A Long-Distance Relationship between RNAi and Polycomb

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RNA interference (RNAi) pathways can result in sequence-specific transcriptional gene silencing on the level of chromatin. In this issue of *Cell*, Grimaud et al. (2006) reveal that the RNAi machinery is required for long-distance physical interactions between chromosomes mediated by the Polycomb repressive complex. These results suggest that the RNAi machinery may regulate higher-order nuclear organization.

RNAi is a highly conserved eukaryotic cellular mechanism that controls gene expression in a sequence-specific manner. RNAi is triggered by double-stranded RNA (dsRNA), which is cleaved by the RNase III enzyme Dicer into ∼21–25 nucleotide fragments. One RNA strand is selected as a guide and loaded into an RNA-induced silencing complex (RISC), which contains an Argonaute protein. RISC activity can lead to either the degradation or translational inhibition of a target mRNA homologous to the dsRNA.

RNAi can also promote transcriptional gene silencing via chromatin modification. The best characterized example comes from the fission yeast *Schizosaccharomyces pombe*, where the RNAi machinery is required for the formation of silent centromeric heterochromatin (Verdel and Moazed, 2005), which is necessary for proper chromosome segregation during mitosis and meiosis. dsRNAs transcribed from pericentromeric repeats are processed into small interfering RNAs (siRNAs) that are bound by the RNA-induced transcriptional silencing (RITS) complex. Similar to RISC in that it contains the single Argonaute protein in *S. pombe*, RITS associates with the site of transcription and recruits the histone H3 lysine 9 methyltransferase, Clr4, and the heterochromatin protein Swi6. An analogous system exists in *Drosophila*, requiring the helicase gene *spindle-E* and the Argonaute genes *aubergine* and *piwi* (Pal-Bhadra et al., 2004).

Distinct from its role in regulating gene expression, the RNAi machinery participates in at least one aspect of nuclear organization. *S. pombe* mutants in which the RNAi machinery is disrupted have defects in telomere clustering, a nuclear configuration thought to be important for chromosome alignment during cell division (Hall et al., 2003). Although the mechanism is not yet understood, short transcripts from subtelomeric regions have been identified in libraries of small RNAs isolated from *S. pombe* (Cam et al., 2005).

In this issue of *Cell*, Grimaud et al. (2006) present an intriguing new example of how the RNAi machinery contributes to nuclear organization—in this case, with a consequence on gene expression. Conserved throughout eukaryotes, Polycomb group (PcG) proteins participate in the transcriptional silencing of homeotic genes crucial for proper development. PcG proteins are recruited to DNA sequence elements termed PcG response elements (PREs) found at or near the promoters of the genes they repress. In *Drosophila*, the *Fab-7* DNA sequence harbors a PRE and a boundary element and regulates expression of *Abdominal-B*, located in the *bithorax* complex, a locus subject to extensive regulation throughout development. Like other PRE elements, insertion of *Fab-7* upstream of a reporter gene on a transgene is sufficient to recruit PcG complexes and results in transcriptional silencing. Interestingly, silencing increases when two copies of the transgene are present, a phenomenon referred to as pairing-sensitive silencing (PSS). Previously, the authors have shown that PSS is dependent on the endogenous copy of *Fab-7* and have demonstrated by fluorescence in situ hybridization (FISH) that *Fab-7* transgenes are juxtaposed physically to the endogenous locus even when located on different chromosomes (Bantignies et al., 2003). Chromosomal pairing is dependent on at least one PcG protein, suggesting the importance of higher-order PcG complex interactions in the process of silencing.

In this study, the authors demonstrate that certain components of the RNAi machinery—*dicer-2*, one of two Dicer genes in *Drosophila*, as well as the Argonaute genes *piwi*, *argonaute1*, and *aubergine*—are required for efficient PSS and long-distance chromosome interactions of the *Fab-7* PRE element. Moreover, *spindle-E* mutants do not affect PSS or chromosomal pairing, and the RNAi machinery does not affect extensively the recruitment of PcG proteins to the *Fab-7* PRE or other PRE sites throughout the genome. These results suggest that this RNAi-dependent mechanism

differs from that of centromeric heterochromatin formation.

