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Review

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Noncoding RNAs and Gene Silencing

Mikel Zaratiegui,[1,2] Danielle V. Irvine,[1,2] and Robert A. Martienssen[1,*]
[1] Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724, USA
[2] These authors contributed equally to this work.
*Correspondence: martiens@cshl.edu
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Noncoding RNA has long been proposed to control gene expression via sequence-specific interactions with regulatory regions. Here, we review the role of noncoding RNA in heterochromatic silencing and in the silencing of transposable elements (TEs), unpaired DNA in meiosis, and developmentally excised DNA. The role of cotranscriptional processing by RNA interference and by other mechanisms is discussed, as well as parallels with RNA silencing in imprinting, paramutation, polycomb silencing, and X inactivation. Interactions with regulatory sequences may well occur, but at the RNA rather than at the DNA level.

Introduction

RNA-mediated gene regulation has its origins in the earliest days of molecular biology, when it was first proposed that sequence-specific noncoding RNA (ncRNA) might interact with promoters to regulate genes (Britten and Davidson, 1969; Jacob and Monod, 1961). Forty years later, our understanding of gene regulation, chromosome organization, and epigenetic mechanisms has undergone something of a revolution with the discovery of RNA interference (RNAi). Important clues to ncRNA regulatory mechanisms came from homology-dependent gene silencing in plants, which can be triggered by transgenes and recombinant viruses (Matzke and Birchler, 2005). Importantly, posttranscriptional (PTGS) and transcriptional gene silencing (TGS) were often intermingled, and at least in some cases DNA methylation seemed to depend on RNA (Wassenegger, 2005). Studies in the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, fungi, and mammalian cells, as well as in plants, allowed genetic and biochemical approaches to uncover transcriptional silencing mechanisms involving RNAi, histone, and DNA modifications (Matzke and Birchler, 2005).

Over the last few years it has become apparent that eukaryotic cells use these mechanisms for a host of functions, including chromosome organization and gene regulation. Transposable elements (TEs) are a major component of most genomes and perhaps a defining component of heterochromatin, and are targeted by these mechanisms. Here we review the proposition that transcriptional control by ncRNA can be mediated via transposons and heterochromatic repeats that are cotranscribed with the target gene, and requires processing of readthrough transcripts by RNAi and other RNA-processing mechanisms. X inactivation, dosage compensation, imprinting, and polycomb silencing all involve ncRNA, and parallels can be drawn even though a role for RNAi has not been demonstrated (see also the Review by P.K. Yang and K.I. Kuroda, page 777 of this issue and the Review by B. Schuettengruber et al., page 735 of this issue).

Heterochromatic Silencing and ncRNA

Heterochromatin has been ascribed important functions in gene regulation and chromosome architecture since the early days of cytogenetics. Heterochromatin decorates centromeres, telomeres, and nuclear organizers and can silence nearby genes in an unstable fashion leading to position effect variegation (PEV) (Lippman and Martienssen, 2004). At the sequence level, heterochromatin is composed of highly repetitive DNA with little or no coding potential, which sets it apart from euchromatin, its gene-rich cousin. These contrasts parallel differences in transcriptional activity: euchromatin is actively transcribed, whereas heterochromatin was thought to be largely silent. Biochemically, heterochromatic DNA is often methylated, while histones are hypoacetylated and methylated on residues associated with transcriptional repression (e.g., H3K9me). These epigenetic marks determine its tight packaging and recruit heterochromatin protein 1 (HP1), a suppressor of PEV. Centromeric heterochromatin is present in organisms as distantly related as fission yeast, plants, insects, and mammals (Figure 1) (Dawe, 2003), as reviewed by C.A. Morris and D. Moazed, page 647 of this issue. A notable exception is the budding yeast, whose centromeres are much reduced. Coincidentally, yeast has lost histone H3K9 methyltransferases and HP1, as well as all trace of the RNAi machinery.

RNAi and Fission Yeast Centromeric Heterochromatin

In fission yeast, the three centromeres share a common general structure: the outer centromeric repeat sequences (*dg* and *dh*), together with the innermost *imr* repeats, symmetrically flank a central region, which is enriched for the centromere-specific histone H3 homolog CENP-A (Figure 1) (Ekwall, 2004). The outer pericentromeric repeats are heterochromatic and coated with the HP1 homologue Swi6 (see Table 1) (Cam et al., 2005), which is required for retention of cohesin in metaphase chromosomes. Unexpectedly, the pericentromeric repeats, hitherto regarded

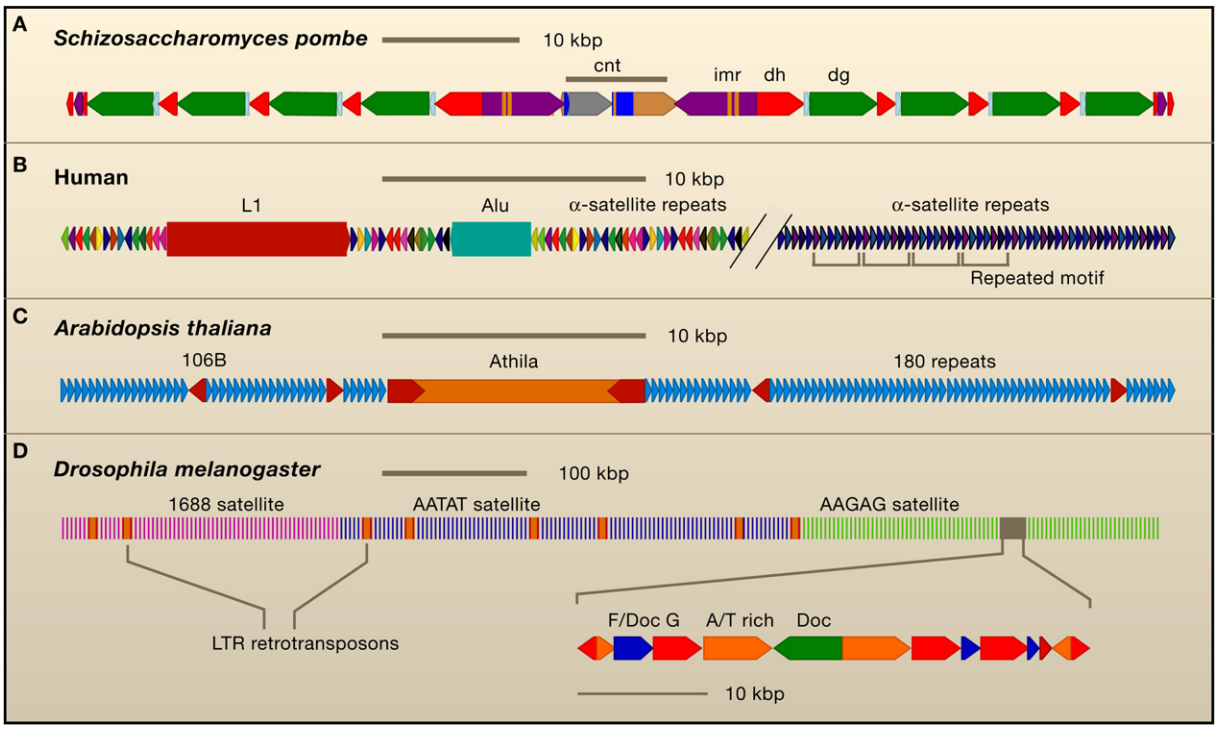

Figure 1. Repeat Structure of the Centromere in Different Organisms

(A) The chromosome III centromere of *S. pombe* consists of repeated dg and dh motifs around a unique central region flanked by inverted imr repeats.

