Combing over heritable gene silencing

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Recent publications suggest that certain proteins of the Polycomb group interact with DNA methyltransferases. This may connect the two systems that are known to mediate somatic inheritance of states of gene expression during development.

The mechanisms that underlie the mitotic inheritance of gene expression patterns during development remain poorly understood, and the relationships between the various epigenetic systems are also unclear. Two recent papers report interactions between DNA methyltransferases (DNMTs) and Polycomb group (PcG) proteins. Viré *et al.*[1] report that a PcG protein EZH2 is required for DNA methylation of target sequences, and Hernández-Muñoz *et al.*[2] report that the DNA methyltransferase DNMT1 is required for proper localization of another PcG protein BMI1 to PcG bodies. The results are consistent with a model of sequential action of epigenetic modifiers in which PcG factors recruit DNA methyltransferases to establish heritable gene silencing. However, the biology of this system is complex, and much remains to be learned of the mechanisms of heritable gene silencing.

In both *Drosophila melanogaster* and mammals, PcG proteins mediate the heritable repression of Hox genes, as shown by homeotic transformations in embryos that lack specific PcG proteins[3]. To date, PcG repression represents the only known form of protein-mediated heritable gene silencing that is involved in normal development. The sequence of DNMT1 had suggested that proteins of the Polycomb or Trithorax groups (Trithorax group proteins antagonize the repressive effects of PcG proteins) might interact with DNA methyltransferases[4]. DNMT1 shares a zinc-binding cysteine-rich region with the mammalian homolog of *Drosophila* trithorax (the mammalian protein is known as ALL1, HRX or MLL), and DNMT1 contains bromo-adjacent homology (BAH) domains that are thought to be involved in the interaction with other chromatin proteins. However, direct evidence of interactions between DNA methyltransferases and PcG or Trithorax group proteins has been lacking.

Viré *et al.*[1] observed binding of EZH2 to DNMT1, DNMT3A and DNMT3B *in vitro* and in co-immunoprecipitation experiments in HeLa cell extracts. They then examined four EZH2 target promoters in the U2OS osteosarcoma cell line and report that EZH2 facilitated (i) the association of DNA methyltransferases with these promoters (shown by chromatin immunoprecipitation) and (ii) DNA methylation of 5′ CpGs (shown by bisulfite genomic sequencing). The observed EZH2-dependent recruitment of all three DNA methyltransferases (DNMT1, DNMT3A and DNMT3B) implies that all have similar functions. Indeed, although DNMT1 and DNMT3 family members are generally designated as maintenance and *de novo* methyltransferases, respectively, DNMT1 has well-established *de novo* activity[5] and all DNMTs have maintenance activity[6,7]. However, the authors also showed that knockdown of any one of the DNMTs by RNA-mediated interference resulted in derepression of *MYT1* (shown by reverse-transcription PCR), which implies that each of the DNMTs has a crucial nonredundant function. If the derepression reported to occur in the DNMT3A and DNMT3B RNA-mediated interference assays was caused by demethylation in DNA, this would seem to contradict the functions of these proteins in the animal, as there is no reported demethylation in DNA from *Dnmt3A*-null mice, which are of normal phenotype at term, and demethylation in *Dnmt3B*-null mice is limited to satellite DNA[8]. The human condition known as ICF syndrome is caused by loss-of-function mutations in *DNMT3B*, but ICF syndrome does not involve substantial developmental abnormalities[9].

The results reported by Viré *et al.*[1] of DNA methylation at four euchromatic loci are surprising. Three of the four loci in the study have CpG islands, clusters of CpG dinucleotides found in most promoter regions[10,11]. However, CpG islands are typically unmethylated[12] except in established lines of cultured cells, and Viré *et al.*[1] do not report the methylation status in normal tissues of the promoters of the genes studied. *In vivo* methylation patterns may be different in tissues and in permanent lines of cultured cells such as the U2OS cell line used by Viré *et al.*[1] DNA methylation is required for the silencing of imprinted genes, interspersed repeat sequences and genes on the inactive X chromosome, but the function of DNA methylation during development in the regulation of specific genes (including the four studied by Viré *et al.*[1]) is not established. The functional relevance of *in vitro* transcriptional-repressor activity of DNMTs can now be assessed in whole-organism studies (conditional alleles of all the effector genes discussed by Viré *et al.*[1] have been constructed), and methods more quantitative than single-point reverse-transcription PCR would help to clarify the magnitude of the effect.

Hernández-Muñoz *et al.*[2] studied the association of the PcG protein BMI1, a component of the Polycomb repressive complex 1 (PRC1) with PcG bodies, which are found at pericentric heterochromatin that is organized around satellite DNA. H3K27 methylation is a modification enriched in pericentric heterochromatin[13], and DNA is heavily methylated within these regions[14]. Hernández-Muñoz *et al.*[2] found that BMI1 localization at PcG bodies required DNMT1 and DNA methylation. In general, PcG bodies are more conspicuous in transformed cell lines[15], including the U2OS osteosarcoma cells used in this study. It will be important to determine whether BMI1 recruitment to other regions of the genome is also dependent on DNA methylation. Euchromatic regions of the mammalian genome are replete with interspersed repeat elements, which are silenced by DNA methylation[16]. Are PcG proteins also involved in silencing these transposable elements?

