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 
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 PHOS gene and drives an antisense transcript is required for the normal kinetics of PHOS 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 mRNAs 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 transcription factor. A model was developed to annotate a set of divergent GAL1ucut promoters that are likely to be transcribed from the 3' end of GAL10 and to identify potential targets for GAL1ucut-directed regulation.

**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.

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