(B) Human centromeres are mainly composed of tandem arrays of α-satellite DNA repeats that span several megabases. A central region (right section) is composed of chromosome-specific higher order motifs composed of related α-satellite repeats, and is flanked by more heterogeneous and disordered repeats interspersed by LINEs and SINEs (left section) adapted from Schueler and Sullivan (2006).

(C) *Arabidopsis* centromeres display long arrays of 180 type satellites interspersed by LTR retrotransposons and LTR-derived 106B repeats (adapted from May et al. [2005]).

(D) *Drosophila* centromeres are composed of long homogeneous satellite regions, with inserted LTR retrotransposons (red and yellow sections), that flank a complex region composed of fragments of TEs arranged in direct and inverted orientations (adapted from Sun et al. [2003]).

as silent, are transcribed from antiparallel promoters by RNA polymerase II (Pol II) and subsequently processed by the RNA-dependent RNA polymerase (RdRP) Rdp1, and Dicer (Volpe et al., 2002). Small RNA duplexes of 22–24 nucleotides are unwound and assembled into the RNA-induced transcriptional silencing (RITS) complex, which is composed of *S. pombe*'s single Argonaute protein (Ago1), Tas3, and the chromodomain protein Chp1 (Verdel et al., 2004). The H3K9 methyltransferase Clr4 is necessary for the processing of the pericentromeric transcripts into siRNA (Verdel and Moazed, 2005). Importantly, loss of RNAi leads to loss of silencing and H3K9me2 from reporter genes embedded within the pericentromeric repeats (Martienssen et al., 2005). The degree of silencing of transgene insertions in the pericentromeric heterochromatin seems to correlate with their positions relative to the heterochromatic transcripts. Ironically, insertions within the transcribed regions are most strongly silenced, and are cotranscribed with the pericentromeric repeat (Irvine et al., 2006).

Biochemical analysis of Rdp1 led to the identification of a second effector complex, RNA-directed RNA polymerase complex (RDRC) that has RNA-directed RNA polymerase activity and physically interacts with components of the RITS complex. The RDRC complex contains Rdp1 and two conserved proteins, Hrr1, an RNA helicase, and Cid12, a member of the poly(A) polymerase family. Both the RITS and RDRC complexes localize to the outer centromeric repeats (Verdel and Moazed, 2005). The chromodomain of Chp1 binds H3K9me2 and this histone modification is necessary for RITS targeting to chromatin, explaining its dependence on Clr4. All of these factors are necessary for siRNA accumulation and heterochromatin modification indicating a close cooperation between the two complexes.

In PTGS, Argonaute proteins bind siRNA via the conserved PAZ domain, which is used as a guide by the PIWI domain to "slice" target messages complementary to the bound siRNA (Song and Joshua-Tor, 2006). Interestingly, the slicing activity of Ago1 is necessary for RDRC localization and heterochromatin assembly in fission yeast, but not for RITS recruitment to the outer centromeric repeats, suggesting that RDRC is recruited to and acts on sliced transcripts (Irvine et al., 2006). The

Table 1. Conserved Proteins Involved in RNAi-Mediated Epigenetic Silencing

S. pombe	*A. thaliana*	*C. elegans*	*Drosophila*	*H. sapiens*	*Neurospora*	*Tetrahymena*
Dcr1	DCL1–DCL4	Dcr-1	Dcr1 and Dcr2	DCR-1	Dcl-2 and Sms-3	Dcl1p
Ago1	AGO1–AGO10	Rde-1, Alg-1, and Alg-2	Ago1 and Ago2	AGO-1–AGO-4	qde-2 and Sms-2	
	–	PRG-1 and PRG-2, and 19 others	Aubergine, Ago3, and Piwi	Piwi-1–Piwi-4		Twi1p
Tas3	–	–	–	–	–	–
Chp1	CMT3	–	HP1	HP1a, b, g	HP1	Pdd1p and Pdd3p
Rdp1	RDR1–RDR6	Ego-1 and Rrf-1–Rrf-3	–	–	qde-1 and sad-1	Rdr1p
Hrr1	SGS2/SDE3	ZK1067.2	GH20028p	KIAA1404	–	–
Cid12	–	Rde-3 and Trf-4	CG11265	POLS	–	–
Swi6	LHP1 or CMT3	Hpl-1, Hpl-2, and F32E10.6	HP1	HP1-α, HP1-β, and HP1-γ	HP1	–
Clr4	SUVH2–SUVH6	–	Su(var)3-9	SUV39H1 and SUV39H2	DIM-5	–
Rik1	DDB1	Ddb-1	Ddb1	DDB1	–	–
Cul4	CUL4	Cul-4	Cul4	CUL4	–	–
Clr8 (Raf1/Dos1)	–	–	–	–	–	–
Clr7 (Raf2/Dos2)	–	–	–	–	–	–
Sir2	SIR2	Sir2-1	Sir2	SIRT1	–	–
Eri1	ERI1	Eri-1	CG6393	THEX1	–	–

chromatin modification machinery includes a network of interactions between Clr4 and the silencing protein Rik1, which has homology with both cleavage and polyadenylation specificity factor (an RNA-binding protein) and DNA damage binding factor Ddb1. Like DDB1 complexes, the Rik1 complex includes the cullin ubiquitin ligase Pcu4, and two novel proteins of unknown function: Clr8 (a.k.a. Raf1/Dos1) and Clr7 (aka Raf2/Dos2). The ubiquitination target is unknown, but this activity is necessary for heterochromatin (Horn et al. [2005], among others). Interaction with the RNAi machineries could recruit the Clr4/Rik1 complexes or else the nascent RNA itself could serve as a scaffold for recruitment (Grewal and Moazed, 2003; Martienssen et al., 2003), coupled to its processing.

Association of H3K9me2 and RITS with centromeric reporter transgenes is exclusively dependent on RNAi. The centromeric repeats, on the other hand, partially retain H3K9me2 and do not lose RITS in the absence of siRNA (Irvine et al., 2006; Sadaie et al., 2004). The H3K14-specific histone deacetylase (HDAC) Clr3 is necessary for this RNAi-independent pathway of H3K9 methylation at pericentromeric repeats (Yamada et al., 2005), but single *clr3* mutants only have defects in histone modification at the mating type (MT) locus (Jia et al., 2004b). The MT locus includes a region (*cenH*) highly homologous to the outer centromeric repeats. This region is transcribed in *clr4* mutants and yields siRNA (Cam et al., 2005; Thon et al., 2005). However, in the absence of RNAi, or in the absence of *cenH*, MT silencing is maintained by an independent pathway mediated by the CREB-related Atf1/Pcr1 DNA-binding complex (Jia et al., 2004a). This redundancy, as well as interaction between Atf1/Pcr1 and the class I HDAC Clr6 (Kim et al., 2004), may account for the role of RNAi in MT silencing in the presence of HDAC inhibitors (Hall et al., 2002). It has also been proposed that RNAi may have a specific role in establishment of silencing, rather than maintenance, based on this effect (Hall et al., 2002). Heterochromatic transcriptional silencing is therefore maintained by the coordinated function of both DNA-binding proteins and RNAi processing.

