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REVIEW

RNA turnover and chromatin-dependent gene silencing

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Abstract Over the last few years, there has been a convergence of two seemingly disparate fields of study: chromatin-dependent gene silencing and RNA turnover. In contrast to RNA turnover mechanisms that operate on a truly posttranscriptional level, we are at the beginning of studies leading the way toward a model in which RNA turnover mechanisms are also involved in chromatin-dependent gene regulation. In particular, data from a variety of organisms have shown that the assembly of silent chromatin coincides with the presence or absence of non-protein-coding RNAs (ncRNAs). These range from long ncRNAs that have been classically implicated in the regulation of dosage compensation and genomic imprinting to small ncRNAs which are involved in heterochromatin assembly via the RNA interference (RNAi) pathway. This raises the question of how common ncRNAs are used to control gene expression at the level of chromatin. It is known at least, that they are present, as recent findings indicate that transcription of eukaryotic genomes is much more widespread than previously anticipated. However, the existence of a ncRNA does not prove its biological significance. Thus, a future challenge will be to distinguish the ncRNAs that are in some way meaningful to the organism from those that arise from the imperfect fidelity of the transcription machinery. Finally, no matter whether functional or not, RNAs transcribed from supposedly silent chromatin seem to be processed rapidly. Recent data from both fission and budding yeast suggest that chromatin-dependent gene silencing is achieved, at least in part, through RNA turnover mechanisms that use components of the RNAi pathway as well as polyadenylation-dependent RNA decay. Hence, silent chromatin is not only controlled transcriptionally, but also on co- and posttranscriptional levels.

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The “hidden transcriptome”

Classically, the transcribed portions of eukaryotic genomes, the transcriptome, have been viewed as the result of RNA polymerases initiating transcription from specific sites that have evolved to produce functional RNA products. This view has been challenged by recent studies in a wide range of eukaryotic organisms that revealed that transcriptional activity of eukaryotic genomes has long been underestimated. Genome-wide expression analyses indicate that eukaryotic transcriptomes are larger and more complex than previously anticipated. The first stage of the ENCODE project demonstrated that more than 90% of the analyzed human genome is transcribed in different cells types (Birney et al. 2007). Similar findings have been reported for mouse and other eukaryotes, suggesting that a big portion of eukaryotic transcripts are nonprotein coding (Carninci et al. 2005; Cheng et al. 2005; Willingham and Gingeras 2006; Chekanova et al. 2007). The latest advances in next-generation sequencing technologies promise to make genome analysis even more comprehensive than has already been achieved by conventional DNA microarrays. The transcriptomes of the budding yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) and the fission yeast *Schizosaccharomyces pombe* (*S. pombe*) have now been sequenced by a next-generation sequencing-based method called “RNA-seq” (Nagalakshmi et al. 2008; Wilhelm et al.

2008), revealing previously unknown transcribed regions of these well-studied model organisms. It turns out that 94% of the fission yeast genome is transcriptionally active, with most of the newly discovered transcripts seeming to be non-protein-coding (Wilhelm et al. 2008). These global surveys beyond doubt set a standard for other organisms, but also raise, yet again, the question of the functional significance of this, until now, unidentified transcriptome.

Not so silent heterochromatin

The concepts of euchromatin and heterochromatin have been proposed by Emil Heitz in the early 20th century (Heitz 1928). Heterochromatin is an epigenetically inherited and conserved feature of eukaryotic chromosomes and is found at various chromosome regions where it functions to silence gene expression, reduce the frequency of recombination, promote long-range chromatin interactions, and ensure accurate chromosome segregation during mitosis (Jia et al. 2004; Pidoux and Allshire 2004; Grewal and Elgin 2007a). Characteristically, heterochromatin is composed of arrays of hypoacetylated nucleosomes that are methylated at lysine 9 of histone H3 (H3K9me) and bound by chromodomain-containing proteins such as the heterochromatin protein 1 (HP1) family of proteins (Grewal and Jia 2007b). Furthermore, reporter genes inserted within or adjacent to heterochromatin are silenced, a conserved phenomenon first described in *Drosophila melanogaster* and known as position effect variegation (PEV; Muller 1930). Importantly, founder cells pass on alternate active (on) and silent (off) states of a reporter gene to their descendants, resulting in a variegated expression pattern (Fig. 1a). A key observation was that the chromosomal region including the reporter gene was physically condensed in the cells in which the gene was "off," but not in the cells in which it was "on" (Zhimulev et al. 1986). Thus, PEV has ever since been thought to be mediated by transcriptional repression of the reporter gene. However, this view has been challenged by a number of recent findings, which suggest that heterochromatin can be a relatively accessible structure, and in some situations, silencing occurs by a mechanism that does not prevent the association of RNA Polymerase II (RNAP II) with promoters situated within heterochromatin.

