

RNA at the steering wheel

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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 (*bx*d) PRE/TRE lies 22 kb upstream of the *Ubx* promoter [10,11]. The *bx*d 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 *tre1*, *tre2* and *tre3* transcripts, associating with the *bxd* PRE/TRE only in third-leg and haltere discs. This results in the enrichment of ASH1-dependent histone modifications such as histone H3 lysine 4 methylation specifically in these tissues.

As ASH1 has been reported to bind single-stranded nucleic acids *in vitro* [12], Sanchez-Elsner *et al.* [8] went one step further and demonstrated that the targeting of ASH1 to the *bxd* PRE/TRE directly depends on its interaction with the noncoding *tre1*, *tre2* and *tre3* transcripts via its SET protein domain, which has been identified as having histone methyltransferase activity. ASH1 turned out to possess a surprising specificity in that it binds *in vitro* sense *tre1*, *tre2* and *tre3* RNAs, but not the antisense counterparts. The interaction was further confirmed by the finding that these RNAs also co-precipitate with chromatin-bound ASH1, and that the association of ASH1 with the *bxd* PRE/TRE is abolished by removing single-stranded RNAs (ssRNAs) through RNase digestion. The authors therefore propose that the specificity of ASH1 targeting to a PRE/TRE might rely on the formation of a complex consisting of ASH1, stretches of ssDNA exposed during ongoing transcription, and the noncoding RNAs generated by this process (Figure 1).

This targeting mechanism is reminiscent of the fly's strategy for compensating for the differences in X-chromosome gene dosage in males and females. In males, the single X chromosome becomes coated with the chromatin-modifying dosage compensation complex (DCC), consisting of six proteins and one of two redundant male-specific noncoding RNAs, *roX1* or *roX2* [13]. Assembly of the DCC presumably takes place on nascent *roX1* or *2* RNAs while they are still tethered to the DNA template on the X chromosome. From these initial nucleation sites, the DCC spreads along the remainder of the X chromosome via more than 30 additional 'entry sites' [14]. The outcome is the chromosome-wide modification of chromatin structure by the histone acetyltransferases present in the complex, inducing a twofold upregulation of transcription [15]. Although the initial recruitment of the DCC and ASH1 seem to rely on very similar mechanisms, the association of ASH1 with the chromatin has to remain fairly restricted to PRE/TREs, as extensive spreading of ASH1 into neighboring regulatory regions would have deleterious consequences. Indeed, over the past few years several other mechanisms for targeting chromatin protein complexes via RNA moieties have become evident, such as the tethering of RNA-induced transcriptional silencing (RITS) complexes to yeast heterochromatin [13,16].

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

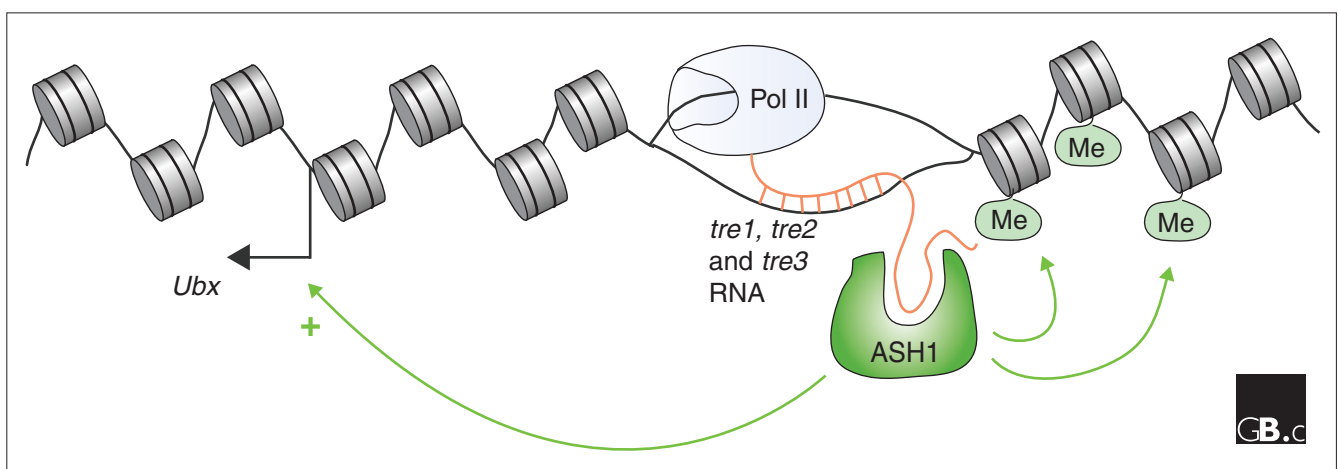


Figure 1

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.

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