Centromeric Heterochromatin in Other Organisms

The centromeres of higher organisms such as insects, mammals, and plants are considerably larger than those of *S. pombe* and are composed of different types of repeats (Figure 1). In mammals, centromeres are composed of megabase size arrays of 171 bp α-satellite repeats interrupted by blocks of LINE elements. RNA and the RNAi machinery have been implicated in the assembly of mammalian centromeric heterochromatin. The localization of mammalian HP1-α to centromeres requires unidentified RNAs (Maison et al., 2002), and it has been shown that transcripts from mouse minor satellite repeats accumulate upon stress or differentiation and localize to

chromocenters (Bouzinba-Segard et al., 2006). Overexpression of noncoding centromeric transcripts impairs centromere function. LINE elements could also contribute to the pool of RNA and are regulated in part by Dicer (Yang and Kazazian, 2006). Importantly, conditional inactivation of Dicer in chicken cells harboring a copy of human chromosome 21 results in the accumulation of α-satellite transcripts (Fukagawa et al., 2004). In a similar dicer knockdown experiment in mouse ES cells, transcripts derived from mouse minor and major satellite repeats were also upregulated and their processing into siRNA was lost (Kanellopoulou et al., 2005). This upregulation was associated with delocalization of heterochromatin proteins and alterations in histone modifications and DNA methylation, implicating the RNAi machinery in pericentromeric heterochromatin assembly in mammals.

Drosophila pericentromeres are composed of very short satellite repeats interrupted by Ty3/gypsy class elements, achieving sizes of hundreds of kilobases and spanning most of the small heterochromatic chromosome 4. Transgene insertions within or juxtaposed to the heterochromatic regions exhibit PEV (Talbert and Henikoff, 2006), like the transgene insertions in fission yeast heterochromatin. Mutant alleles of the Argonaute homologs *piwi*, *aubergin*e, and the helicase *homeless* suppress silencing of these transgene insertions and proper HP1 localization, resembling suppressors of PEV (Pal-Bhadra et al., 2004). *Ago2* mutant embryos exhibit defects in centromeric chromatin structure, such as CID/CENP-A and HP1 delocalization (Deshpande et al., 2005). Similarly, defects in centromeric heterochromatin assembly are displayed in *Drosophila dfmr1* mutants (Desphande et al., 2006). dFMR1, related to the Fragile X mental retardation protein in humans, is found in a complex with *Ago2* (Ishizuka et al., 2002).

The centromeres of plants are composed of tandem arrays of thousands of short (100–400 bp) satellite repeats and retroelements. In *Arabidopsis*, these repeats include the satellite cen180 (170–180 bp), 106B repeats (400 bp), which are derived from Athila2 retroelement LTRs, and full-length Athila (gypsy) retrotransposons. The satellite repeats are methylated by the DNA methyltransferases (DNMTs) MET1 and CMT3 (Cao et al., 2003). These repeats are transcribed, and transcripts remain in the nucleus and are processed into siRNA with the participation of RDR2 and DCL3 (see Table 2) (May et al., 2005). Transcripts from a subtype of cen180 repeats are silenced by type I histone deacetylase HDA6 and by the DNMT MET1, and appear to initiate within Athila retrotransposons, while other RNAi-dependent cotranscripts initiate in the repeats. This cotranscription of repeats and retrotransposons is reminiscent of cotranscription between repeats and reporter genes in fission yeast.

In summary, the function of heterochromatin in many eukaryotes depends on nonprotein coding and transposon-derived transcripts processed by RNAi. Heterochromatin modification machineries are recruited in part by this processing. In conjunction with RNAi-independent chromatin modification, dynamically silenced and condensed heterochromatin provides the structural requirements for centromere formation and function. The widespread presence of TEs at eukaryotic centromeres may reflect their importance in heterochromatin formation or function. Alternatively, the centromeres can be viewed as ''transposon farms,'' recruiting and domesticating retrotransposons that integrate within them.

RNA-Mediated Silencing of Transposable Elements

Barbara McClintock deduced the existence of transposable, or ''controlling'' elements in maize more than half a century ago (McClintock, 1951). Since then TEs have been found in every eukaryotic genome, and in many cases constitute a majority of the sequence content. Active transposition is potentially mutagenic, and transpositions occurring in the germline are transmitted to the offspring, with cumulative defects in subsequent inbred generations. Additionally, the insertion of a TE can affect the proper expression and processing of nearby genes (Lippman et al., 2004). The presence of TEs thus influences development, for example at the *agouti* locus in mice (Morgan et al., 1999). LINE elements have been proposed to act as ''booster regions'' to silence genes on the inactive X chromosome (see P.K. Yang and K.I. Kuroda, page 777) and could use a similar mechanism. LINE activity can have unpredictable consequences and could even contribute to behavior by transposition in the central nervous system (Muotri et al., 2005).

At the macroscopic level, polymorphic TE distribution and silencing could account for an unknown amount of intraspecies variability. In its most extreme form, unleashed transposition in the germline could be responsible for speciation itself. This was first proposed for hybrid dysgenesis in *Drosophila* and has recently been demonstrated in sunflower (Ungerer et al., 2006). TEs, and their epigenetic baggage, are therefore major players in evolution, and it is no surprise that genomes have acquired mechanisms to regulate TEs, especially in the germline. As a consequence, the vast majority of TEs are silent and inactive. This silencing is brought about by both transcriptional and posttranscriptional mechanisms.

Transposons were first discovered in plants, and TE regulation is well understood in plant models such as *Arabidopsis thaliana* (see Table 2). Plant TEs are methylated and associated with H3K9me2 and small RNA, indicating RNAi, DNA, and histone modification pathways are responsible for TE silencing. Twenty-four nucleotide siRNAs are derived from most TEs by DCL3 and depend on RdRP action by RDR2. However, most TEs are not upregulated in *dcl3* and *rdr2* mutants, while many are reactivated (and siRNA is lost) in mutants of the CpG DNMT *met1* or the Swi2/Snf2 ATPase *ddm1* (Lippman et al., 2003). Transcriptional silencing downstream of DNA methylation requires the HDAC *hda6/sil1* and a distantly related Swi2/Snf2 ATPase, MOM1 (Mittelsten Scheid et al., 2002). When *met1* and *ddm1* are backcrossed to wild-type strains, most TEs remain active (resembling ''presetting''