Many of the RNAs newly identified in genome-wide gene expression surveys seem to originate from supposedly silent chromatin; and particular RNAs derived from heterochromatic regions have been reported in a broad range of species (Rouleux-Bonnin et al. 1996, 2004; Lorite

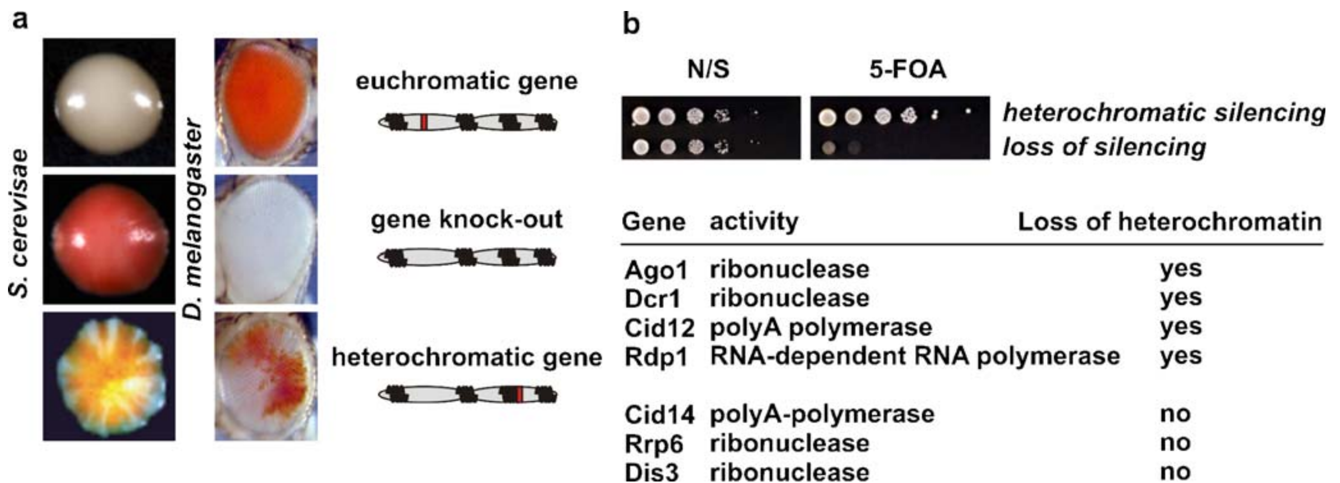


Fig. 1 Chromatin-dependent gene silencing. **a** Two examples demonstrating variegated expression of a gene upon packaging into a heterochromatic structure. In *S. cerevisiae*, cells expressing the wild type *ade2*$^{+}$ gene from its endogenous, euchromatic locus produce colonies that are white, whereas those lacking the *ade2*$^{+}$ gene appear red. In *Drosophila melanogaster*, expression of the "*white*" locus, normally located in euchromatin, confers on the eye a red pigmentation. The eye of a "*white*" mutant appears white. In both organisms, juxtaposition of the *ade2*$^{+}$ or *white* genes with heterochromatin results in silencing of the particular gene without changing the underlying coding sequence. Although inherited, the packaging state of these genes (euchromatic versus heterochromatic) can switch at a low frequency. This results in a variegating phenotype in a clonal population of cells (position effect variegation, PEV). **b** In *S. pombe*, heterochromatic gene silencing is lost in RNA turnover mutants. Depicted is a "silencing" assay. This assay is based on 5-fluorotic acid (5-FOA) which is toxic to cells expressing the *ura4*$^{+}$ gene. The cells shown harbor a *ura4*$^{+}$ gene inserted into centromeric heterochromatin. Classic mutants showing loss of silencing are genes involved in heterochromatin assembly such as histone modifying enzymes or heterochromatin proteins. More recently, it has become evident that mutants defective in RNA turnover mechanisms also show a loss of silencing phenotype. Whereas loss of silencing in RNAi mutants (Ago1, Dcr1, Cid12, Rdp1) is attributed to heterochromatin assembly defects, heterochromatin is not affected in mutants defective in nuclear RNA surveillance. (Image courtesy of S. Gasser, Friedrich Miescher Institute for Biomedical Research, Basel (*S. cerevisiae*); Jonathan Schneiderman, Harvard Medical School, Boston (*D.m.)*; Yukiko Shimada, Friedrich Miescher Institute for Biomedical Research, Basel (*S. pombe*))

et al. 2002; Volpe et al. 2002; Azzalin et al. 2007; Houseley et al. 2007; Li et al. 2008; Pezer and Ugarkovic 2008; Vasiljeva et al. 2008). Remarkably, certain genes are even found to depend on heterochromatin for their activity (Yasuhara and Wakimoto 2006; Smith et al. 2007). Furthermore, analysis of RNAP II occupancy in organisms ranging from yeast to humans show that RNAP II associates with the vast majority of the analyzed genome, including numerous "heterochromatic" regions (Breiling et al. 2001; Dellino et al. 2004; Steinmetz et al. 2006; Guenther et al. 2007). Finally, heterochromatin has little or no effect on RNAP II occupancy at reporter genes inserted into fission yeast heterochromatic loci (Buhler et al. 2006). It is possible that heterochromatin stalls polymerases along the reporter gene, thereby preventing RNA synthesis. However, transcription run-on experiments showed that RNA is produced from these loci (Buhler et al. 2006; Bühler, unpublished). Importantly, expression of these reporter genes is efficiently silenced, unless specific RNA-decay pathways are impaired (Buhler et al. 2007; Murakami et al. 2007; Wang et al. 2008) (Fig. 1b). This strongly suggests that PEV can be achieved, at least in part, through degradation of heterochromatic RNAs rather than shutting off transcription (see below).

These data suggest that heterochromatin, as many other supposedly nontranscribed regions of eukaryotic genomes, is not necessarily silenced at a transcriptional level (Fig. 3). This raises the question of whether active transcription is a prerequisite for proper functioning of silent chromatin structures or whether such transcription simply reflects inaccuracy of transcriptional control.

