Minireview

RNA at the steering wheel
Sabine Schmitt and Renato Paro

Address: Zentrum für Molekulare Biologie Heidelberg (ZMBH), University of Heidelberg, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany.

Correspondence: Renato Paro. Email: paro@zmbh.uni-heidelberg.de

Published: 26 May 2006

Abstract

Expression of the genetic information encoded in our genomes is usually regulated by proteins interacting with the DNA. In some cases, however, noncoding RNAs transcribed from DNA control elements cooperate with histone-modifying enzymes to regulate gene expression, as has recently been shown for noncoding RNA originating from Polycomb- and Trithorax-group response elements.

The large majority of DNA sequences of most eukaryotic genomes do not possess any coding capacity. Nevertheless, many of these sequences are transcribed, generating a vast amount of noncoding RNA [1,2]. We are still far from fully understanding the role of this seemingly nonproductive large-scale transcription. In some situations, however, the process of intergenic transcription and the noncoding RNAs generated have been linked to the epigenetic control of gene expression. Intergenic transcripts appear to influence the way chromatin - the complex of DNA and the histones that package it - controls gene expression [3].

A prominent example of this type of epigenetic gene regulation is the mechanism of cellular memory, well studied in the fruit fly Drosophila melanogaster. Cellular memory is the inheritance of a cell’s gene-expression program through cell division, and this memory is a prerequisite for the maintenance of cell fates throughout development. In Drosophila, cis-regulatory DNA element called PRE/TREs consisting of Polycomb group response elements and Trithorax group response elements are central components of the mechanism for establishing cellular memory. Polycomb group (PcG) and Trithorax group (TrxG) proteins are targeted to these elements, promoting the formation of repressive (via PcG) or transcriptionally competent (via TrxG) chromatin structures [4]. In tissues where a PRE/TRE-controlled gene is active, transcription through the PRE and TRE sequences can be observed [5,6]. This suggests that the transcription acts as an anti-silencing mechanism, preventing the PcG proteins from repressing their target genes [7]. A recent study by Sanchez-Elsner et al. [8] now provides a first insight into the molecular basis of this anti-silencing mechanism. The authors show that the noncoding RNAs originating from a PRE/TRE stay associated with the element and anchor a histone-modifying enzyme, thus directly controlling the establishment of an epigenetically activated chromatin structure.

Sanchez-Elsner et al. [8] use the regulation of the Drosophila homeotic gene Ultrabithorax (Ubx) as a model to investigate the function of noncoding RNAs generated by transcription through PRE/TREs. Ubx specifies the identities of cells that give rise to the haltere and the third leg of the adult fly. Once established in appropriate segments during embryogenesis, the maintenance of Ubx expression depends on the continuous presence of functional TrxG proteins, in particular the histone methyltransferases ASH1 (Absent, Small and Homeotic discs) and TRX (Trithorax) [9]. The targeting of these proteins to Ubx depends on two PRE/TREs, one of which (bithorax, bx) is located within the third intron of Ubx, while the bithoraxoid (bxd) PRE/TRE lies 22 kb upstream of the Ubx promoter [10,11]. The bxd PRE/TRE is in close proximity to one of two characterized enhancers that drive Ubx expression in imaginal discs [10], the larval clusters of cells from which adult external structures will form during metamorphosis. Using the technique
of rapid amplification of cDNA ends, Sanchez-Elsner et al. [8] identified three capped and polyadenylated transcripts originating from the bxd PRE/TRE in sense orientation with respect to its target gene Ubx. These noncoding RNAs, named tre1 (949 nucleotides), tre2 (1,109 nucleotides), and tre3 (350 nucleotides), are transcribed in a pattern overlapping the expression of Ubx, which is highly active in third-leg and haltere imaginal discs, but absent, for example, in wing disc tissue. The histone methyltransferase ASH1 follows the distribution of noncoding wing disc tissue. The histone methyltransferase ASH1 leg and haltere imaginal discs, but absent, for example, in