Table 2. *Arabidopsis* Proteins Implicated in RNA-Directed DNA Methylation

Protein	Locus Name	Function
AGO4	Argonaute 4	RNAi component
RDR2	*R*NA-*d*irected *R*NA polymerase	RNAi component
DCL2–DCL4	Dicer	RNAi components
MET1	Methyltransferase 1	CpG maintenance DNA methylation
CMT3	Chromomethylase 3	CpNpG maintenance DNA methylation
DDM1	*D*ecrease in *D*NA *m*ethylation	SNF2-like chromatin remodeler
DRM1 and DRM2	*D*omains *r*earranged *m*ethyltransferase	de novo CpN DNA methylation
SUVH4 (KYP)	KRYPTONITE	H3K9 methyltransferase
HDA6 (SIL1)	Histone deacetylase 6	Histone deacetylase
DRD1	*d*efective in *R*NA-*d*irected DNA methylation 1	SNF2-like chromatin remodeler
NRPD1a (SDE4)	*S*ilencing *d*efective 4	RNA Pol IVa component
NRPD2a (DRD2)	*d*efective in *R*NA-*d*irected DNA methylation 2	RNA Pol IVa and IVb component
NRPD1b (DRD3)	*d*efective in *R*NA-*d*irected DNA methylation 3	RNA Pol IVb component

of TEs in maize). The few TEs that are resilenced do not lose siRNA in the mutant, suggesting that siRNAs are needed in *cis* to initiate transcriptional silencing. Consistent with this model, *hda6* mutants lose TE silencing but not siRNA, and silencing is readily reestablished after a backcross (Lippman et al., 2003).

Non-CpG methylation is mediated by the redundant DNMT3 homologs DRM1 and DRM2, and by the CpNpG methyltransferase CMT3, which may act downstream of RNAi (Lippman et al., 2004). The 24 nt siRNAs are bound by AGO4, and in *ago4* mutants CpNpG and CpNpN methylation is lost from some TEs, which also lose H3K9me2, mediated by the Su(Var)3-9 homolog KYP/SUVH4 (Chan et al., 2006; Ebbs and Bender, 2006; Lindroth et al., 2004; Tran et al., 2005). H3K9me2 can, in collaboration with H3K27me2, recruit CMT3 and contribute to DNA methylation in this way (Lindroth et al., 2004). TE activation is greatly enhanced in *cmt3 met1* double mutants (Kato et al., 2003), suggesting that RNAi-dependent and RNAi-independent mechanisms might contribute to TE silencing and methylation in a partially redundant fashion (Lippman et al., 2003) reminiscent of the relationship between RNAi and HDACs in heterochromatic silencing in *S. pombe*.

Like DNA methylation, redundant genes control RNAi and histone modification in plants. For example, while relatively few TEs are activated in *dcl3* mutants, *dcl3 dcl2* and *dcl3 dcl4* double mutants have phenotypic defects on inbreeding that resemble *ddm1*, indicating they may regulate TE silencing (Gasciolli et al., 2005). *rdr2* mutants do not have these defects, despite almost complete loss of detectable 24 nt siRNA (Lu et al., 2006), indicating perhaps that amplification of siRNA by *rdr2* is not an absolute requirement for TE silencing in plants. In maize, silencing of the MuDR TIR class transposon can be induced by an endogenous inverted repeat that generates siRNA. Silencing is lost in mutants in the maize homolog of *RDR2* (*mop1*—described in detail below), but this loss occurs stochastically during development and requires inbreeding for full effect (Slotkin et al., 2005). Histone H3K9 methyltransferases SUVH4, SUVH5, and SUVH6 are also redundant in *Arabidopsis*, with individual enzymes specialized for TEs, inverted repeats, and centromeres (Ebbs and Bender, 2006). While triple mutants have substantial reduction in H3K9me2, H3K9me1, and CpNpG methylation, additional SUVH homologs have roles in heterochromatic silencing, and cause dramatic phenotypes when overexpressed, suggesting that redundancy in this pathway is even more widespread (Fischer et al., 2006).

TE silencing in mammals is also associated with DNA methylation, mediated by the de novo Dnmt3 family (related to DRM1/2) and the maintenance Dnmt1 (related to MET1) in the male germline (Bestor and Bourc'his, 2004). siRNAs derived from several TEs are detectable in mouse oocytes (Watanabe et al., 2006) and LINE element (L1)-derived siRNAs are detectable in some human cell lines (Yang and Kazazian, 2006). Interestingly, knockdown of DCR-1 in this system elicited a small increase of endogenous L1 transcripts, but the increase in transposition of an exogenous L1 TE was much higher. Perhaps in mammals as in plants, RNAi has an early role in silencing later reinforced by DNA methylation.

In model organisms without DNMT1 or DDM1 homologs, such as *C. elegans* and *D. melanogaster*, RNAi silences TEs in the germline, so that over generations this leads to a reduction in TE copy number and effective silencing in the adult. But at least in *C. elegans*, TEs are highly active in somatic cells, which are notoriously capable of RNAi. This indicates that germline cells must express specific silencing factors required for TE silencing. Large-scale reverse genetic screens have been performed for mutants defective in RNAi on the one hand, and transposon activity on the other (Kim et al., 2005;

Vastenhouw et al., 2003). As expected, the genes in each screen overlap, but only one Argonaute gene (*ppw-2*) is required for TE silencing on its own. However, redundancy is a major problem: for example, the two *piwi* homologs in *C. elegans* are genetically redundant (Cox et al., 1998).

In *Drosophila*, Ago1 and Ago2 target 20%–40% of the many TE families expressed in somatic cultured cells though silencing is relatively modest (Rehwinkel et al., 2006). Argonaute homologs, *piwi*, and *aub* are hardly expressed in these cells (Saito et al., 2006), and knockdown has little effect (Rehwinkel et al., 2006). Instead, Piwi expression is enriched in the *Drosophila* germline, as it is in *C. elegans*, zebrafish, and mammals. There are no piwi proteins in plants and fungi.

In *Drosophila*, *piwi* and *aubergine* are essential for germline development, and maternally contributed Piwi protein is part of the polar granule, a germline organelle. Null mutants fail to develop primordial germ cells, and overexpression leads to abundant and proliferating germline stem cells (Megosh et al., 2006). In mammals, Piwi null mutants are male sterile because of defective sperm differentiation (Girard et al., 2006; Parker and Barford, 2006). Piwi-bound siRNAs (piRNAs) have been sequenced from several organisms and are distinct from both siRNA and miRNA (Parker and Barford, 2006). Whereas miRNA and siRNA range between 21 and 26 nt, piRNAs are larger (between 26 and 31 nt) and lack 2′ and/or 3′ OH termini (Aravin et al., 2006; Girard et al., 2006; Grivna et al., 2006). piRNA biogenesis is independent of Dcr1 or Dcr2 in *Drosophila* (Vagin et al., 2006), and Piwi proteins are prominent in flies and mammals (that have no RdRP) but are absent from plants and fungi (which have multiple RdRPs). piRNA may therefore be generated by cleavage and replication of the precursor in a manner that depends on Piwi itself, but not on Dicer or RdRP. The rat homolog RIWI is associated with a RecQ helicase whose *Neurospora crassa* homolog is also involved in RNAi (Lau et al., 2006). piRNA in flies depends on at least three different helicase genes (Kavi et al., 2005), *lighten-up*, *homeless* (*spindle-E*), and *armitage* (Vagin et al., 2006). *piwi*- and *aub*-associated small RNAs match multiple transposons, including *HeT-A*, *roo*, *gypsy*, and *hoppel (1360)* (Saito et al., 2006; Vagin et al., 2006). In the mouse genome, 17%–20% of MIWI-associated piRNAs match SINE, LINE, and LTR retrotransposons, indicating a role in TE silencing (Aravin et al., 2006; Girard et al., 2006). However, the remaining piRNAs map to about 140 clusters of unique genome sequences that have not previously been identified as TEs. The largest clusters are present in syntenic regions in human and rat genomes and in gene and repeat poor regions, but, unlike miRNAs, piRNAs are not conserved. The clustered piRNAs are derived from only one strand resembling rasiRNA in *Drosophila* derived from the *Suppressor of Stellate* (*Su[Ste]*) locus, and from retrotransposons (Vagin et al., 2006).