Transcription-dependent establishment of epigenetic modifications

Although it may seem paradoxical, there is growing evidence that transcription can be a prerequisite for the assembly and maintenance of silent chromatin. However, one major difficulty is to distinguish effects of transcription per se from functions of the resulting transcripts. It is possible that RNAP II transcribes noncoding DNA to remodel chromatin and that the resulting ncRNA simply reflects a nonfunctional by-product (Fig. 2, I). Indeed, genes can be activated by transcription through promoter regions making DNA sequences more accessible to the transcription machinery (Hirota et al. 2008). It has also been demonstrated that genes can be silenced as a consequence of transcription interference (Martens et al. 2004; Hongay et al. 2006). Alternatively, one could envisage models in which an RNA is actively involved in recruiting modifying activities to assemble a higher-order chromatin structure (Fig. 2, II and III). This can introduce heritable variation into gene expression without altering the DNA sequence itself, forming the foundation of epigenetic phenomena. Although little is known about the underlying mechanisms linking RNA to chromatin, there is growing evidence that this link may play a major role in epigenetics (Bernstein and Allis 2005; Fig. 2).

Classic examples where RNA is linked to chromatin-dependent processes include dosage compensation and genomic imprinting in mammals. In the case of genomic imprinting, only one of the two alleles of a gene is expressed, dictated by its paternal or maternal origin. The approximately 70 imprinted genes in mammals often exist in clusters containing imprinting control regions that determine expression (Tycko and Efstratiadis 2002; Verona et al. 2003). Most imprinted clusters also encode for at least one imprinted ncRNA, suggesting that these RNAs could play a functional role. Studies of the two well-characterized imprinting clusters, *Igf2r* and *Kcnq1*, have clearly demonstrated that transcription of *Air* and *Kcnq1ot1* RNAs, respectively, is critical for the imprinted expression of the genes along these clusters (Sleutels et al. 2002; Mancini-Dinardo et al. 2006). Whether other ncRNAs associated with other imprinted clusters have a function is not known. Dosage compensation accounts for uneven numbers of X-chromosomes found in males and females by equalizing the output of gene expression. Dosage compensation in both *Drosophila* and mammals has been well studied and is intimately linked to ncRNAs leading to changes in chromatin structures that either repress (mammals) or activate (*Drosophila*) X-linked genes. In mammals, X inactivation is initiated by a master control locus, the X-inactivation center (Xic), and the noncoding RNA (Xist) it produces. Xist RNA accumulates on the chromosome from which it was produced and is responsible for inducing *cis*-limited silencing of the more than 1,000 genes on the X chromosome (Penny et al. 1996; Marahrens et al. 1998; Wutz and Jaenisch 2000). The Xist RNA has been the subject of intense investigation for almost two decades, but its mechanism of action as a ncRNA still remains mysterious. Although no functional protein partners have been identified, the evidence suggests that Xist RNA may act at multiple levels, including nuclear compartmentalization, chromatin modulation, and recruitment of Polycomb group proteins (Masui and Heard 2006). Most recent data suggest that RNAi and nuclear RNA degradation pathways may also contribute to X inactivation in mammals, an intriguing link that awaits further investigation (Ciaudo et al. 2006; Ogawa et al. 2008).

ncRNAs have also been implicated in chromatin-dependent gene regulation in other organisms. In *S. cerevisiae*, noncoding antisense RNA has been implicated in transcriptional silencing of the Ty1 retrotransposons (Berretta et al. 2008). Antisense transcription also regulates chromatin-dependent

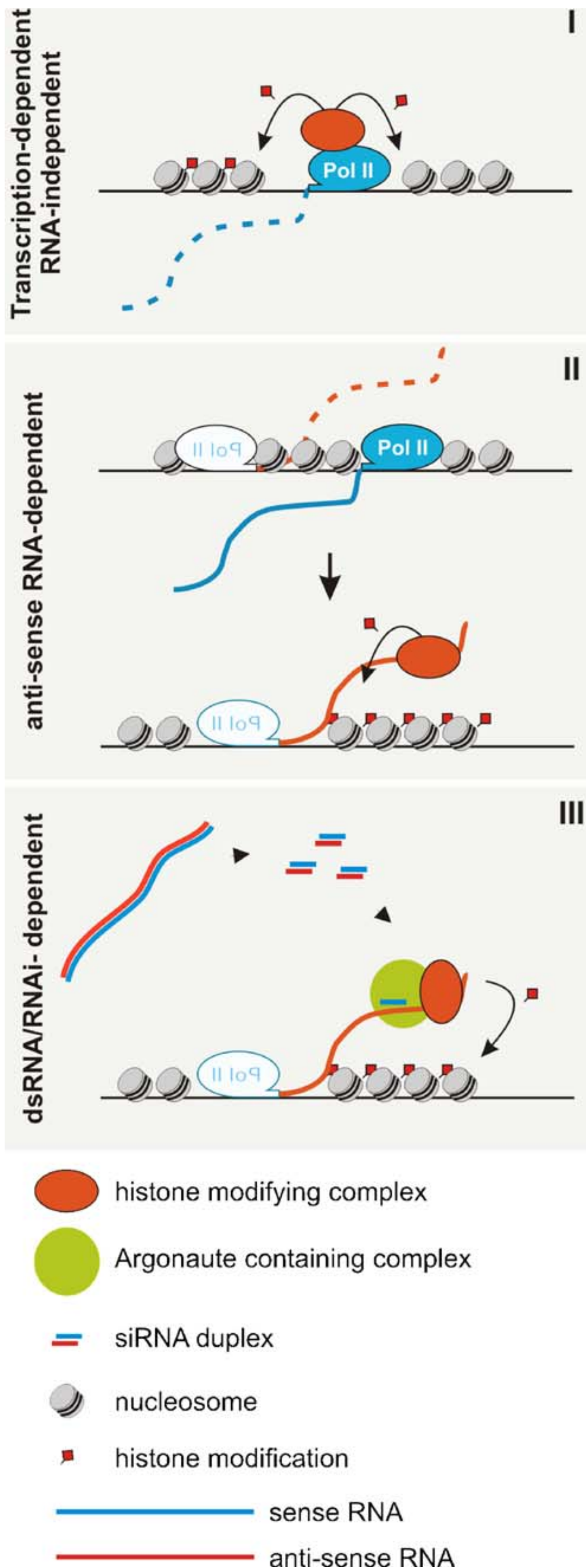
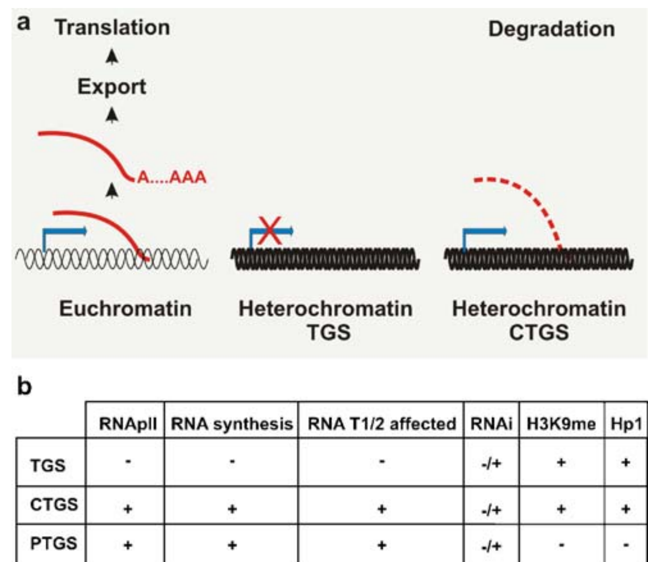