The data from these papers suggest a model in which PRC2 and PRC3 recruit DNMTs to establish DNA methylation marks, which in turn recruit PRC1 to maintain a repressive state. Each aspect of the model awaits testing in a developing organism. Evidence to date is not altogether consistent with a crucial role for these interactions. Mice deficient for Eed, a PRC2 component and Ezh2 binding partner, show loss of imprinting at a subset of paternally repressed loci, but DNA methylation is still present and methylation abnormalities have been reported only at imprinted loci[17]. These results indicate a relationship between PcG proteins and DNMTs that is not as simple as that offered in the schematic of Viré *et al.*[1], but the scheme does pose testable hypotheses.

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The current findings raise questions about the evolutionary conservation of the mechanisms by which PcG proteins mediate gene silencing. Although the PcG proteins are evolutionarily conserved, there may be differences among their mechanisms. In *Drosophila*, PcG proteins are recruited to their target loci by specific regulatory sequences termed Polycomb response elements (PRE). No PRE has been identified in mammals[3]. In *Drosophila*, the products of gap and pair-rule genes target large complexes of PcG proteins to Hox loci in the early embryo, but there is no evidence of a similar mechanism in mammals. In addition, the new findings imply that in mammals, gene silencing by PcG proteins involves DNA methylation, but this is highly unlikely in *Drosophila* owing to the absence of Dnmt1 and Dnmt3 homologs or any appreciable DNA methylation[18].

Finally, the findings by Viré *et al.*[1] and Hernández-Muñoz *et al.*[2] have potential implications for altered nuclear structure in cancer. Cancer-cell genomes often show global hypomethylation with hypermethylation at specific loci. Hypermethylation of the promoter regions of tumor-suppressor genes correlates with transcriptional repression, but it is not known which change occurs first[19]. It is possible that PcG recruitment of DNMTs could result in the observed hypermethylation. Gene repression at hypermethylated sequences could be mediated by PRC1 components, possibly including BMI1, and the loss of repressive states in hypomethylated regions could be due to the failure to recruit PRC1 components. Before these issues can be addressed, we will need a clear understanding of the functional significance of the interactions between PcG proteins and DNA methyltransferases.

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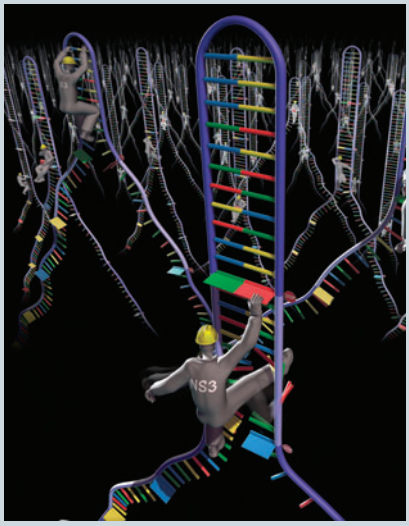
Helicase à go-go-go



Helicases use the energy derived from NTP hydrolysis to pry apart the hydrogen bonds between base pairs. These enzymes have been characterized in various ways, but most methods report only their bulk macroscopic properties. The ability to follow helicase activity at the single-molecule level can provide insights into how the hydrolysis of NTP is directly coupled to translocation, unwinding of the duplex and processivity.

In a recent study, Dumont *et al.* (*Nature* **439**, 105–108, 2006) use a single-molecule apparatus with high spatial and temporal resolution to examine the activities of hepatitis C virus NS3, an RNA helicase. Monomeric NS3 and ATP were placed in a chamber in which a 60-base-pair (bp) RNA hairpin with long single-strand arms was held by optical tweezers. Initially, NS3 binds the single-stranded region of the hairpin and translocates in the 3′→5′ direction. When NS3 encounters the duplex region and begins unwinding, the length of the RNA (reflected in the distance between the beads at the ends of the RNA) increases. Once the enzyme completes unwinding and dissociates, the hairpin re-forms, enabling multiple measurements from a single RNA molecule. In the illustration, NS3 is depicted as a workman climbing up a base-paired ladder, disrupting the steps as he moves.

Surprisingly, the unwinding traces are not monotonic but alternate between periods of rapid strand separation and pauses. This behavior is nonrandom; the rapid unwinding corresponds to a distance of 11±3 bp, which is consistent with the enzyme's step size as obtained from previous bulk measurements (the pause is shown as a stop sign every 11 steps). In rare instances, backward movement over the same distance was observed. As individual unwinding events were recorded, information about the steps involved in unwinding could be extracted. For example, exit from a pause requires ATP binding, as expected, but it also has an ATP-independent component, the nature of which remains to be determined. The rate at which unwinding occurs between pauses (with v_{max} = 51 bp s^{-1}) is dependent on ATP concentration. This result suggested that there are substeps within each 11-bp translocation, and careful examination of the tracings revealed the existence of 3.6-bp substeps, which has not been seen before. This observation is consistent with three cycles of ATP binding and hydrolysis per 11-bp unwinding event. Finally, processivity experiments indicated that NS3 is limited by its ability to translocate rather than its ability to separate strands and that reannealing competes with its translocation.

Overall, the kinetic properties determined by the single-molecule approach approximate the bulk measurements, with some of the differences attributed to the form (monomeric versus dimeric) of NS3 used. The authors propose a model for NS3 helicase activity, taking into account that there are two RNA-binding sites per monomer. In this model, one site is thought to contact the duplex 11 bp ahead of the enzyme after the paused enzyme binds ATP. The other (lagging) RNA-binding site is thought to act in duplex opening, translocating forward 3.6 bp during each of the three sequential ATP-binding and hydrolysis reactions. Other enzymes that disrupt base-pairing, such as polymerases, may also be studied using a variant of this assay. This may reveal some of the secrets of these molecular motors.

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