Silencing of the protein-coding *Stellate* genes in the *Drosophila* germline is required for male fertility. The *Stellate* genes, present in ~200 copies on the X chromosome, are silenced by homologous heterochromatic Y linked *Su(Ste)*. Fragments of *Stellate* and *Su(Ste)* are capable of conferring PEV to nearby reporter genes in *cis*, resembling heterochromatin (Aravin et al., 2004). Mutations in *aubergine* and *homeless/spindle-E* result in the loss of the *Su(Ste)* rasiRNAs and impair *Stellate* silencing. Antisense *Su(Ste)* transcripts escape silencing (Aravin et al., 2001), reminiscent of fission yeast centromeres where the reverse strand escapes silencing and maintains siRNA levels (Djupedal et al., 2005; Volpe et al., 2002).

Both *piwi* and *aubergine* have significant roles in transposon silencing in the germline of *Drosophila* (Kavi et al., 2005). P element silencing occurs in the germline of flies derived from eggs that harbor P elements (P cytotype). Cytotype can be conferred by single P elements when they are inserted into tandemly repeated telomere-associated sequences (TASs) that lie adjacent to the *HeT-A* transposons at each chromosome end. Posttranscriptional silencing depends on *aubergine* (Reiss et al., 2004) and it has been suggested that small RNAs (piRNAs) homologous to the TAS repeats might be processed and extend to the P element insertion sequences and associated transgenes, accounting for silencing (Kavi et al., 2005). In the I-R system of hybrid dysgenesis, a non-LTR retrotransposon (the *I* element) is also posttranscriptionally silenced in the germline by *aubergine* and the presence of *I* elements at a specific site in heterochromatin (Vagin et al., 2006). Finally, the *flamenco* locus silences *gypsy* transposons posttranscriptionally, requiring *piwi* and resulting in 27–30 nt small RNA (Sarot et al., 2004). It has been proposed that silencing may be mediated by *gypsy* fragments found at the *flamenco* locus, which may also silence other retrotransposons (Kavi et al., 2005; Robert et al., 2001).

RNA-Dependent DNA Methylation

DNA methylation in plants and animals requires both de novo methylation (of unmethylated DNA) and maintenance methylation (of hemimethylated DNA), and RNA has been implicated in guiding DNA methylation patterns. In *Arabidopsis thaliana*, forward screens have been used to identify mutants in RNA-dependent DNA methylation (RdDM) (Table 2): in each case, an unlinked inverted repeat was used to silence the promoter of a target gene, which was used to screen for mutants. These screens recovered mutants in histone methylation (*kyp/suvh4*) and deacetylation (*hda6*) as well as maintenance DNA methylation at CpG (*met1*) and CpNpG (*cmt3*) motifs. The activity of MET1 could maintain silencing even when the initial *trans*-acting trigger was removed (Matzke and Birchler, 2005), while the maintenance activity of CMT3 and SUVH4 was less stable, resulting in variegated plants in the absence of the inverted repeat (Ebbs and Bender, 2006; Melquist and Bender, 2004). Unlike symmetric CpG methylation, non-CpG methylation requires active signals to target regions of replicated DNA. *DRM1* and *DRM2* are redundant and were not recovered in forward screens, but reverse genetics showed that they are required for asymmetric CpNpN methylation and for

RdRM (Cao et al., 2003). siRNA levels from the inverted repeats were unaffected in these mutants, indicating they acted downstream (Cao et al., 2003). The RNA-silencing proteins RDR2 and DCL3 are required for production of endogenous 24 nt siRNA, but *DCL2*, *DCL3*, and *DCL4* are redundant in overall siRNA production (Gasciolli et al., 2005; Henderson et al., 2006), and inverted repeats do not require RDR2 to generate dsRNA, explaining perhaps why RNAi mutants were not recovered in RdDM screens. Only in a screen that targeted the endogenous gene SUPERMAN (*SUP*) for silencing was a mutant in RNAi recovered (*ago4*) (Cao et al., 2003). AGO4 binds siRNAs from most TEs (Qi et al., 2006) and has RNA cleavage (slicer) activity, but also has noncatalytic roles in RdDM of some, but not all, TEs (Qi et al., 2006).

Interestingly, mutants in *MET1*, *DDM1*, and *DRM2* lose siRNA from the same subset of transposons and tandem repeats, as does *ago4* (Lippman et al., 2003; Zilberman et al., 2004). This loss of siRNA is correlated with a loss of H3K9me2 (Chan et al., 2005; Lippman et al., 2003) and it is possible that siRNA and AGO4 are bound to the chromosome via methylated histones, as is the case in fission yeast. Isolation of *AGO4*, the chromodomain *CMT3*, and the H3K9 methyltransferase *KYP* in the same screen raises parallels with the RITS complex of fission yeast, which contains Ago1 and the chromodomain Chp1 and depends on H3K9me for its association with the chromosome. Unlike fission yeast, however, loss of *CMT3* or *KYP* does not result in loss of siRNA in *Arabidopsis*, although redundancy may be an issue (Ebbs and Bender, 2006). Perhaps siRNA guides KYP and CMT3 on the one hand, and DRM1/2 on the other, by mechanisms requiring catalytic and noncatalytic AGO4, respectively.

Forward screens for RdDM did not recover mutants in *DDM1*, but a seed-specific screen recovered another SNF2-like chromatin remodeler gene, *DRD1* (*d*efective in *R*NA-directed *D*NA methylation), and large subunits of plant-specific putative Pol IV, *NRPD1b*, and *NRPD2* (Kanno et al., 2004). Another Pol IV large subunit, *NRPD1a*, was identified in a screen for mutants in PTGS (Herr et al., 2005). Two functionally distinct Pol IV complexes have been proposed: Pol IVa (containing NRPD1a and NRPD2), which is required for siRNA along with RDR2 and DCL3; and Pol IVb (containing NRPD1b and NRPD2), which is required for RdDM along with AGO4 and DRM1/2 but is not required for siRNA (Kanno et al., 2005). Instead, Pol IVb may convert RNA signals into chromatin modifications downstream of siRNA, along with DRD1 (Huettel et al., 2006).

