuclear lamina association in relation to other mechanisms, such as PRC-mediated H3K27me3 deposition, RBM15-mediated m<sup>6</sup>A methylation (15), and silencing at non-Xist regulated regions, to determine the degree to which lamina association is required for these events. For example, Xist could be tethered to another nuclear compartment, such as the nucleolus, via the BoxB/ $\lambda$ N system, and the XCI assayed for silencing and PRC-mediated histone modifications to test the absolute necessity for lamina association in these processes. Further, assessing the effect of LBR depletion on the positioning behavior of both the active X chromosome and inducible Xist transgenes integrated on autosomes would help dissect the specificity of the proposed mechanism.

The work of Chen *et al.* is an important step towards establishing how the heterogeneous nuclear environment functions as a transcriptional regulator, as well as suggesting additional mechanisms of how Xist in particular, and lncRNAs in general, may establish developmentally regulated transcriptional silencing. As a similar process has recently been shown in *C.elegans*, this is likely an evolutionarily conserved mechanism coupling dosage compensation, developmentally regulated silencing, and the nuclear lamina (16). These findings have broad applicability to translational cancer research, particularly in breast cancer, where the Xist transcript shows increased transcriptional activity in cultured cells and patient samples (17). As the cooperative nature of HDAC3 complexes and Xist have recently been shown to regulate AKT phosphorylation in human breast cancer samples through a PHLPP1-dependent interaction (18), the connection to specific cancer-regulating mechanisms is clear. While this provides an informative network of interactions at the nuclear lamina involving Xist and HDAC3 leading to cancer-related outputs (*Figure 1*), many connections are indirect and merit further investigation. The ability to tether or release genomic regions via lncRNAs to the nuclear periphery or other compartments may become a useful tool in modulating gene expression across larger genomic regions, or provide a fine-tuning mechanism in genomic-based therapies. Further experiments, utilizing a suite of imaging and biochemical tools, will clarify this complex set of mechanisms and should point the way towards improved clinical outcomes through diagnostic and therapeutic strategies that leverage our growing knowledge of functional nuclear organization.

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