Fig. 2 Models for the role of transcription in epigenetic chromatin modification. *I* Transcription-dependent modifications. Chromatin modifying complexes are recruited co-transcriptionally by the transcription machinery. The synthesized RNA can be regarded as a nonfunctional by-product that has to be discarded. *II* Antisense RNA-dependent modifications. An antisense RNA serves as an assembly platform in order to recruit chromatin modifying complexes. This can occur via *cis* elements or the formation of a particular secondary structure. The modifying complexes recruited by the RNA place epigenetic marks on the locus, which then control transcription of the sense RNA. In this model, the chromatin state can be controlled via regulation of the stability of the antisense RNA (see text). *III* RNAi-dependent modifications. In contrast to model II where the RNA itself recruits the modifying activities, siRNAs act as guide molecules to target the modifying complexes to the RNA. siRNAs are generated by the RNAi pathway from longer dsRNA. dsRNA can be generated by bidirectional transcription, transcription of inverted repeats or by an RNA-dependent RNA polymerase that converts a ssRNA into dsRNA. See Fig. 4b for a model of RNAi-dependent heterochromatin formation in *S. pombe*

silencing of the *PHO84* gene during chronological aging (Camblong et al. 2007). The *PHO84* antisense RNA turns out to be a ncRNA whose levels are kept low by the nuclear exosome, an RNAse complex with 3′–5′ exonucleolytic activity. This allows full expression of *PHO84* sense mRNA. However, this ncRNA accumulates at the expense of *PHO84* mRNA under stress conditions, coinciding with reduced binding of the exosome component Rrp6 to the *PHO84* gene. These data suggest that the *PHO84* ncRNA could act to recruit histone deacetylases (HDACs) to inhibit sense transcription and that this is regulated by the exosome via



	RNApII	RNA synthesis	RNA T1/2 affected	RNAi	H3K9me	Hp1
TGS	-	-	-	-/+	+	+
CTGS	+	+	+	-/+	+	+
PTGS	+	+	+	-/+	-	-

Fig. 3 Chromatin-dependent gene silencing mechanisms operate at a transcriptional and/or posttranscriptional level. **a** Silencing of heterochromatin can be achieved by either shutting off transcription (TGS) or by degradation of heterochromatic RNAs (CTGS). In contrast to classic posttranscriptional gene silencing (PTGS), CTGS depends on the status of chromatin from which the gene is transcribed and is therefore referred to as "co-transcriptional". **b** Criteria to differentiate between TGS, CTGS, and PTGS

modulating the abundance of the ncRNA (Camblong et al. 2007). Similarly, the involvement of both HDACs and the exosome in gene silencing has also been described in *S. pombe* (Yamada et al. 2005; Nicolas et al. 2007).

