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# Long Noncoding RNAs in Development: Solidifying the Lncs to *Hox* Gene Regulation

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Long noncoding RNAs (lncRNAs) are pervasively expressed in mammals, although their functions during development remain poorly understood. In this issue of *Cell Reports*, Delpretti et al. and Li et al. suggest essential roles for lncRNAs in coordinating *Hox* gene expression.

It is estimated that up to three quarters of the human genome is transcribed, although the vast majority of expressed RNAs do not encode proteins (Djebali et al., 2012). A major class of noncoding transcript comprises a functionally diverse class of molecules collectively referred to as long noncoding RNAs (lncRNAs). LncRNAs have been implicated in a wide variety of biological processes, including X chromosome inactivation, genomic imprinting, embryonic development, and metastasis. Many lncRNAs display highly restricted expression profiles during development, suggesting key roles in controlling gene expression in specific cellular contexts (Ponting et al., 2009). One of the most well characterized lncRNAs in mammals is encoded by the *Xist* gene, which is essential for X chromosome inactivation (Lee and Bartolomei, 2013). *Xist* accomplishes this, in part, through recruitment of Polycomb repressive complexes, which transcriptionally silence genes through chromatin modifications. From studies of *Xist* and other lncRNAs, it has been suggested that one mechanism of lncRNA action is to provide recruitment platforms for transcriptional complexes that would otherwise have limited selectivity in target gene recognition.

The contribution of lncRNAs to embryonic development is potentially significant, given that gene expression must be finely tuned to ensure proper tissue patterning and differentiation. A global picture of lncRNAs function has emerged from analyses of their roles in embryonic stem cells (ESCs). An assessment of over 200 lncRNAs in ESCs demonstrated that the vast majority of targeted genes are regulated in *trans*, with apparently no systematic relationship between the locus of lncRNA expression and the gene target (Guttman et al., 2011). Considering that there are likely to be thousands of lncRNAs expressed during embryonic development, resolving the regulatory logic of lncRNA function will be a challenging task.

One family of transcription factors that is critical in embryonic development, and that appears to be regulated by lncRNAs, is encoded by genes within the *Hox* clusters (Figure 1A). During development, *Hox* genes are regulated through modifications of histones and chromatin structure (Noordermeer and Duboule, 2013). A seminal study by Chang and colleagues demonstrated that a lncRNA transcribed from an intergenic region within the human *HOXC* cluster, termed *HOTAIR*, regulates *Hox* genes in *trans*, specifically by repressing genes in the *HOXD* cluster (Rinn et al., 2007). *HOTAIR* appears to accomplish this by recruiting Polycomb repressive complexes, an activity reminiscent of the role of *Xist* in X chromosome inactivation. Subsequent studies from Chang and colleagues demonstrated that *HOTAIR* recruits both Polycomb repressive and LSD1 complexes (Figure 1B) (Tsai et al., 2010). Additionally, the function of lncRNAs is not limited to gene repression, as a lncRNA expressed from the distal end of the *HoxA* cluster appears to facilitate the activation of *HoxA* genes in *cis* (Wang et al., 2011).

A follow-up study analyzing the function of *Hotair* raised significant questions about its role in Hox-dependent developmental patterning. As a surrogate for *Hotair* mutation, mice in which the entire *HoxC* gene cluster was deleted, including the *Hotair* gene (Schorderet and Duboule, 2011), were analyzed by the Duboule group. Deletion of the *HoxC* cluster led to a modest 2-fold increase in *HoxD* expression in embryos, while the chromatin landscape at derepressed *HoxD* genes was not appreciably affected. Notably, removal of the *HoxC* cluster did not appear to cause major skeletal transformation, which would be predicted from altered *Hox* expression. However, the *HoxC* cluster produces dozens of noncoding RNAs, and removal of the entire gene cluster may remove additional lncRNAs that could oppose *Hotair*. Thus, the analysis of *HoxC* cluster mutants left open the possibility that compensatory interactions may have masked the true function of *Hotair* during development.