The template for Pol IV is unknown, but methylated DNA (Onodera et al., 2005) or double-stranded RNA (dsRNA) (Pontes et al., 2006; Vaughn and Martienssen, 2005) has been proposed. In fission yeast, several Pol II subunits are required for heterochromatic silencing. Perhaps in plants Pol IVb fulfills the silencing role, using DNA as a template, while Pol IVa has become specialized in siRNA production, using dsRNA as a template (Vaughn and Martienssen, 2005). This idea has received support from cytological studies, in which Pol IVb, AGO4, and siRNA are all localized in nucleolar bodies putatively identified as Cajal bodies (Li et al., 2006), distinct from chromatin where Pol IVa is localized (Pontes et al., 2006). AGO4 interacts with the CTD of Pol IVb (Li et al., 2006), reminiscent of the reported interaction between Ago1 and Rpb1 in *S. pombe* (Schramke et al., 2005) and in humans (Kim et al., 2006).

siRNA-directed DNA methylation (Morris et al., 2004) and histone modifications H3K9me2 and H3K27me3 (Ting et al., 2005) have been reported in human cells and lead to TGS when directed against promoters, in some cases via Argonaute and Pol II (Janowski et al., 2006; Kim et al., 2006; Weinberg et al., 2006). Again, RdDM and RNAi-dependent histone modification may be distinct mechanisms.

Role of Transposons and Tandem Repeats in Imprinting

Imprinted genes in higher plants and mammals are expressed from only one allele in a manner that depends on the parent of origin. For example, the homeobox gene *FWA* and the polycomb gene *MEDEA* are only expressed from the maternal chromosome of *Arabidopsis* seeds. Expression depends on the DNA glycosylase DEMETER, which demethylates the promoter of each gene in the maternal gametophyte, and *demeter* seeds abort because expression of imprinted genes is lost (Gehring et al., 2006). This phenotype is suppressed by mutants in *met1* and appears to be restricted to the endosperm, a terminally differentiated tissue that does not contribute to the next generation (Jullien et al., 2006). The *FWA* promoter is composed of a SINE element and two sets of tandem repeats, generated after SINE integration (Lippman and Martienssen, 2004). These repeats are the targets of DNA methylation and correspond to siRNA (Chan et al., 2006; Lippman and Martienssen, 2004). Silencing of *FWA* in somatic tissues is relieved in *met1* but not in RNAi mutants, in which only non-CG methylation is lost. But de novo silencing of *FWA* transgenes depends on *RDR2*, *AGO4*, *DCL3*, *POLIV*, and *DRD1*, indicating it occurs by RdDM (Chan et al., 2004). Conversely, transgenic tandem repeats can (partially) silence hypomethylated *fwa* in *trans* (Chan et al., 2006), in a manner that resembles paramutation—a *trans*-acting silencing mechanism that also depends on tandem repeats and RdRP (see the Essay by V. Chandler, page 641 of this issue). This raises the possibility that the silencing of imprinted genes might involve RNAi (Martienssen et al., 2004).

Meiotic Silencing of Unpaired DNA

While *S. pombe* has no DNA methylation, in the filamentous ascomycete *Neurospora crassa* DNA methylation is found at TEs and repeats, and depends on H3K9me2, but neither depends on RNAi (Freitag et al., 2004). Nonetheless, two RNA-silencing phenomena, quelling and meiotic silencing of unpaired DNA (MSUD), have been

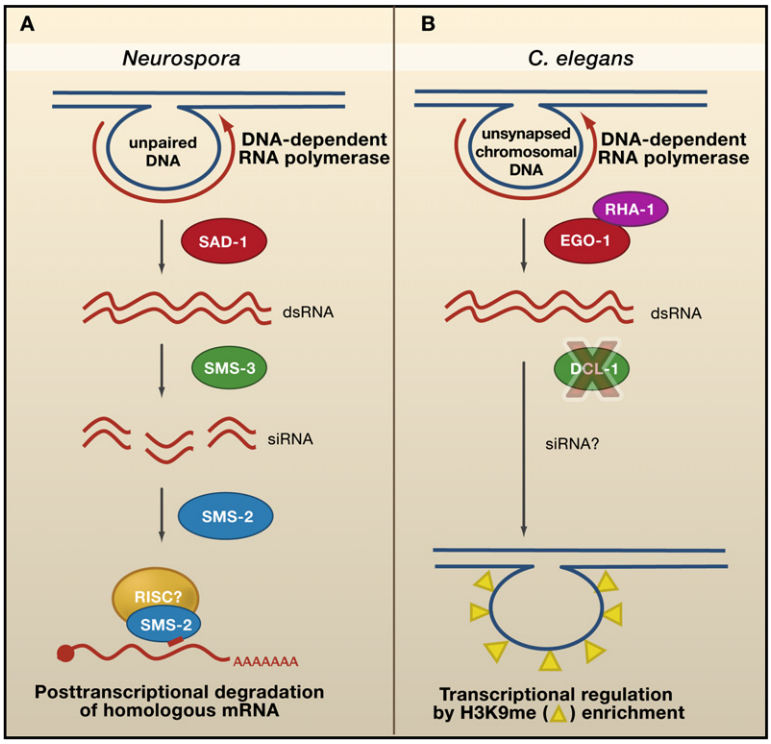

Figure 2. Models of MSUD RNA-Silencing Pathways

(A) During the sexual phase of the *Neurospora* life cycle, any transcribed region of DNA that has failed to pair in the homolog pairing stage is silenced in a process called MSUD. In this pathway, unpaired DNA is transcribed by an unknown DNA-dependent RNA polymerase to produce a transcript that is processed into dsRNA by the RdRP Sad-1. siRNAs produced by Sms-3 dicing of dsRNA are incorporated into a putative RISC complex containing Sms-2, a Paz/Piwi domain-containing protein. The RISC complex guided by siRNA acts in the degradation of homologous mRNA.

(B) During meiosis in *C. elegans*, unsynapsed chromosomes and chromosomal regions are silenced by a mechanism with parallels to MSUD. Unpaired chromosomal regions are transcribed and the transcripts are processed into dsRNA by the germline-specific RdRP ego-1. The single *C. elegans* dicer, DCL-1, is not involved in processing these dsRNAs. It is unknown whether siRNAs are produced from these dsRNAs (by an unidentified enzyme) or whether the long RNA transcripts themselves act as guides for H3K9 methylation of the unpaired regions.

identified (Nakayashiki, 2005). Quelling is an siRNA-directed PTGS mechanism that occurs during the vegetative phase of growth and represses the expression of transgenes that occur in more than one copy. Several quelling-defective (*qde*) genes have been identified, all of which belong to the RNAi pathway (Table 1). MSUD is distinct from quelling and was first revealed through studies of the *Ascospore maturation-1* (*Ams-1*) gene (Shiu et al., 2001). *Neurospora* is haploid in its vegetative state but has a transient diploid stage during the sexual phase, when two haploid nuclei of opposite MT fuse. The diploid nucleus immediately undergoes meiosis, followed by one mitotic division to yield eight linearly arranged haploid ascospores. MSUD senses and silences any gene that is unpaired during the homolog pairing stage and all homologous copies of that gene, irrespective of whether they are paired. Critical for MSUD is that a region of the unpaired gene is transcribed during meiosis (Lee et al., 2004).