RNAi-dependent heterochromatin assembly

The mechanism of recruitment of chromatin modifying activity by the ncRNAs described above is likely to involve site-specific RNA-binding proteins (Fig. 2, II). In contrast, chromatin modifying activities can also be targeted to chromatin via the RNAi pathway in a broad spectrum of eukaryotic organisms (Zaratiegui et al. 2007; Fig. 2, III). In *A. thaliana*, dsRNA promotes the methylation of homologous DNA regions. DNA methylation as well as histone H3K9 methylation require a member of the Argonaute family of proteins, which play key roles in RNAi-related pathways (Mette et al. 2000; Henderson and Jacobsen 2007). In *D. melanogaster*, multiple copies of alcohol dehydrogenase transgenes induce transcriptional silencing of the transgenes as well as of the endogenous alcohol dehydrogenase gene, a phenomenon that requires Piwi, an Argonaute homolog, and Polycomb, a chromodomain protein involved in the transcriptional inactivation of many developmental regulators (Pal-Bhadra et al. 1999, 2002). In *C. elegans*, silencing of transgene arrays requires components of the RNAi pathway and several proteins involved in transcriptional gene silencing (Grishok et al. 2005; Robert et al. 2005). Moreover, Piwi and other RNAi components were shown to contribute to the formation of centromeric heterochromatin in *D. melanogaster* (Pal-Bhadra et al. 2004). Similarly, in *Tetrahymena thermophila*, programmed DNA elimination specified by H3K9 methylation requires another Argonaute family member, Twi1 (Mochizuki et al. 2002; Taverna et al. 2002).

The link between RNAi and epigenetic mechanisms has been extensively studied in the fission yeast *Schizosaccharomyces pombe* (*S. pombe*), where small interfering RNAs (siRNAs), together with long ncRNAs, are essential for the formation of heterochromatin at pericentric DNA repeats (Grewal and Elgin 2007a; Fig. 4). The key components of RNAi, Dicer, and Argonaute, and an RNA-dependent RNA polymerase, are conserved in *S. pombe*. Deletion of the genes encoding any of these proteins (Dcr1, Ago1, and Rdp1, respectively) results in loss of H3K9 methylation and HP1 localization at centromeres. Moreover, siRNAs corresponding to centromeric repeats have been identified (Reinhart and Bartel 2002; Cam et al. 2005; Buhler et al. 2008). Biochemical analysis of the *S.*

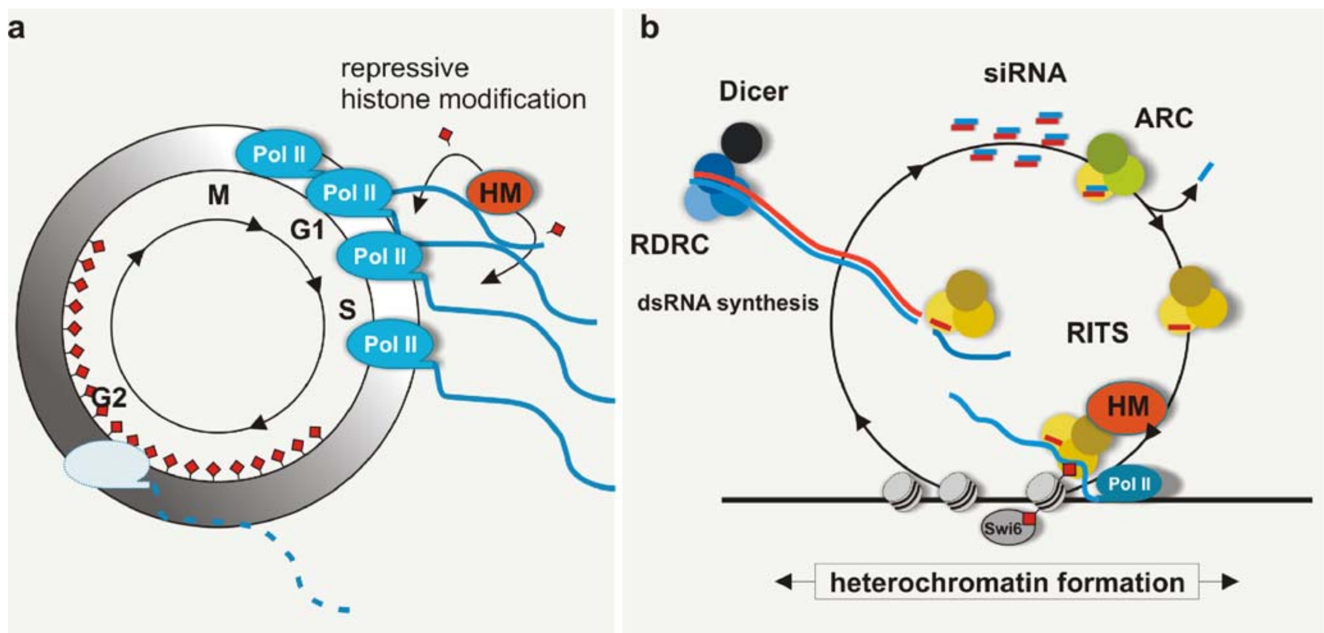


Fig. 4 Cell cycle-specific transcriptional activity within heterochromatin. **a** Studies of fission yeast and mammals demonstrated that transcription of pericentromeric repeats is under cell cycle control. During DNA replication (S phase), a more accessible chromatin structure permits RNA polymerase II to transcribe centromeric DNA. The resulting RNA is thought to recruit histone-modifying activities in order to assemble a more restrictive, heterochromatic structure upon exit of S phase. In G2, heterochromatin is kept silent by either TGS, CTGS, or both (the lengths of the indicated cell cycle phases roughly resemble the ones from *S. pombe*). **b** While still enigmatic in mammals, in *S. pombe*, the G1/S phase-specific pericentromeric transcripts most likely recruit the RNAi machinery to modify histones and silence expression of the same loci according to the nascent transcript model. This model proposes that the Argonaute-containing RITS complex mediates heterochromatin formation by associating with nascent RNAs via siRNA base pairing, and with methylated H3K9 via the chromodomain of its Chp1 subunit. See text for details. *HM* histone-modifying complex

