PNAS LETTER

Lppnx lncRNA: The new kid on the block or an old friend in X-inactivation choice?

Rafael Galupa[a,1]

Dear Editor

I would like to raise a few points for reflection and discussion based on the recently published manuscript by Hierholzer et al. (1).

Is the *Lppnx* locus described in this study different from the *Linx* locus described previously? (2, 3) The *Lppnx* promoter deletion (0.6 kb) is contained within the deletion of the *Linx* promoter (2 kb), with very similar effects on the expression of its associated transcript and on *Xist* and choice of X to be inactivated (3). Similarly, characterization of the *Lppnx* transcript shows that it is all equivalent to the *Linx* transcript described previously (2): Expression occurs in both sexes and is associated with pluripotency (restricted to ICM in vivo and down regulated upon differentiation of ES cells ex vivo); transcripts are more abundant in the nucleus than cytosol and have no protein-coding potential.

Is the *Lppnx*/*Linx* RNA important for *Xist* regulation? The authors favor such hypothesis based on a promoter deletion; however, deleting the promoter of a lncRNA locus can also eliminate important genomic cis-regulatory elements (4). We have previously complemented experiments of *Linx* promoter deletion with a *Linx* promoter inversion, which showed that the absence of *Linx* transcription and transcript did not lead to skewed *Xist* expression ratios and choice patterns, contrary to the promoter deletion (3). Thus, before further investigations are pursued, it is preliminary (and maybe misleading) to state that the lncRNA underlies the effects reported.

Moreover, the mechanisms proposed by the authors are not incompatible with a genomic cis-regulatory element, namely the chromatin contacts with the *Xist-intron1* region and the loading of pluripotency factors at this region and others. It remains unclear based on the data presented whether such chromatin contacts are significant (statistically and/or biologically), and it will be interesting to investigate whether the effects on *Xist-intron1* are in *cis* as expected.

Importantly, the fact that deletion of the *Xist-intron1* region in *Lppnx*-deficient ES cells rescues the expected *Xist* ratios does not indicate that *Lppnx*/*Linx* acts via *Xist-intron1*. Several elements are known to affect *Xist* ratios and can do so independently of each other (3, 5, 6); if a positive "skewer" is deleted on the same chromosome in which a negative skewer was previously deleted (or vice versa), their effects are expected to rescue each other's, and this does not mean that they act via each other.

Finally, is the *Lppnx*/*Linx* locus the elusive *Xce* locus (7, 8)? The authors narrowed down the *Xce* locus to an 80-kb region (without reporting how) and, through genetic dissections, showed that only *Lppnx*/*Linx* and not the other loci present within the large 80-kb region (*Cdx4* and *Chic1*) has an impact on *Xist* expression. Interestingly, when deleting the *Lppnx*/*Linx* promoter, the authors observed different effects on *Xist* expression depending on which *Xce* allele harbored the deletion. This could suggest that the *Xce* effects are determined by more than the *Lppnx*/*Linx* locus itself.

Author affiliations: [a]Molecular, Cellular and Developmental Biology Unit, Centre de Biologie Intégrative, University of Toulouse, CNRS, UPS, 31062 Toulouse, France

Author contributions: R.G. wrote the paper.

The author declares no competing interest.

[1]Email: rafael.galupa@univ-tlse3.fr.

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