In embryonic cells, PcG proteins reside in approximately 50 to 100 nuclear foci termed PcG bodies, which have been proposed to be concentrated areas of transcriptional repression possibly containing multiple PcG complexes bound to distinct PREs. Using FISH coupled with immunofluorescence, Grimaud et al. (2006) provide compelling evidence that PcG bodies do indeed correspond to PRE-containing loci. Furthermore, the authors determine that several RNAi proteins also localize to distinct nuclear foci, and a subset of these foci colocalize with PcG bodies, raising the possibility of physical association between PcG proteins and the RNAi machinery. Although extensive biochemical studies of PcG complexes have not identified associated RNAi components, this does not preclude the possibility of transient physical interactions between PcG proteins and the RNAi machinery. Given their distinct localization patterns, an important future question is whether the individual RNAi proteins reside in the same or different nuclear compartments, which may provide insight into their specific nuclear functions. Recently, PcG bodies have been suggested to act as centers for sumoylation; in fact, the human PcG protein Pc2 is a SUMO E3 ligase (Kagey et al., 2003). Additionally, the *Caenorhabditis elegans*-specific PcG protein SOP-2 is modified by sumoylation and is capable of binding RNA (Zhang et al., 2004a, 2004b). It is therefore tempting to speculate that a functional relationship may exist between PcG-dependent sumoylation and RNAi.

In support of a direct role for the RNAi machinery in PSS and long-distance chromosome interactions, the authors show that *Fab-7* is transcribed in both sense and antisense directions, providing a potential source of dsRNA. In addition, 21–23 nucleotide RNAs corresponding to *Fab-7* are detected, and their levels decrease in *piwi* and *dicer-2* mutants. Unexpectedly, these RNAs are produced from a region of *Fab-7* that corresponds not to the PRE itself but the nearby boundary element present in the transgene. These results are consistent with the finding that PcG protein recruitment to PREs is mostly unaffected and raises the possibility that the boundary element or its associated proteins may be involved in this RNAi-dependent mechanism. Given that insertion of multiple transgenes into the genome can result in their silencing as well as silencing of a homologous gene by a mechanism referred to as cosuppression, it is important to note that transcription of *Fab-7* and small RNAs are only detectable when the *Fab-7* transgene is present. In at least one case in *Drosophila*, silencing of multiple transgene copies is PcG dependent, and recruitment of PcG proteins to these transgenes is observed (Pal-Bhadra et al., 1997). The RNAi machinery and the production of siRNAs have also been implicated in cosuppression in a number of organisms.

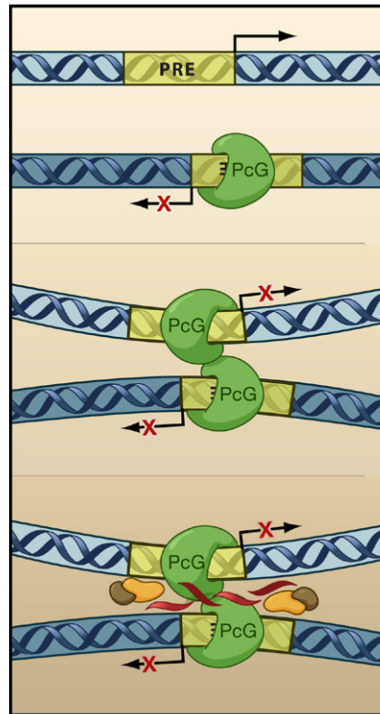

Figure 1. Model for How RNAi Promotes PcG-Dependent Long-Distance Interaction between PRE-Containing Loci

(Top) One PRE-containing locus is transcriptionally active, whereas a second PRE-containing locus on a different chromosome is bound by a PcG complex (green) and is transcriptionally silent. No interaction between the two loci is observed.

(Middle) Once both PRE-containing loci become transcriptionally repressed by PcG complexes, they are capable of establishing a long-distance interaction in the context of a PcG body.