pombe RNAi proteins resulted in the identification of three main RNAi effector complexes: the RNA-induced transcriptional silencing (RITS) complex, the argonaute siRNA chaperone (ARC) complex, and the RNA-directed RNA polymerase complex (RDRC) (Motamedi et al. 2004; Verdel et al. 2004; Buker et al. 2007). Both RITS and ARC contain siRNAs bound to Argonaute. The siRNAs found in ARC are mostly double-stranded, suggesting that ARC is a precursor complex involved in siRNA maturation. The RITS complex contains single-stranded siRNAs (Buker et al. 2007), which have been proposed to act as specificity factors for association with chromatin. In principle, siRNAs could target specific chromatin regions by base pairing with either DNA or nascent RNAs (Grewal and Moazed 2003). Studies over the past years have provided support for a model in which siRNAs act as guide molecules to target histone modifying enzymes to chromatin via base pairing between siRNA and pre-mRNA as RNAP II synthesizes the RNA transcript (Figs. 2 and 4b). Direct support for this model comes from artificial tethering of RITS to the transcript of a normally euchromatic gene. Tethering of the RITS complex to *ura4*$^+$ RNA via a site-specific RNA-binding protein (N protein of phage λ) results in heterochromatin assembly and silencing of the cognate *ura4*$^+$ gene. This tethering also results in the generation of *ura4*$^+$-specific siRNAs, and silencing requires proteins associated with both RNAi and heterochromatin (Buhler et al. 2006).

Long thought to be restricted to the assembly of centromeric heterochromatin, the *S. pombe* RNAi machinery has recently also been implicated in a mechanism by which transcription termination at convergent gene pairs is regulated by cohesin (Gullerova and Proudfoot 2008). Importantly, read-through transcription is detectable in the G1 phase of the cell cycle, whereas during G2 transcription of the convergent genes is properly terminated, unless the RNAi pathway is impaired. The data suggest a model in which read-through transcription at convergent gene pairs during G1 causes the formation of dsRNA, which feeds into the RNAi pathway and assembles a heterochromatic structure (Fig. 4). Heterochromatin between the two genes would then recruit cohesin which acts as a roadblock to prevent read-through in G2 (Gullerova and Proudfoot 2008). Although the generality of this mechanism remains to be established, it is tempting to speculate that a fraction of the “hidden transcriptome” may serve to control transcription by depositing regulatory, epigenetic marks and may function in a cell cycle-dependent manner (Figs. 2 and 4).

Cell cycle-specific transcriptional activity within heterochromatin

In fission yeast, studies of RNAi-mediated heterochromatin assembly demonstrate that what has long been thought to be transcriptionally silent is intrinsically dependent on transcription. In this case, transcription of heterochromatin is important for the generation of siRNAs, and the synthesis of nascent chromatin-bound RNAs that serve as templates to recruit chromatin-modifying activities (Fig. 4b). However, it has been unclear how the requirement for transcription in heterochromatin assembly could be reconciled with the silencing of heterochromatic genes. One possibility to explain this apparent paradox is that a trigger round of transcription is required to initiate heterochromatin assembly. Once assembled, transcription would be shut off by restricting RNAP II’s access to DNA (Fig. 4a). Consistent with this, regulated transcription termination between convergent gene pairs suggests a cycle of events involving transient formation of heterochromatin during G1 in a transcription/RNAi-dependent manner (Gullerova and Proudfoot 2008).

Similar results demonstrate that transcription of pericentromeric heterochromatin is under cell cycle control in yeast and mammalian cells (Chen et al. 2008; Kloc et al. 2008; Lu and Gilbert 2008). Two populations of RNAP II-dependent transcripts derived from mouse pericentric heterochromatin major (γ) satellite repeats accumulate at different times during the cell cycle. A small RNA species seems to be synthesized exclusively during mitosis and rapidly eliminated during mitotic exit. A more abundant population of large, heterogeneous transcripts is induced late in G1 phase, with highest rates of transcription in early S phase (Lu and Gilbert 2007). Similar cell cycle-dependent regulation of heterochromatin transcription has now been shown in *S. pombe*, where transcripts corresponding to heterochromatic pericentric DNA repeats accumulate in S phase (Chen et al. 2008; Kloc et al. 2008). Concomitant with the accumulation of heterochromatic RNAs in S phase, heterochromatic marks such as H3K9me, and binding of the heterochromatin protein HP1 decreased. Other marks that are associated with actively transcribed genes as well as an increase in RNAP II occupancy were detected in S phase, indicating that the pericentric heterochromatin structure becomes more permissive to transcription (Chen et al. 2008; Fig. 4a).

The fission yeast studies nicely support a model for pericentric heterochromatin formation in which a more accessible chromatin structure permits RNAP II to transcribe centromeric DNA, which in turn recruits the RNAi machinery (Fig. 4). Although evidence for an RNAi-like, transcription coupled heterochromatin assembly pathway in mammals remains elusive, it is certainly intriguing that in both species the period of highest transcription takes place during S phase, raising the possibility that there may be a conserved transcription-coupled silencing mechanism at eukaryotic centromeres. Conceptually, this suggests that such transcription is not noise, but is induced in response to

cellular proliferation and is actively involved in the inheritance of epigenetic marks from mother to daughter cells. It remains to be investigated how frequently such cell cycle-dependent transcription is used by eukaryotes in order to control gene expression in a chromatin-dependent manner and to what extent the RNAi pathway may be involved (Kanellopoulou et al. 2005; Murchison et al. 2005; Lu and Gilbert 2008).

