NON-CODING RNA

X chromosome inactivation unravelled

The long non-coding RNA (lncRNA) *Xist* (X inactive-specific transcript) is required for the transcriptional silencing of one X chromosome in each cell, in a process known as X chromosome inactivation (XCI) that occurs during mammalian female development. Owing to technical limitations, little is known about the mechanism of transcriptional silencing during XCI. McHugh *et al.* now describe the use of RNA antisense purification followed by quantitative mass spectrometry (RAP–MS) — a novel approach for identifying proteins that directly interact with lncRNAs *in vivo* — to identify *Xist*-interacting proteins that have a role in XCI.

RAP–MS involves crosslinking lncRNAs and proteins *in vivo*, followed by stringent, antisense-mediated purification of the directly interacting proteins. SILAC (stable isotope labelling by amino acids in culture) is then used for the quantitative comparison of purified proteins by mass spectrometry between experimental and control RNA purifications. The authors performed RAP–MS on male mouse embryonic stem (ES) cells induced to express *Xist* and identified a highly specific and reproducible set of ten proteins that directly interact with *Xist*. Of these proteins, knocking down the genes encoding scaffold attachment factor A (SAFA; also known as HNRNPU), SMRT- and HDAC1-associated repressor protein (SHARP; also known as SPEN or MSX2-interacting protein) and lamin-B receptor (LBR) largely abolished the silencing of XCI-affected genes in the male ES cells as well as in differentiating female ES cells. These three proteins are therefore required for *Xist*-mediated gene silencing. SAFA was previously shown to tether *Xist* to the inactive X chromosome, SHARP is known to interact with the histone deacetylase 3 (HDAC3) activator SMRT (silencing mediator of retinoic acid and thyroid hormone receptor; also known as NCOR2), and LBR anchors heterochromatin to the inner nuclear membrane.

> “*Xist* binds to SHARP to recruit SMRT and activate HDAC3 … resulting in gene silencing”

The initiation of XCI includes the localization of *Xist* along the X chromosome and the exclusion of RNA polymerase II (Pol II) from the chromosome. The depletion of SAFA resulted in diffused localization of *Xist* in the nucleus, in agreement with previously published data, suggesting that SAFA acts to localize *Xist* to genomic DNA. Depleting SHARP led to the retention of Pol II at *Xist*-coated X chromosomes, indicating that SHARP might be required for initiating transcriptional silencing following *Xist* localization, possibly by recruiting the transcriptional co-repressors SMRT and HDAC3. Indeed, depleting SMRT or HDAC3 (but not other HDACs) abrogated *Xist*-dependent gene silencing. Another feature of XCI is the recruitment of Polycomb repressive complex 2 (PRC2), which initiates maintenance of chromatin compaction and gene silencing by catalysing histone H3 lysine 27 trimethylation. The authors found that although PRC2 is not required for initiating XCI, depleting SHARP or HDAC3 resulted in the loss of PRC2 at inactivated X chromosomes, suggesting that SHARP and HDAC3 may also have a role in establishing a repressive epigenetic state following the initiation of XCI.

In summary, the data support a model in which SAFA tethers *Xist* to X chromosomes. *Xist* binds to SHARP to recruit SMRT and activate HDAC3, which directs the removal of histone acetylation marks, resulting in gene silencing and chromatin condensation. Following the initiation of XCI, *Xist* recruits PRC2 to the X chromosome in a HDAC3-dependent manner to maintain the epigenetic inactive state. The data also show that RAP–MS is highly specific and efficient, and it could thus be used for identifying directly interacting proteins of any lncRNA.

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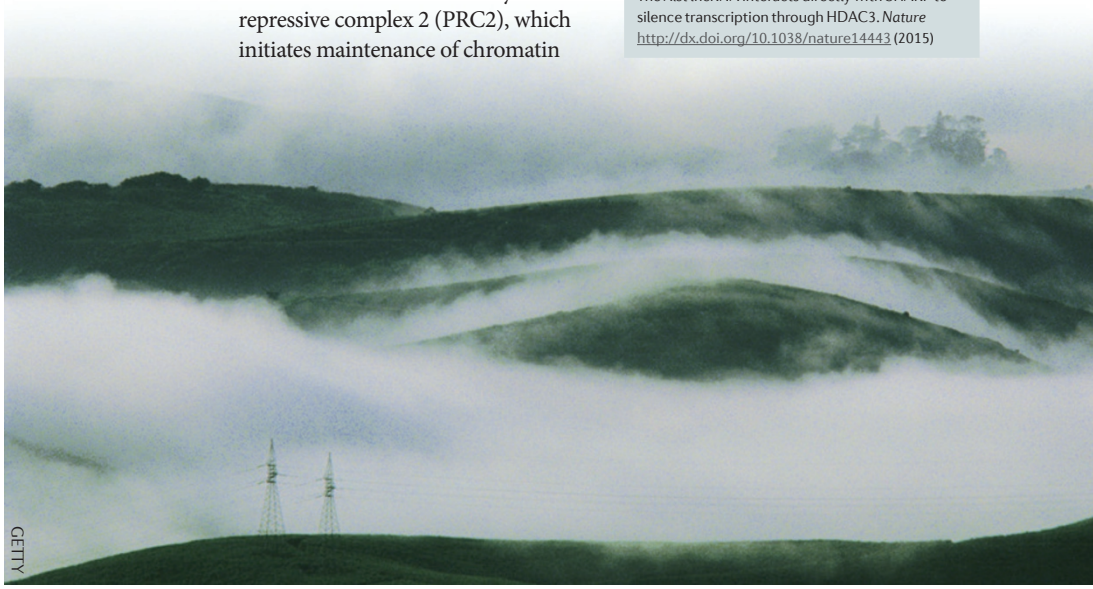
Nature Reviews Molecular Cell Biology

This article is modified from the original in *Nature Rev. Mol. Cell Biol.* (http://dx.doi.org/10.1038/nrm3998)

ORIGINAL RESEARCH PAPER McHugh, C. A. *et al.* The *Xist* lncRNA interacts directly with SHARP to silence transcription through HDAC3. *Nature* http://dx.doi.org/10.1038/nature14443 (2015)



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