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# ncRNA transcription makes its mark

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**A recently recognized strategy for gene regulation involves transcription of a non-coding RNA (ncRNA) transcript that overlaps the gene targeted for regulation. In many cases, it seems that it is the act of transcription itself rather than the ncRNA transcript that mediates regulation. A paper in this issue of the *EMBO Journal* shows one mechanism by which these transcription events regulate transcription; elongating RNA polymerases direct a set of regulatory histone modifications that modulate expression of an overlapping gene.**

A major surprise of the past few years has been the discovery of significant transcription activity across entire eukaryotic genomes, showing a large class of ncRNAs that are often rapidly degraded (Yazgan and Krebs, 2007). In a number of cases, these ncRNAs have been found to regulate gene expression. Most of these regulatory ncRNAs function through RNAi-mediated pathways of gene repression. However, some ncRNAs regulate gene expression in *cis*. In these cases, the act of transcription itself, rather than the RNA product of transcription, mediates regulation of an overlapping gene.

The proposed mechanisms for regulation in *cis* include promoter occlusion or transcriptional interference by RNA polymerases transcribing ncRNAs (Yazgan and Krebs, 2007). Other genes show regulated transcription start-site choice from a single promoter, giving rise to either a coding transcript or an ncRNA (Jenks *et al*, 2008; Kuehner and Brow, 2008). A cryptic promoter that lies at the 3′ end of the *PHO5* gene and drives an antisense transcript is required for the normal kinetics of *PHO5* activation (Uhler *et al*, 2007). Transcription of a series of ncRNAs upstream of the *Schizosaccharomyces pombe fbp1+* promoter is required for its induction when cells are shifted to inducing conditions (Hirota *et al*, 2008). Passage of RNA polymerase II through the *fbp1+* promoter during transcription of these ncRNAs promotes the formation of open chromatin, allowing the transcription factor access to the *fbp1+* promoter during induction.

At present, reports by Houseley *et al* (2008) and by Pinskaya *et al* (2009) provide compelling evidence that transcription of ncRNAs influences post-translational modifications of histones that facilitate the repression of overlapping genes.

Chromatin immunoprecipitation (ChIP) experiments carried out by Houseley *et al* showed a surprising pattern of Set1-dependent histone H3K4 trimethylation across the well-characterized *GAL1–10* gene locus (Figure 1). A significant peak of this histone methylation mark, normally associated with the 5′ end of transcribed genes, was found within the 3′ end of *GAL10* when cells were grown in glucose medium (*GAL1-10* repressing conditions). These observations led Houseley *et al* to identify and characterize a set of ncRNAs that are transcribed from the 3′ end of *GAL10* across the promoter region shared by the divergent *GAL1* and *GAL10* genes, which they named *GAL10* ncRNAs.

Pinskaya *et al* observed that cells lacking Set1 induced *GAL1–10* expression more rapidly than wild-type cells when cells were shifted to galactose medium, although the final, fully induced levels of *GAL* mRNA were unchanged. The increased expression of *GAL1–10* in *set1* cells correlated with TBP occupancy at the *GAL1–10* promoter, suggesting that Set1 regulates transcription initiation at *GAL1–10*. Furthermore, an H3K4A mutant showed a similar induction phenotype, indicating a role for H3K4 methylation in *GAL1–10* induction. Subsequent experiments identified a set of ncRNA transcripts similar to those reported by Houseley *et al*, which they named *GAL1ucut* (*GAL1* upstream cryptic unstable transcripts).

Both groups mapped the *GAL1ucut* promoter to a location in the 3′ end of *GAL10* near a pair of binding sites for the Reb1

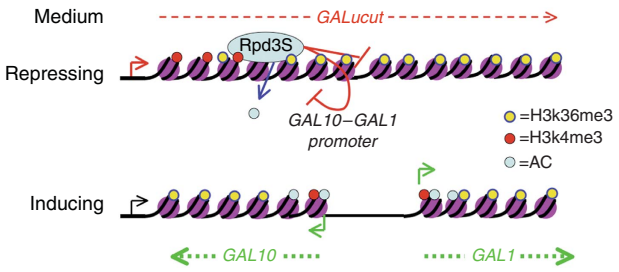


**Figure 1** Model for ncRNA-based regulation of *GAL1–GAL10* expression. Cells grown in glucose (repressing conditions) transcribe an ncRNA, *GAL1ucut*, from the 3′ end of *GAL10*. This directs H3K4 methylation at the 5′ end of the *GAL1ucut* and H3K36 methylation across the *GAL1–10* locus. The Rpd3S histone-deacetylase complex is recruited to and represses *GAL1–10* expression. Subsequent deacetylation of histones at the *GAL1–10* promoter might inhibit the recruitment of TBP and RNA polymerase II either directly or indirectly by inhibiting chromatin-remodelling events that are necessary for the binding of these factors. The physiological relevance of this mechanism might be to delay *GAL1–10* induction in the presence of galactose until all the available glucose is utilized.

transcription factor. Mutation of *REB1*, or of the Reb1 sites in *GAL10,* abolished *GAL1ucut* expression. Furthermore, both groups found an inverse relationship between *GAL1ucut* and *GAL1–10* expression. *GAL1ucut* is expressed under conditions that repress *GAL1–10*, and as *GAL1–10* is induced *GAL1ucut* declines. Curiously, Houseley *et al* did not observe an effect of *GAL1ucut* on *GAL1–10* expression when cells were shifted to a medium with high levels of galactose. Rather, they observed that *GAL1ucut* antagonized the induction kinetics and final levels of *GAL1–10* in a medium with low levels of both glucose and galactose. The basis for the difference in observations between the groups is not obvious, but both agree that *GAL1ucut* is used to attenuate *GAL1–10* expression.

