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EPIGENETICS

X inactivation by titration

> A recent study has implicated an X-encoded long non-coding RNA in X-chromosome counting

X-chromosome inactivation (XCI) in mammals compensates for the imbalance in X chromosome number between females (XX) and males (XY). How the number of X chromosomes is counted by the cell is a long-standing problem. A recent study has implicated an X-encoded long non-coding RNA (lncRNA) in X-chromosome counting that titrates an autosomal transcription factor away from the X chromosome to inactivate it.

Mammalian XCI is known to occur when the X to autosome ratio (X/A) is ≥1, leading to the expression of inactive X specific transcript (*Xist*), which is an lncRNA that silences the X chromosome. However, a full understanding of how the cell calculates the X/A ratio through X-encoded numerators and autosomally encoded denominators has been elusive.

Sun *et al.* postulated that the X-encoded lncRNA *Jpx*, which is known to activate the transcription of *Xist*, is a good candidate for an X-encoded numerator. In support of this hypothesis, transgene experiments in mouse embryonic stem cells (ESCs) showed that *Xist* displays a dosage-dependent response to *Jpx* expression, so *Xist* expression is switched on only when the levels of *Jpx* are high enough.

The authors then chose to investigate CTCF as a potential autosomal regulator of XCI initiation — that is, as an autosome-encoded denominator of the X/A ratio — as putative binding sites for the CTCF transcription factor had previously been identified in the *Xist* promoter. They used chromatin immunoprecipitation (ChIP) to show that CTCF binds to the promoter site P2 in ESCs prior to XCI. However, on differentiation in female cells, CTCF binding at this site is depleted in the future inactive X chromosome. Furthermore, CTCF transgene studies indicated that CTCF was a direct *Xist* repressor.

Sun *et al.* were then able to show a link between *Jpx* and CTCF: *Jpx* overexpression relieved the effects of CTCF overexpression. Furthermore, they showed direct binding of *Jpx* to CTCF *in vivo*. Finally, ChIP confirmed that when CTCF was overexpressed, the binding of CTCF to P2 was increased, and *Xist* upregulation was compromised. However, when *Jpx* alone was overexpressed, CTCF binding to *Xist* was reduced approximately by half.

The authors' data point towards a model in which, in order to initiate XCI, *Jpx* is activated and titrates repressive CTCF away from the *Xist* promoter through competitive binding; this is the first time that such a function has been reported for an lncRNA. The allele-specific nature of these interactions suggests that further regulatory factors are yet to be found.

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ORIGINAL RESEARCH PAPER Sun, S. *et al.* *Jpx* RNA activates *Xist* by evicting CTCF. *Cell* **153**, 1537–1551 (2013)