How is the action of tre1, tre2 and tre3 RNAs confined to the bxd PRE/TRE? Sanchez-Elsner et al. [8] propose that these RNAs remain associated with the chromatin at their site of production, as they are only present in the chromatin-bound fraction of ASH1 and not in the soluble pool. The production and tethering of tre1, tre2 and tre3 RNAs to the chromatin is, however, independent of ASH1, as it is not inhibited by its absence. This is an intriguing result, because it also implies

![Figure 1](image.png)

**Figure 1**
RNA polymerase II (Pol II) transcription through the bxd PRE/TRE leads to the generation of noncoding tre1, tre2 and tre3 RNAs. These RNAs remain tethered to their site of origin, presumably via base pairing with the unwound single-stranded DNA. The histone methyltransferase ASH1 specifically interacts with tre1, tre2 and tre3 RNAs and thus becomes targeted to the PRE/TRE. Through its SET domain, ASH1 catalyzes the trimethylation (Me) of lysines 4 and 9 on histone H3, and of lysine 20 on histone H4. This leads to the activation of the chromatin structure and, as a consequence, the stimulation of Ubx expression.
that the intergenic promoters are regulated by a different set of factors than the target gene promoters, which require the continuous presence of functional ASH1 protein [9].

The most surprising finding from the work of Sanchez-Elsner et al. [8] is the observation that sense tre1, tre2 and tre3 transcripts expressed ectopically from a transgene can associate with the endogenous bxd PRE/TRE locus in trans. This results in the recruitment of ASH1 and, as a consequence, in the expression of Ubx in tissues in which this gene is normally kept silent. By contrast, antisense transcription of tre1, tre2 and tre3 RNAs does not have this activating effect, but as the authors mention, attenuates Ubx expression in its normal domains. Activating trans-effects for other PRE/TREs have not been observed so far. The PRE/TRE Frontoabdominal-7 (Fab-7) controls the homeotic gene Abdominal-B (AbdB); ectopic expression of Fab-7 from a transgene in all tissues throughout development does not affect the epigenetic state of the endogenous Fab-7 site, as the expression of the AbdB target gene remains normal [7]. In this case, however, large transcripts encompassing the entire PRE/TRE were produced, suggesting that the precise size and structure of the bxd transcripts used by Sanchez-Elsner et al. [8] were crucial for tethering ASH1 to the element.

The results presented by Sanchez-Elsner et al. [8] provide the first direct link between transcription through a PRE/TRE and the activation of chromatin via the interaction of noncoding RNAs with the histone methyltransferase ASH1 placing activating marks on histones. Once a PRE/TRE has been switched into the epigenetically activated mode, how is this information transmitted from mother to daughter cells during proliferation? Covalently modified histones presumably play a pivotal role in this process: during DNA replication, they may become distributed among daughter strands in the same semiconservative way as newly synthesized DNA [17]. In addition, modifications such as methylated lysine 4 of histone H3 (which is also set by ASH1 [18]) have been shown to survive mitosis, and might thus function to signal the activated state into the following cell cycle [19]. In the context of the bxd PRE/TRE, the recruitment of ASH1 to the chromatin depends crucially on the presence of tre1, tre2 and tre3 RNAs. This raises the intriguing possibility that noncoding RNAs, tethered to other target loci, might also serve as epigenetic ‘bookmarks’ to ensure the re-targeting of ASH1 to a PRE/TRE, thus ensuring the full reestablishment of epigenetic activation once the cells have traversed critical stages of the cell cycle.

Acknowledgements
We thank C. Beisel and M. Tariq for discussions and critical reading of the manuscript. S.S. is funded by a PhD fellowship of the Boehringer Ingelheim Fonds. The research of R.P. is funded by grants from the Deutsche Forschungsgemeinschaft.

