Transcription coactivator and IncRNA 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 (Psip1), 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 Psip1 (Psip1/p75) in recruiting the Trithorax/mixed lineage leukemia (MLL) complex to expressed Hox genes [18]. Interestingly, loss of Psip1 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 Psip1 might function as a transcriptional regulator of Hottip expression. Following up on this possible connection, the authors showed that knockdown of Psip1/p52, the p52 isoform of Psip1, or Hottip, led to down-regulation of multiple 5’ HoxA genes; knockdown of
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 IncRNA 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 IncRNA 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

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**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 (IncRNA) in distal limb cells. Hottip IncRNA 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.

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