(Bottom) After pairing is established, sense and antisense transcription in the vicinity is stimulated, and the RNAi machinery (orange) associated with the PcG body produces siRNA (red) from dsRNAs. These siRNAs are bound possibly by PcG proteins or unknown proteins (brown) to stabilize chromosomal pairing and to maintain silencing of both loci.

A critical issue to address is whether RNAi-dependent long-range chromosomal interactions apply only to transgenes or also exist for natural PRE elements, most of which share limited homology. Strikingly, the authors demonstrate that two distant PRE-containing loci on the same chromosome colocalize frequently to the same PcG body and that RNAi mutants have a modest effect on these long-range interactions. The presence of a transgene has no effect on this pairing. Furthermore, pairing of these PRE-containing loci is observed only in a tissue where both loci are transcriptionally repressed by PcG proteins and not in a tissue in which one locus is transcriptionally active. These results suggest that RNAi may control endogenous as well as transgenic long-distance interactions mediated by PcG complexes.

The authors observe that chromosomal pairing in RNAi mutants is disrupted in larval but not earlier embryonic stages, and they conclude that RNAi is not required for the establishment of chromosomal pairing but is necessary for its maintenance throughout development. However, one possible explanation for this result is that the maternal contribution of RNAi proteins or transcripts must be depleted in the RNAi mutants as development progresses before effects on chromosomal pairing are apparent. The finding that only later stages are affected may explain why RNAi mutants do not display

homeotic developmental phenotypes or overall disruption of PcG body formation.

The Grimaud et al. (2006) study raises a number of mechanistic questions regarding how the RNAi machinery promotes chromosomal pairing of PRE-containing loci. First, it remains an open question as to how PcG complexes associate to form nuclear bodies. As there is limited sequence homology among PRE elements, it seems likely that protein-protein interactions between PcG proteins or associated factors mediate formation of these complexes. A second question is whether PcG bodies are static or dynamic structures. Because PcG proteins dissociate from chromatin during mitosis, these contacts would have to be reestablished each cell cycle. Pairing of two endogenous PRE-containing loci to the same PcG body was found to occur in approximately one-fourth of cells examined, suggesting that any given PRE could associate with different PREs situated throughout the genome. Grimaud et al. (2006) propose that, once long-distance contact is established, the increased local concentration of PRE-containing loci in PcG bodies that are associated with the RNAi machinery could stimulate transcription of dsRNA and siRNA production (Figure 1). These RNAs are postulated to act as a molecular glue that stabilizes interactions between PcG complexes to promote transcriptional silencing. Determining the fate of these siRNAs and what proteins may recognize them should elucidate this fascinating new mechanism by which the RNAi machinery affects nuclear organization.

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TOPping up ATR Activity

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The nuclear protein kinase ATR is a key regulator of genome integrity that functions at checkpoints for damaged or incompletely replicated DNA. In this issue of *Cell*, Kumagai et al. (2006) shed light on the molecular mechanism that controls ATR. They report that a physical interaction between ATR and a distinct domain of TopBP1 greatly enhances ATR kinase activity.

Maintenance of genomic integrity is among the fundamental requirements of life, guarding against developmental errors as well as devastating diseases such as cancer (Kastan and Bartek, 2004). All eukaryotes share a network of cellular pathways that sense and signal diverse types of DNA damage or the presence of incompletely replicated DNA and through downstream effectors respond by cell cycle arrest, DNA repair, or the elimination of damaged cells by apoptosis. Central to the DNA-damage response are two phosphoinositide 3-kinase related kinases: ATM (ataxia-telangiectasia mutated) and ATR (ataxia-telangiectasia and Rad3-related; Bakkenist and Kastan, 2004). Despite some crosstalk between these two upstream kinase modules, their labor is largely divided, in that ATM responds primarily to DNA double-strand breaks (DSBs), whereas ATR is crucial in the response to DNA replication stress and a broader spectrum of DNA lesions. Given their pivotal roles in