

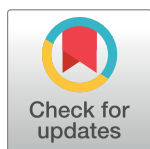
PERSPECTIVE

Transcription coactivator and lncRNA duet evoke Hox genes

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Mammalian genomes are pervasively transcribed [1, 2] to produce thousands of long noncoding RNAs (lncRNAs) [3–5], transcripts that are more than 200 nucleotides in length that do not code for proteins. Although only a handful of functional lncRNAs have been well characterized to date, recent work suggests that some lncRNAs have crucial roles in the control of gene expression during both normal development and disease, through multiple mechanisms [6, 7]. As new lncRNAs are being discovered at a rapid pace, their molecular mechanisms are continuing to be enriched and diversified. For example, a few lncRNAs have been shown to affect expression of nearby genes through recruitment of protein regulatory complexes [8–10], while it is suggested that others function akin to enhancers and local regulators *in cis* [11, 12]. In fact, such local gene-regulatory mechanisms have been invoked to explain the observation that lncRNA expression is often correlated with the expression of nearby genes—the so-called “guilt by association” [13].

The *cis*-acting mechanisms of lncRNAs are largely unknown. Whether loci encoding lncRNAs function via their lncRNA transcripts or DNA elements is often unclear. An important question in the field centers around distinguishing between at least 2 possible mechanisms—either the function of many of the lncRNAs that appear to regulate genes *in cis* depends on the RNAs themselves or their effect is mediated by enhancer-like activity of underlying DNA elements in the lncRNA locus, the act of transcription, and/or splicing of lncRNAs [14, 15]. Furthermore, the molecular components regulating the expression of lncRNAs remain mostly unexplored. An understanding of the possible commonalities of the disparate underlying mechanisms may facilitate instructive and predictive models of lncRNA function.

Pradeepa et al. [16] set out to elucidate 1 mechanism of lncRNA regulation and function using the *HoxA* locus as their model. The Bickmore group had previously shown that positive cofactor 4 (PC4) and splicing factor 2 (SF2) interacting protein (Psp1), also known as lens epithelium-derived growth factor (LEDGF), played an important role in the regulation of Hox genes [17] and had more recently demonstrated the role of the p75 isoform of Psp1 (Psp1/p75) in recruiting the Trithorax/mixed lineage leukemia (MLL) complex to expressed Hox genes [18]. Interestingly, loss of Psp1 led not only to reduced binding of the MLL complex and loss of histone 3 lysine 4 trimethylation (H3K4me3) at the distal *HoxA* genes, it also resulted in complete loss of expression of the lncRNA *HoxA* transcript at the distal tip (*Hottip*), transcribed in an antisense direction away from the distal end of the 5' *HoxA* cluster [10]. This suggested that Psp1 might function as a transcriptional regulator of *Hottip* expression. Following up on this possible connection, the authors showed that knockdown of Psp1/p52, the p52 isoform of Psp1, or *Hottip*, led to down-regulation of multiple 5' *HoxA* genes; knockdown of

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p52 also strongly down-regulated *Hottip* expression. Consistent with these observations, MLL occupancy was significantly reduced across posterior *HoxA* genes upon knockdown of p52 or *Hottip* compared to controls.

The authors next took advantage of CRISPR-Cas9 technology to create gene-body deletions of *Hottip*. Loss of *Hottip* expression led to decreased expression of posterior (*HoxA13*, *HoxA11*, and *HoxA10*) and increased expression of anterior (*HoxA2*, *HoxA6*, and *HoxA7*) *HoxA* genes. To find direct genomic targets of *Hottip*, chromatin isolation by RNA purification [19] (ChIRP) was performed, which demonstrated specific occupancy of *Hottip* RNA over the promoters of *HoxA13* and *HoxA11*. Importantly, ectopic activation of full-length *Hottip* via dCas9-mediated transcriptional activation showed specific induction of posterior (*HoxA13*, *HoxA11*, and *HoxA10*) but not anterior *HoxA* or *HoxD* genes. Additionally, premature termination of *Hottip* RNA by insertion of a synthetic polyadenylation cassette downstream of the *Hottip* transcription start site significantly reduced *HoxA13* and *HoxA11* mRNA levels in vitro and in vivo, firmly establishing a role for the intact *Hottip* lncRNA molecule and distinguishing its requirement from the act of transcription at the *Hottip* locus in the regulation of gene expression *in cis*. Taken together, the study provides a solid mechanism for the control of posterior *HoxA* gene transcription through activation of *Hottip* lncRNA by *Psip1/p52* (Fig 1).