Co-transcriptional gene silencing

The studies described above suggest that heterochromatin restricts access to pericentric DNA repeats in G2 and thus silences these regions transcriptionally (Fig. 4a). Consistent with this, earlier transcriptional run-on experiments with exponentially growing *S. pombe* cultures, where about 80% reside in G2, have shown that transcription of the "forward" strand is inhibited by pericentric heterochromatin. However, the "reverse" strand seems to be transcribed as efficiently in wild-type cells as in heterochromatin-deficient cells (Volpe et al. 2002). Furthermore, RNAP II occupancy at reporter genes that have been inserted at the silent mating type locus or pericentromeric DNA repeats does not substantially increase in cells in which heterochromatin is disrupted and silencing of the reporter genes abolished (Buhler et al. 2007; M. Bühler and Y. Shimada, unpublished data). This suggests that transcriptional gene silencing (TGS) mechanisms might cooperate with RNA decay mechanisms in order to keep heterochromatic regions silent. Importantly, this RNA degradation seems to be different from classical posttranscriptional gene silencing (PTGS), since it depends on the status of the chromatin from which the RNA is transcribed and is therefore referred to as co-transcriptional gene silencing (CTGS; Buhler et al. 2006, 2007; Buhler and Moazed 2007) (Fig. 3).

At heterochromatic loci where RNAi is essential for silencing, RNA degradation could theoretically be mediated by the RNAi machinery (Buhler et al. 2007; Figs. 1 and 3). Consistent with this idea, recombinant fission yeast Ago1 has siRNA-guided endonucleolytic activity ("slicer" activity), and siRNAs originating from centromeric RNAs as well as centromeric reporter gene insertions have been detected (Irvine et al. 2006; Buhler et al. 2007; Buker et al. 2007). Therefore, it is possible that centromeric transcripts, including those originating from centromeric reporter gene insertions, are "sliced" by an Ago1-containing complex (Fig. 4b). This RNA decay is distinct from RNAi-mediated PTGS since it is allele-specific (Fig. 3b). Cells expressing two alleles of a gene, one heterochromatic and the other euchromatic, only silence the former (Buhler et al. 2006). In contrast, siRNA-loaded RISC acts in *trans* to induce degradation of cytoplasmic target RNAs during PTGS (Zamore 2001). CTGS is the simplest model to explain allele specificity, in which the Ago1-containing RITS complex targets nascent transcripts and mediates their degradation.

Silencing of heterochromatin at other loci, however, can function independently of the RNAi pathway. Furthermore, highly unstable ncRNAs from heterochromatic regions can be detected in *S. cerevisiae*, which has entirely lost the RNAi pathway (Wyers et al. 2005; Houseley et al. 2007; Vasiljeva et al. 2008). This suggests that CTGS is likely to be a conserved RNA turnover mechanism that can also function independently of the RNAi pathway to keep heterochromatin silent (Fig. 3). Importantly, recent work demonstrated that the nucleolytic activity can be provided by the exosome (Houseley et al. 2006, 2007; Buhler et al. 2007; Murakami et al. 2007; Vasiljeva et al. 2008; Wang et al. 2008; Fig. 1b). Whatever the exact mechanism of degradation, this seems to be mediated by a specialized polyadenylation complex referred to as TRAMP (Trf4-Air1/Air2-Mtr4 polyadenylation) that plays central roles in surveillance mechanisms that monitor RNA quality (Fig. 5). At least two TRAMP complexes exist in *S. cerevisiae*. Each comprises a noncanonical polyA-polymerase (either Trf4 or Trf5), a putative RNA-binding protein (either Air1 or Air2), and a putative helicase (Mtr4) (Lacava et al. 2005; Vanacova et al. 2005; Wyers et al. 2005). In *S. pombe*, the Trf4/5 homologue Cid14 forms an equivalent TRAMP complex with Air1 and Mtr4 (Buhler et al. 2007; Fig. 5a). A high degree of conservation of all known yeast TRAMP subunits suggests that humans have functional TRAMP complexes as well (Houseley and Tollervey 2008). Importantly, exosome and TRAMP mutant yeast strains are the first examples that show loss of heterochromatic gene silencing, without any obvious defects in heterochromatin formation (Fig. 1b). This further corroborates the concept of CTGS as a heterochromatic gene silencing pathway acting downstream of heterochromatin assembly (Fig. 3).

The role of polyadenylation in CTGS.

Polyadenylation in Bacteria and Archaea stimulates RNA degradation and is conceptually similar to poly-ubiquitylation in targeting proteins for degradation (Slomovic et al. 2008). A similar mechanism has been proposed for eukaryotes. In this case, TRAMP complexes would add short polyA-tails to aberrant RNAs (Fig. 5b). Such short polyA-tails serve as a good, unstructured substrate for the exosome to start degradation of the aberrant RNA (Houseley et al. 2006). Strains lacking the exosome component Rrp6 accumulate TRAMP-specific polyadenylated precursors of many ncRNAs in *S. cerevisiae* (Wyers et al. 2005). Furthermore, silencing of certain heterochromatic genes in *S. pombe*

