RNA WORLD

Antisense transcripts get involved

Links between long non-coding RNAs (lncRNAs) and gene silencing have been made since the identification of the X chromosome-inactivation transcript (*XIST*) in the early 1990s; what has remained enigmatic is the mechanism by which lncRNAs block gene expression. Two recent papers now show that two different antisense transcripts silence imprinted gene clusters via specific interactions with chromatin and chromatin-modifying proteins.

AIR and *KCNQ1OT1* are antisense transcripts normally expressed from paternally inherited chromosomes and are involved in silencing in *cis* clusters of genes surrounding the growth factor receptor gene *IGF2R* and the potassium-channel gene *KCNQ1*, respectively. When Pandey *et al.* reduced the stability of the mouse *Kcnq1ot1* transcript by inserting destabilizing 3′-UTR elements, they found that silencing was impaired, providing strong evidence that the lncRNA molecule itself, rather than just the act of antisense transcription, is functionally important.

Furthermore, by immunoprecipitation of RNA-associated chromatin they revealed that *Kcnq1ot1* interacts with the promoters of imprinted genes in the *Kcnq1* domain in the placenta, where these genes are silenced, but not in the fetal liver, where they are active. The authors also showed that the antisense transcript interacts with the histone methyltransferase G9a and two components of the Polycomb complex, EZH2 and SUZ12. Both G9a and the Polycomb complex lay down gene-silencing histone modifications that have been mapped to the repressed imprinted genes. Together, these observations provide a mechanistic basis for how *Kcnq1ot1* RNA could direct lineage-specific gene silencing via chromatin association and protein-complex recruitment.

Nagano and colleagues used RNA/DNA fluorescence *in situ* hybridization (FISH) to examine localization of the murine *Air* transcript at the imprinted solute carrier gene *Slc22a3*, which lies over 230 kb from *Igfr2* (and the *Air* promoter). They found a placenta-specific developmentally regulated association of the antisense transcript with the silenced gene that corresponded to repressive histone modifications. RNA immunoprecipitation by these authors also demonstrated interaction between the antisense transcript and G9a, and they showed that *Air* is required to silence *Slc22a3* by targeting G9a to the promoter. The silencing effect of G9a was also confirmed, as mice lacking this protein switched on *Slc22a3*.

Having looked at separate loci where lncRNAs had been implicated in imprinted gene silencing, these two papers suggest that the antisense RNAs have a direct, functional involvement in lineage-specific transcriptional repression, mediated via epigenetic modifications. The close similarities between the findings for these two transcripts suggest that other lncRNAs, including *XIST*, might function via specific interactions with chromatin. It now seems clear that future research will look beyond the act of transcription to the RNA molecules themselves to understand gene-silencing phenomena, but questions remain about how lncRNAs can regulate genes in a locus- and tissue-specific manner.

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ORIGINAL RESEARCH PAPERS Pandey, R. R. *et al.* *Kcnq1ot1* antisense noncoding RNA mediates lineage-specific transcriptional silencing through chromatin-level regulation. *Mol. Cell* **32**, 232–246 (2008) | Nagano, T. *et al.* The *Air* noncoding RNA epigenetically silences transcription by targeting G9a to chromatin. *Science* 6 Nov 2008 (doi:10.1126/science.1163802)