Several interesting and important questions remain. *Psip1* binds to 5' *HoxA* genes but somehow still requires *Hottip* RNA to activate these genes. Thus, the concept of transcription coactivator using an enhancer-like RNA to amplify or expand its reach may apply to other

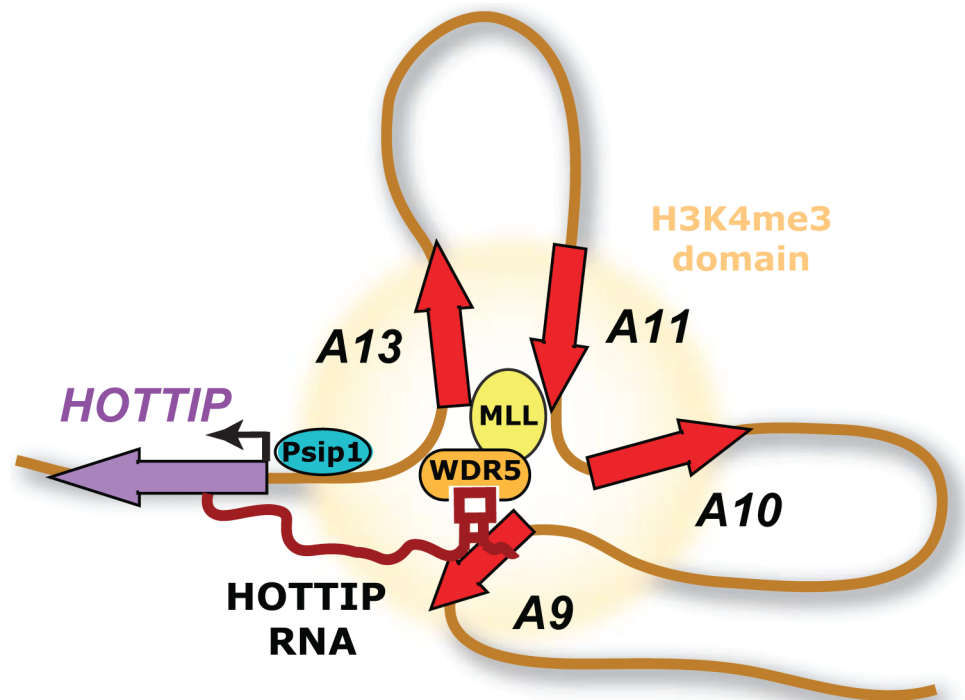


Fig 1. Model of positive cofactor 4 (PC4) and splicing factor 2 (SF2) interacting protein (*Psip1*) action. *Psip1* activates the transcription of *Hottip* long noncoding RNA (lncRNA) in distal limb cells. *Hottip* lncRNA in turn stimulates the transcription of 5' *HoxA* genes by enforcing H3K4me3 chromatin modification. *Hottip*'s selective effect on 5' *HoxA* genes is due to the chromosome looping that brings 5' *HoxA* genes into the vicinity of *Hottip* RNA emanating from the *Hottip* DNA locus. This mechanism transmits the spatial information in DNA looping into chemical information in chromatin modification and gene activation. Abbreviations: WDR5, WD repeat-containing protein 5; MLL, mixed lineage leukemia.

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pairs as well. These findings also provide substantial insights into the regulation of *Hottip*, the expression of which is dysregulated in a number of human cancers. *Hottip* appears to act locally near its site of transcription, but the mechanism through which *Hottip* RNA localizes specifically to the distal *HoxA* genes *in cis* is unknown. It is possible that *Hottip* takes advantage of the existing 3-dimensional chromosomal structure at the distal HoxA loci [10] to affect the local transcription landscape. This would be an attractive mechanism given the now-appreciated highly conserved hierarchical organization of the genome [20], in which fundamental structural units serve to guide regulatory elements or, in this case, specific RNA transcripts to their cognate promoters. These interactions could provide insight into the way in which the 3-dimensional organization of the genome reflects alterations in lineage and stage-specific transcriptional programs that govern cell fate, with lncRNAs such as *Hottip* acting as well-timed molecular switches. This may represent a fundamental property of mammalian gene regulatory networks, whose mechanisms for specificity and the general applicability represent key areas for future investigation.