References
1. Stolc V, Gauhar Z, Mason C, Halasz G, van Batenburg MF, Rifkin SA, Hua S, Herreman T, Tongpraisit W, Barbano PE, et al.: A gene expression map for the euchromatic genome of Drosophila melanogaster. Science 2004, 306:655-660.
2. Carninci P, Kasukawa T, Katayama S, Gough J, Frith MC, Maeda N, Oyama R, Ravasi T, Lenhard B, Wells C, et al.: The transcriptional landscape of the mammalian genome. Science 2005, 309:1559-1563.
3. Bernstein BE, Kamal M, Lindl-Blad-Toh K, Bekiranov S, Bailey DK, Huebert DJ, McMahon S, Karlsson EK, Kulbokas EJ, 3rd, Gingeras TR, et al.: Genomic maps and comparative analysis of histone modifications in human and mouse. Cell 2005, 120:169-181.
4. Garcia S, Paro R: Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. Annu Rev Genet 2004, 38:413-443.
5. Rank G, Prestel M, Paro R: Transcription through intergenic chromosomal memory elements of the Drosophila bithorax complex correlates with an epigenetic switch. Mol Cell Biol 2002, 22:8026-8034.
6. Bae E, Calhoun VC, Levine M, Lewis EB, Drewell RA: Characterization of the intergenic RNA profile at abdominal-A and Abdominal-B in the Drosophila bithorax complex. Proc Natl Acad Sci USA 2002, 99:16847-16852.
7. Schmitt S, Prestel M, Paro R: Intergenic transcription through a polycomb group response element counteracts silencing. Genes Dev 2005, 19:697-708.
8. Sanchez-Elsner T, Gou D, Kremmer E, Sauer F: Noncoding RNAs of trithorax response elements recruit Drosophila Ash1 to Ultrabithorax. Science 2006, 311:1118-1123.
9. Klymenko T, Muller J: The histone methyltransferases Trithorax and Ash1 prevent transcriptional silencing by Polycomb group proteins. EMBO Rep 2004, 5:373-377.
10. Chan CS, Rastelli L, Pirrotta V: A Polycomb response element in the Ubx gene that determines an epigenetically inherited state of repression. EMBO J 1994, 13:2553-2564.
11. Christen B, Bienz M: Imaginal disc silencers from Ultrabithorax: evidence for Polycomb response elements. Mech Dev 1994, 48:255-266.
12. Krajewska WA, Nakamura T, Mazo A, Canani E: A motif within SET-domain proteins binds single-stranded nucleic acids and transcribed and supercoiled DNAs and can interfere with assembly of nucleosomes. Mol Cell Biol 2005, 25:1891-1899.
13. Andersen AA, Panning B: Epigenetic gene regulation by non-coding RNAs. Curr Opin Cell Biol 2003, 15:281-289.
14. Park Y, Kelley RL, Oh H, Kuroda MI, Miller VH: Extent of chromatin spreading determined by roX RNA recruitment of MSL proteins. Science 2002, 298:1620-1623.
15. Akhtar A, Becker PB: Activation of transcription through histone H4 acetylation by MOF, an acetyltransferase essential for dosage compensation in Drosophila. Mol Cell 2000, 5:367-375.
16. Verdel A, Jia S, Gerber S, Sugiyama T, Gygi S, Grewal SIS, Mazoed D: RNAi-mediated targeting of heterochromatin by the RITS complex. Science 2004, 303:672-676.
17. Tagami H, Ray-Gallet D, Almouzni G, Nakatani Y: Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis. Cell 2004, 116:51-61.
18. Beisel C, Imhof A, Greene J, Kremmer E, Sauer F: Histone methylation by the Drosophila epigenetic transcriptional regulator Ash1. Nature 2002, 419:857-862.
19. Kouskouti A, Talianidis I: Histone modifications defining active genes persist after transcriptional and mitotic inactivation. EMBO J 2005, 24:347-357.