In a study reported in this issue, Li, Liu, and colleagues engineered a specific deletion of the *Hotair* gene (Li et al., 2013). They found that targeted disruption of *Hotair* led to the derepression of hundreds of genes, including genes within the *HoxD* cluster, consistent with the group's previous findings in human fibroblast cells. As a consequence *Hotair* mutants are characterized by homeotic transformation of spinal vertebrae and defects in the limb. The authors also found that removal of *Hotair* causes a gain of chromatin marks associated with gene activation (H3K4me3) and a loss of Polycomb repressive marks at *HoxD* gene loci. Importantly, the authors did not observe changes in the neighboring *Hoxc11* and *Hoxc12* genes, indicating
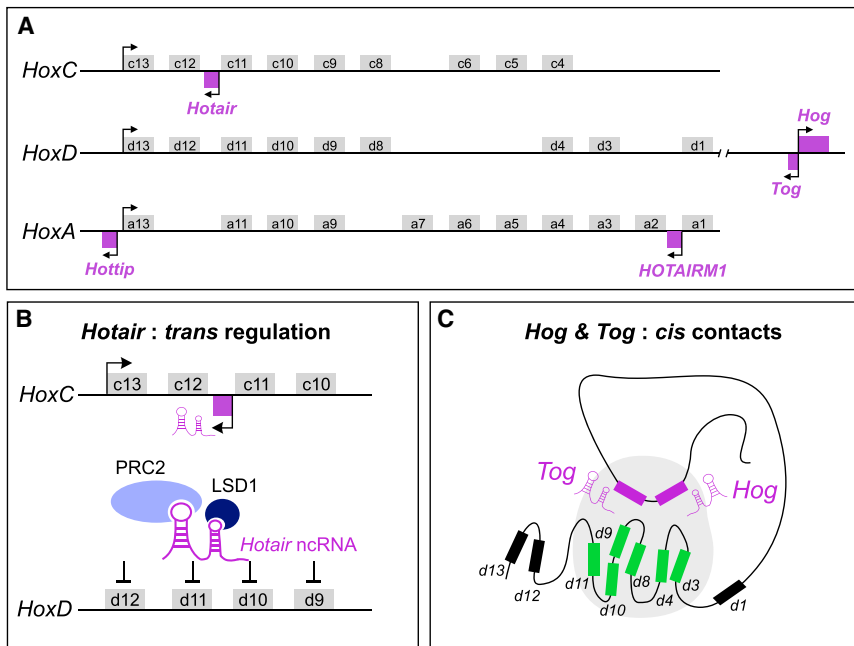
**Figure 1. Regulatory Interactions Between LncRNAs and *Hox* Genes**

(A) LncRNAs implicated in the regulation of *Hox* gene expression are indicated in purple. *Hox* loci also produce many additional lncRNAs, but for simplicity, these are not shown.

(B) *Hotair* represses posterior *HoxD* genes in *trans* through the recruitment of PRC2 and LSD complexes. In *Hotair* mutants, *HoxD* are derepressed, and mice display skeletal deformalities.

(C) The locus that produces *Hog* and *Tog* physically associates in *cis* with genes active in the *HoxD* cluster. These *HoxD* genes are expressed in the caecum (indicated in green), where *Hog* is specifically expressed. Other enhancer contacts are not shown.

that the defects are not due to removal of *cis* regulatory sequences, supporting the idea that *Hotair* functions in *trans*. Although the phenotypes of *Hotair* mutants are somewhat subtle, they nevertheless provide compelling evidence that *Hotair* is indeed essential in regulating the patterns of *Hox* and other genes during development.

Targeting of *Hotair* to *HoxD* genes relies on its specific profile of expression, raising the question of how the patterns of lncRNAs are regulated during development. The study by Delpretti et al. sheds light onto this question, through a comparative analysis of the function of *Hox* genes in the development of the caecum, a part of the digestive system required for the metabolism of cellulose in herbivores and omnivores (Delpretti et al., 2013). The authors identified two novel lncRNAs, which they named *Hotdog* (*Hog*) and *Twin of Hotdog* (*Tog*),

which are located in a gene desert in proximity to the *HoxD* gene locus. *Hog* and *Tog* share a common transcription start region and are transcribed from opposite DNA strands.

Interestingly, expression of *Hog* is specific to the caecum, and its locus physically associates with genes in the *HoxD* cluster (Figure 1C). When *Hog* and *Tog* are displaced from their normal position, *HoxD* genes are no longer expressed in the caecum. Conversely, when *Hoxd9–Hoxd11* genes are deleted, *Hog* and *Tog* are no longer expressed. These observations suggest that the physical contacts between the *Hog* and *Tog* genes and the *HoxD* locus are critical in regulating the profile of *HoxD* genes in caecum. While the study does not address the specific functions of these lncRNAs, it is tempting to speculate that, like *Hotair*, *Hog* and *Tog* play a direct role in defining a chromatin landscape that confers tissue specific

gene expression and are not simply by-products of enhancer activity.

Although there remain significant questions concerning the biological functions of most lncRNAs, the two studies in this issue of *Cell Reports* collectively provide important insights into their roles in controlling gene regulation during development. While the study by the Chang group closes an important chapter on *Hotair* function, the work by the Duboule group opens up the possibility that additional *Hox*-associated lncRNAs have selective roles in regulating *Hox* gene expression in tissue-specific contexts. It will be interesting to see whether the associations between lncRNAs and *Hox* genes are conserved in other developmentally regulated noncoding RNAs.

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