References

1. Mattick JS. RNA regulation: a new genetics? *Nat Rev Genet*. Nature Publishing Group; 2004; 5: 316–323. <https://doi.org/10.1038/nrg1321> PMID: 15131654
2. Kapranov P, Cheng J, Dike S, Nix DA, Duttagupta R, Willingham AT, et al. RNA Maps Reveal New RNA Classes and a Possible Function for Pervasive Transcription. *Science*. 2007; 316: 1484–1488. <https://doi.org/10.1126/science.1138341> PMID: 17510325
3. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. *Nat Rev Genet*. 2009; 10: 155–159. <https://doi.org/10.1038/nrg2521> PMID: 19188922
4. Ponting CP, Oliver PL, Reik W. Evolution and functions of long noncoding RNAs. *Cell*. 2009; 136: 629–641. <https://doi.org/10.1016/j.cell.2009.02.006> PMID: 19239885
5. Wilusz JE, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. *Genes & Development*. 2009; 23: 1494–1504. <https://doi.org/10.1101/gad.1800909> PMID: 19571179
6. Wang KCK, Chang HYH. Molecular mechanisms of long noncoding RNAs. *Molecular Cell*. 2011; 43: 904–914. <https://doi.org/10.1016/j.molcel.2011.08.018> PMID: 21925379
7. Fatica A, Bozzoni I. Long non-coding RNAs: new players in cell differentiation and development. *Nat Rev Genet*. 2014; 15: 7–21. <https://doi.org/10.1038/nrg3606> PMID: 24296535
8. Lee JT. Lessons from X-chromosome inactivation: long ncRNA as guides and tethers to the epigenome. *Genes & Development*. 2009; 23: 1831–1842. <https://doi.org/10.1101/gad.1811209> PMID: 19684108
9. Nagano T, Mitchell JA, Sanz LA, Pauler FM, Ferguson-Smith AC, Feil R, et al. The Air Noncoding RNA Epigenetically Silences Transcription by Targeting G9a to Chromatin. *Science*. 2008; 322: 1717–1720. <https://doi.org/10.1126/science.1163802> PMID: 18988810
10. Wang KC, Yang YW, Liu B, Sanyal A, Corces-Zimmerman R, Chen Y, et al. A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. *Nature*. 2011; 472: 120–124. <https://doi.org/10.1038/nature09819> PMID: 21423168
11. Ørom UA, Derrien T, Beringer M, Gumireddy K, Gardini A, Bussotti G, et al. Long noncoding RNAs with enhancer-like function in human cells. *Cell*. 2010; 143: 46–58. <https://doi.org/10.1016/j.cell.2010.09.001> PMID: 20887892
12. Guil S, Esteller M. Cis-acting noncoding RNAs: friends and foes. *Nat Struct Mol Biol*. 2012; 19: 1068–1075. <https://doi.org/10.1038/nsmb.2428> PMID: 23132386
13. Rinn JL, Chang HY. Genome Regulation by Long Noncoding RNAs. *Annu Rev Biochem*. 2012; 81: 145–166. <https://doi.org/10.1146/annurev-biochem-051410-092902> PMID: 22663078
14. Bassett AR, Akhtar A, Barlow DP, Bird AP, Brockdorff N. Considerations when investigating lncRNA function *in vivo*. *Elife*. 2014.
15. Engreitz JM, Haines JE, Perez EM, Munson G, Chen J, Kane M, et al. Local regulation of gene expression by lncRNA promoters, transcription and splicing. *Nature Publishing Group*. 2016; 539: 452–455. <https://doi.org/10.1038/nature20149> PMID: 27783602
16. Pradeepa MM, McKenna F, Taylor GCA, Bengani H, Grimes GR, Wood AJ, et al. (2017) Psp1/p52 regulates posterior *Hoxa* genes through activation of lncRNA *Hottip*. *PLoS Genet* 13(4): e1006677. <https://doi.org/10.1371/journal.pgen.1006677> PMID: 28384324

17. Sutherland HG, Newton K, Brownstein DG, Holmes MC, Kress C, Semple CA, et al. Disruption of *Ledgf/Psip1* results in perinatal mortality and homeotic skeletal transformations. *Mol Cell Biol. American Society for Microbiology*; 2006; 26: 7201–7210. <https://doi.org/10.1128/MCB.00459-06> PMID: [16980622](https://pubmed.ncbi.nlm.nih.gov/16980622/)
18. Pradeepa MM, Grimes GR, Taylor GCA, Sutherland HG, Bickmore WA. *Psip1/Ledgf p75* restrains *Hox* gene expression by recruiting both *trithorax* and *polycomb* group proteins. *Nucleic Acids Res.* 2014; 42: 9021–9032. <https://doi.org/10.1093/nar/gku647> PMID: [25056311](https://pubmed.ncbi.nlm.nih.gov/25056311/)
19. Chu CC, Qu KK, Zhong FLF, Artandi SES, Chang HYH. Genomic maps of long noncoding RNA occupancy reveal principles of RNA-chromatin interactions. *Molecular Cell.* 2011; 44: 667–678. <https://doi.org/10.1016/j.molcel.2011.08.027> PMID: [21963238](https://pubmed.ncbi.nlm.nih.gov/21963238/)
20. Lupiáñez DG, Spielmann M, Mundlos S. Breaking TADs: How Alterations of Chromatin Domains Result in Disease. *Trends Genet.* 2016; 32: 225–237. <https://doi.org/10.1016/j.tig.2016.01.003> PMID: [26862051](https://pubmed.ncbi.nlm.nih.gov/26862051/)