The semidominant mutant *Sad-1* is deficient in MSUD (Shiu et al., 2001) and encodes an RdRP, while *Sms-2* and *Sms-3* encode Argonaute and Dicer, respectively (Figure 2). *Sad-1* and *Sms-2* are paralogues of the *Neurospora* quelling components *qde-1* and *qde-2*, indicating that a distinct set of silencing protein components seem to be involved in the MSUD and quelling pathways (Nakayashiki, 2005). But neither DNA methylation nor chromatin modification are implicated in MSUD (Shiu and Metzenberg, 2002). MSUD-like phenomena have also been observed in *C. elegans* and mouse, but unlike in *Neurospora*, these processes involve chromatin modifications. During meiosis in *C. elegans*, unpaired chromosomes and chromosomal regions accumulate high levels of H3K9me2 and can be silenced (Figure 2) (Bean et al., 2004). The RdRP *ego-1* is required, but Dicer and Drosha are not (Maine et al., 2005). Meiotic silencing of unsynapsed chromosomes (MSUC) in mice affects the XY body in males as well as unsynapsed autosomes in both the male and female germlines (Turner et al., 2005). Recently, a link between MSUC, the chromatin remodeling machinery, and MIWI has been identified via XY body protein interactions (Costa et al., 2006). But it remains to be seen whether the MIWI pathway, which unlike MSUD does not require RdRP, is involved in MSUC.

DNA Elimination in Protozoa

In ciliated protozoans, developmentally regulated genome-wide DNA elimination occurs during the sexual process of conjugation. Like all ciliates, the unicellular *Tetrahymena thermophila* contains two functionally distinct nuclei: a diploid germline micronucleus (Mic) and a polyploid macronucleus (Mac). The Mac serves as the somatic nucleus providing all transcription during vegetative growth, while the Mic is transcriptionally inert until conjugation. Both types of nuclei develop from the same zygotic nucleus, which is formed by fusion of two haploid micronuclei during conjugation. Differentiation of a new Mac is accompanied by extensive deletion of Mic-specific DNA sequences, known as internal eliminated sequences (IESs), followed by ligation of the flanking Mac-destined sequences (Matzke and Birchler, 2005). Approximately 6000 individual IESs,

comprising ~15% of the *T. thermophila* Mic genome, are removed (Mochizuki and Gorovsky, 2004b).

Nongenic, heterogeneous, bidirectional transcription of the Mic genome occurs during the early stages of conjugation, and injection of dsRNAs homologous to Mac sequences that are normally retained triggers elimination of these sequences during conjugation (Yao et al., 2003). Conjugation-specific small RNAs ~26–31 nt in length, referred to as "scan RNAs" (scnRNAs), appear before DNA elimination (Mochizuki and Gorovsky, 2004a) and are required for both H3K9me and accumulation of chromodomain proteins on IESs in the Mac (Liu et al., 2004). Twi1p, a *piwi* homolog, interacts with scnRNAs and is required for their accumulation and for DNA elimination (Mochizuki and Gorovsky, 2004a). Dcl1p, one of three *T. thermophila* Dicer-like proteins, is required for processing dsRNA precursors resulting from Mic bidirectional transcription into scnRNAs and for H3K9me of IESs (Mochizuki and Gorovsky, 2004a, 2005).

Similarity between Twi1 and Piwi, as well as the size of scnRNA (26–30 nt), suggests this system belongs in the piRNA class of RNAi-mediated germline silencing found in flies and mammals. However, Dcl1p is required and IES transcripts are double stranded, unlike piRNA precursors in animals. MSUD also occurs in the germline, and it is tempting to make parallels with "pairing" of Mac and Mic genomes via scnRNA, but these analogies await further investigation.

Common Themes in ncRNA-Mediated Silencing

Transcription and Processing of ncRNA

Most ncRNAs implicated in epigenetic silencing are, or are suspected to be, synthesized by Pol II. However, the most striking differences between ncRNAs important for epigenetic silencing and other products of Pol II, such as mRNA and some snURNAs, lie in their processing. Pre-mRNAs and pre-snURNAs undergo a series of precise and coordinated modifications such as splicing and 5′ and 3′ end processing, and are actively transported between the nuclear and cytoplasmic compartments. On the other hand, Pol II ncRNAs are often retained near the site of transcription (Motamedi et al., 2004) and can evade normal pre-mRNA processing (Seidl et al., 2006), leading many to label them "aberrant" RNAs. This has become a catch-all phrase for any Pol II RNA that does not look like a mature mRNA, and it is thought that their aberrant nature is what recruits the RNAi machinery for proper disposal (Gazzani et al., 2004; Herr et al., 2006). Interestingly, similar splicing and 3′ end processing factors that inhibit RNAi silencing in *Arabidopsis* (Herr et al., 2006) seem to be required for RNAi in *C. elegans* (Kim et al., 2005).

By preventing splicing and 3′ end RNA processing (that results in transcript release), RNAi may promote read-through transcription of ncRNA from one strand, to silence transcription from the other. This could be the case for the centromeric transcripts in *S. pombe*, and for several examples of antiparallel noncoding transcripts in the mouse, such as *Tsix* (at the X inactivation center) and *Air* (at the imprinted locus *Igf2r*). The *Air* ncRNA mediates the imprinting of the *Igf2R* locus (Figure 3) and has multiple forms, including a large spliced and polyadenylated mRNA. This mature mRNA is, however, efficiently exported out of the nucleus, where it is expected to be of little use in nuclear silencing. The function of the *Air* ncRNA could therefore be dependent on the simple act of transcription. Other unstable forms of *Air* are retained in the nucleus and evade the splicing process (Seidl et al., 2006). Analogously, splicing of the Tsix transcript is completely dispensable for Xist silencing (Figure 3) (Sado et al., 2006). It is the "aberrant" nuclear forms that could be mediating the epigenetic silencing functions, which can hardly be considered aberrant from this perspective.

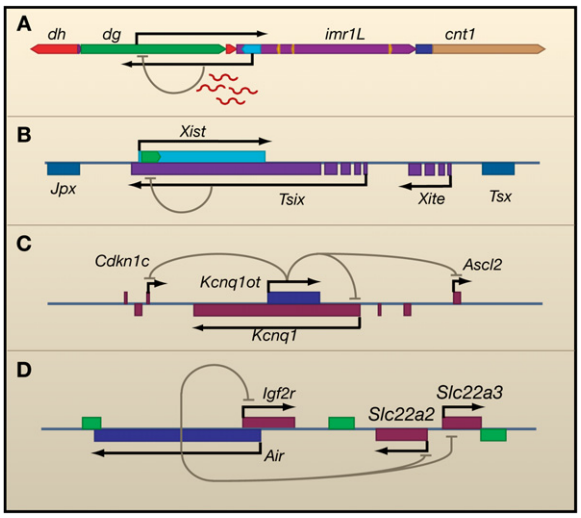

Figure 3. Silencing Transcripts at the *S. pombe* Centromere and Different Transcript-Mediated Silencing Systems

(A) *S. pombe dg-imr* transcript in centromere IL. The forward promoter is silenced by constitutive transcription and processing into siRNA of the reverse strand.

(B) The X inactivation center has several noncoding transcripts, and transcription of *Tsix* silences the Xist promoter.

(C and D) Paternal locus transcription of noncoding transcripts Kcnq1ot and Air influences the expression of overlapping and non-overlapping genes in the imprinted gene cluster at the telomeric end of mouse chromosome 7 and the Igf2r locus, respectively. Paternally expressed genes are colored blue, maternally expressed genes are colored red, and ubiquitously expressed genes are colored green.