Both groups argue that *GAL1ucut* acts *in cis*. First, Houseley *et al* formed a heterozygous diploid yeast strain in which one of the two *GAL1–10* loci lacked the *GAL1ucut* promoter. They observed no attenuation of *GAL1–10* expression in this strain. Second, both groups found that *GAL1ucut* RNA was stabilized by mutations affecting RNA degradation pathways used to target ncRNA, and Pinskaya *et al* showed that this stabilization had no effect on *GAL1–10* induction.

Earlier work has shown that Rpd3S histone-deacetylase complex is recruited to the body of protein-coding genes by H3K36-methylated nucleosomes (Lee and Shilatifard, 2007). This serves to inhibit intragenic transcription from cryptic promoters that might otherwise be activated by the passage of transcription elongation complexes. Houseley *et al* observed that histone modifications, which are the hallmarks of this Rpd3S-mediated intragenic repression mechanism, methylation of histone H3K36 and subsequent histone deacetylation, were found across the repressed *GAL1–10* locus. Furthermore, these marks were dependent on *GAL1ucut* transcription, and deletion of the Eaf3 subunit of the Rpd3S complex relieved glucose repression to a level similar to that observed when the *GAL1ucut* promoter was deleted.

Pinskaya *et al* also found a role for Rpd3S in *GAL1ucut* function. As H3K4-methylated histones can be recognized by proteins with the PHD domain (Mellor, 2006), Pinskaya *et al* systematically tested yeast strains lacking different PHD proteins for an effect on *GAL1–10* induction. They found that loss of Rco1, a component of the Rpd3S complex, mimicked the effects of *set1* mutations on *GAL1–10* expression. In addition, they used ChIP to show that Rpd3 is recruited to the repressed *GAL1–10* locus and that this is abolished by H3K4A and *set1* mutations. Interestingly, they did not observe any effect of a mutation deleting *SET2*, which encodes the H3K36 methyltransferase (Lee and Shilatifard, 2007), on *GAL1–10* induction kinetics, suggesting that the effects of *GAL1ucut* transcription might be mediated primarily through H3K4 methylation.

Although the different observations regarding the effects of *GAL1ucut* on induction kinetics and expression in low levels of glucose still need to be resolved, these papers indicate that cryptic transcription events might be used to set the chromatin-modification state of overlapping sequences. This regulatory strategy might be used more widely; both groups present preliminary observations, suggesting that ncRNA might regulate expression of other yeast genes. Furthermore, in higher eukaryotes, ncRNA are implicated in genomic imprinting (Edwards and Ferguson-Smith, 2007) and the function of some enhancers (Drewell *et al*, 2002). Perhaps these transcription events serve to establish epigenetic marks that influence the function of the overlapping regulatory elements.

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## Conflict of interest

The authors declare that they have no conflict of interest.

## References

Drewell RA, Bae E, Burr J, Lewis EB (2002) Transcription defines the embryonic domains of cis-regulatory activity at the Drosophila bithorax complex. *Proc Natl Acad Sci USA* **99:** 16853–16858

Edwards CA, Ferguson-Smith AC (2007) Mechanisms regulating imprinted genes in clusters. *Curr Opin Cell Biol* **19:** 281–289

Hirota K, Miyoshi T, Kugou K, Hoffman CS, Shibata T, Ohta K (2008) Stepwise chromatin remodelling by a cascade of transcription initiation of non-coding RNAs. *Nature* **456:** 130–134

Houseley J, Rubbi L, Grunstein M, Tollervey D, Vogelauer M (2008) A ncRNA modulates histone modification and mRNA induction in the yeast GAL gene cluster. *Mol Cell* **32:** 685–695

Jenks MH, O'Rourke TW, Reines D (2008) Properties of an intergenic terminator and start site switch that regulate IMD2 transcription in yeast. *Mol Cell Biol* **28:** 3883–3893

Kuehner JN, Brow DA (2008) Regulation of a eukaryotic gene by GTP-dependent start site selection and transcription attenuation. *Mol Cell* **31:** 201–211

Lee JS, Shilatifard A (2007) A site to remember: H3K36 methylation a mark for histone deacetylation. *Mutat Res* **618:** 130–134

Mellor J (2006) It takes a PHD to read the histone code. *Cell* **126:** 22–24

Pinskaya M, Gourvennec S, Morillon A (2009) H3 lysine 4 di-and and tri-methylation deposited by cryptic transcription attenuates promoter activation. *EMBO J* **28:** 1697–1707

Uhler JP, Hertel C, Svejstrup JQ (2007) A role for noncoding transcription in activation of the yeast PHO5 gene. *Proc Natl Acad Sci USA* **104:** 8011–8016

Yazgan O, Krebs JE (2007) Noncoding but nonexpendable: transcriptional regulation by large noncoding RNA in eukaryotes. *Biochem Cell Biol* **85:** 484–496



 