Noncoding RNAs could therefore undergo a specialized processing, or lack thereof, in competition with the pre-mRNA-processing machinery. In both *Air* and *Tsix*, truncation and release of the regulatory ncRNA by introduction of a premature poly(A) signal results in loss of silencing, associated with changes in histone modification. RNAi might prevent such processing and release by slicing off the 5′ cap, which would be expected to inhibit 3′ end processing (Flaherty et al., 1997), but other means could be just as effective. One modification undergone by long ncRNA is nonsense-mediated decay (NMD). Knockdown of NMD and exosome factors (Eif1, Rent1, and Exosc10) impairs

Xist upregulation during XCI (Ciaudo et al., 2006). This impairment occurs at the posttranscriptional level and presumably impacts *Tsix*. This effect was also observed on the *H19* transcript present in the imprinted *IGFII* locus (Figure 3), but not on other ncRNAs (Ciaudo et al., 2006). There could also be interplay between posttranscriptional and transcriptional mechanisms: the presence of a preterminal stop codon in an immunoglobulin-μ minigene, which makes the transcript a target for NMD, leads to its transcriptional downregulation by H3K9me (Buhler et al., 2005). The mechanism involved could be suppressed by overexpression of the exonuclease Eri1, hinting at the involvement of RNAi in this system.

Silencing In cis *and In* trans

An interesting consequence of the peculiar biogenesis of these ncRNAs is that it precludes them from nuclear-cytoplasmic transport, and this could limit their scope of action to the point of origin. miRNAs are exported from the nucleus, and this is a requirement of their maturation pathway. The possibility therefore exists that heterochromatic siRNA could be transported out of the nucleus and act in *trans*. The phenomenon of DNA elimination in *Tetrahymena* does have a cytoplasmic stage for the siRNA, but the mechanisms that would transport this signal remain to be found. However, this does not seem to be the case in *S. pombe*, where the action of siRNA from an RITS-targeted mRNA was restricted in *cis* (Buhler et al., 2006). Only by mutation of the siRNA exonuclease Eri1 could silencing in *trans* be detected. Likewise, the likelihood of transgene silencing depends on the number of copies, and could point to a similar requirement for high abundance of siRNA. In *Drosophila*, *trans*-silencing *Su(Ste)* rasiRNAs are generated from a highly repetitive source, and reach concentrations similar to those of ribosomal RNA. In paramutation, RdRP-dependent RNA signals must act in *trans*, but siRNA have proved difficult to detect. The confined action of ncRNA is reminiscent of the classical view of heterochromatin as a region of the nucleus unfavorable to transcription, which could be determined by the local concentration of effector ncRNA. In one of the most dramatic examples of heterochromatin, the Barr body, the inactivated X chromosome is coated by the Xist ncRNA (see P.K. Yang and K.I. Kuroda, page 777).

Gene Silencing by Cotranscription with ncRNA

The silencing brought about by ncRNAs can bring nearby genes under their influence. In many cases where TEs silence neighboring genes, this correlates with cotranscription from TE promoters. Also, imprinted loci are often associated with ncRNA that overlap some of the imprinted genes. Cotranscription may be a commonplace device for spreading of heterochromatin marks, and it is tempting to include PEV where transgenes are inserted in or near heterochromatic domains (Irvine et al., 2006; Talbert and Henikoff, 2006). PEV and cotranscriptional silencing bear many striking resemblances: PEV occurs in *cis*, over a limited distance within reach of Pol II transcription, and brings the target genes under the influence of the mechanisms that regulate the silencing element, such as histone modification and RNAi. The cotranscriptional slicing of heterochromatic transcripts also provides an attractive model that explains how the RNAi machinery could silence elements far from the origin of the siRNA, as is the case in PEV (Irvine et al., 2006).

Recent reports have demonstrated a physical interaction between Ago1 and the large subunit of Pol II, responsible for recruitment of the pre-mRNA-processing machineries (Kim et al., 2006; Schramke et al., 2005). Argonaute is limited to binding nascent transcripts by the cognate sequence of the loaded siRNA, but the partners that recruit it to heterochromatin in *S. pombe* (Chp1) have no such sequence requirements. Argonaute could therefore hitch a ride with Pol II, promoting the deposition of heterochromatic marks on the chromatin that Pol II transcribes. As the nucleosomes become methylated with silencing marks, the binding of downstream effectors such as Chp1 could stabilize and perpetuate Argonaute binding to the silenced regions, as well as the silencing marks themselves.

This implies that an ncRNA transcript that yields siRNA transforms Pol II into a silencing complex, and possibly displaces the normal pre-mRNA-processing machinery from the CTD, inhibiting splicing and 3′ end processing. A similar model could be proposed for the plant silencing polymerase Pol IV, although its substrate is still unknown. The Pol II CTD recruits chromatin modification machinery, such as Set2, that deposits epigenetic marks associated with active transcription and elongation in euchromatic genes (Li et al., 2003). It could also potentially recruit the silencing machinery in the case of ncRNA. One of the signals that could switch Pol II from a pre-mRNA processing to a silencing complex is the slicing of the nascent transcript by Argonaute associated with siRNA (Irvine et al., 2006). Alternatives, including "oozing" and intranuclear localization, have also been proposed; it is likely that a combination of these mechanisms contributes to the phenomena collectively known as PEV (Talbert and Henikoff, 2006).

The involvement of ncRNA in silencing is not limited to TEs, and transcription of ncRNA can regulate developmentally important genes in very sophisticated ways. In animals, ncRNAs from polycomb response elements (PRE) and trithorax response elements (TRE) are transcribed at the stage at which polycomb confers mitotic "transcriptional memory" to targets such as the hox genes (B. Schuettengruber et al., page 735). Transcription of the ncRNA impacts this memory (Sanchez-Elsner et al., 2006; Schmitt et al., 2005), and *piwi* may be involved (Grimaud et al., 2006). In plants, polycomb silencing and histone modification silences the transcription factor gene *FLC* in response to long periods of cold, imparting a memory of winter so that the plants flower next spring (Sung and Amasino, 2005). This process requires RNA-binding proteins, splicing factors, and a CPSF-like 3′ processing factor, which inhibits RNAi (Herr et al., 2006; Quesada et al., 2005). It is tempting to speculate that readthrough

transcription and slicing of ncRNA might contribute to polycomb silencing, but a direct role for RNAi awaits further investigation.

Concluding Remarks

What is the potential scope of action of ncRNAs? We have discussed their participation in chromosomal structure in the heterochromatic regions, and genome surveillance roles like TE silencing, DNA elimination, and MSUD. However, there is an emerging awareness that noncoding regions of the genome, such as intergenic regions, have the potential to be transcribed and show certain levels of conservation (Furuno et al., 2006; Khaitovich et al., 2006). Additionally, transcription in antisense orientation to protein-coding genes is of an unexpected prevalence (Timmons and Good, 2006). Elucidation of the mechanisms affected by this not so ''aberrant'' transcription will no doubt shed light into how they contribute to the epigenetic landscape.

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