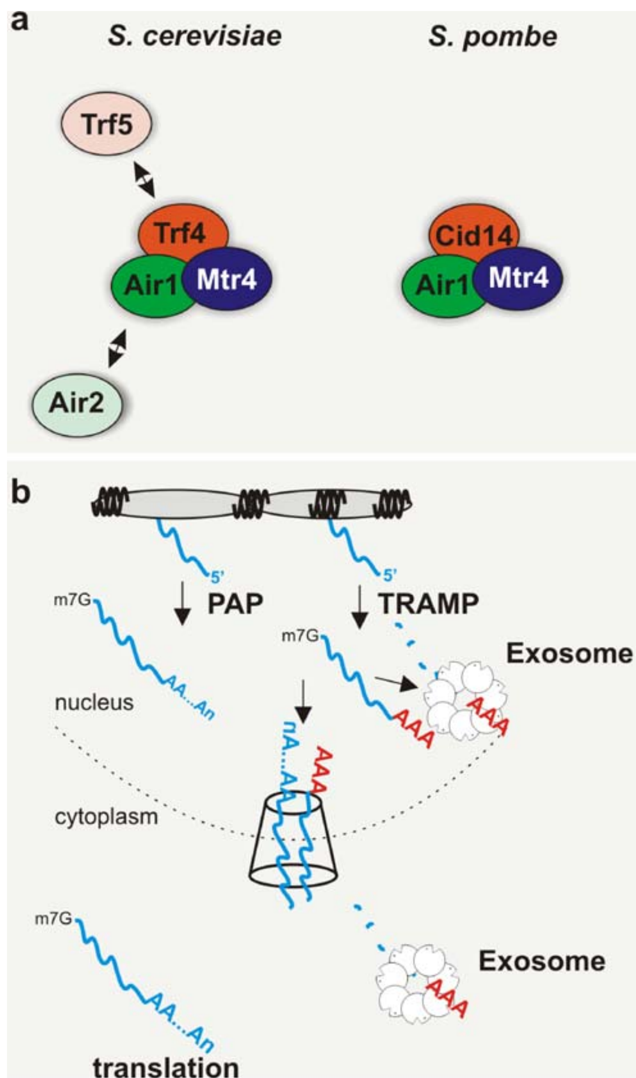


Fig. 5 RNA degradation mediated by TRAMP. **a** Trf4/5-Air1/2-Mtr4 polyadenylation (TRAMP) complexes have been purified from *S. cerevisiae* and *S. pombe*. Cid14 is the functional *S. pombe* homolog of *S. cerevisiae* Trf4/5. Both Trf4/5 and Cid14 have been demonstrated to possess polyadenylation activity. **b** Model for heterochromatic gene silencing mediated by TRAMP and the exosome. RNAs transcribed from heterochromatic regions are identified by TRAMP and marked as aberrant with a short polyA tail. This serves as a signal for the exosome to degrade the RNA. The site of degradation remains elusive. *PAP* canonical polyA polymerase

depends on the polyadenylation activity of Cid14 (Buhler et al. 2007). Therefore, I propose a model in which heterochromatin interferes with normal RNA processing to generate aberrant RNAs. These are tagged with short polyA-tails by TRAMP and targeted for degradation by the exosome. Whereas "marking" of heterochromatic transcripts by TRAMP is most likely to occur at the site of transcription (Houseley et al. 2007), degradation may occur elsewhere (Fig. 5b). In principle, heterochromatic RNAs could be degraded at the site of transcription, which is referred to as *cis*-PTGS (Grewal and Jia 2007b). This is supported by findings in *S. cerevisiae* that TRAMP and the exosome component Rrp6 can be co-transcriptionally recruited to the locus of unstable ncRNAs and mRNA transcripts with defects in 3′ end processing, respectively (Hilleren et al. 2001; Houseley et al. 2007). Furthermore, the *Drosophila* exosome was shown to stably associate with RNAP II (Andrulis et al. 2002). However, it cannot be ruled out that RNAs are marked as "aberrant" before they leave the site of transcription and therefore could be degraded elsewhere, possibly even in the cytoplasm (Fig. 5b). A short polyA-tail added by TRAMP could serve as such a mark and would thus be an elegant solution to couple cytoplasmic degradation to nuclear transcription. It should be noted, however, that TRAMP-mediated degradation can also function independently of polyadenylation, as the catalytic activity of Trf4 is dispensable for the degradation of certain substrates in *S. cerevisiae* (Houseley et al. 2007; Rougemaille et al. 2007). It turns out that polyadenylation is particularly important for the degradation of structured RNAs (Vanacova et al. 2005). Hence, it is also possible that TRAMP subunits themselves, rather than a polyA-tail, mark their substrates for degradation and that polyadenylation is only required for the degradation of highly structured substrates.

Nuclear RNA surveillance-maintaining genomic integrity

RNA turnover mechanisms are part of an elaborate eukaryotic surveillance system that evolved to monitor the quality of the transcriptome (Doma and Parker 2007). Translation-dependent mechanisms such as nonsense-mediated mRNA decay act in the cytoplasm to control the quality of open-reading frames and thereby prevent the production of potentially malfunctioning proteins. What about RNAs that cannot be translated? Many newly identified ncRNAs in genome-wide studies appear to be short-lived and are not detected by conventional RNA analyses as they seem to be processed by nuclear RNA turnover pathways. Studies of yeast have established that these RNA surveillance mechanisms are important for genomic stability. Double mutants of Trf4 and topoisomerase I show defects in rDNA condensation and mitotic segregation (Sadoff et al. 1995; Castano et al. 1996; Wang et al. 2000; Edwards et al. 2003). Similarly, *S. pombe* Cid14 mutants have abnormal nucleoli and suffer from defects in chromosome segregation (Win et al. 2006). Similar effects have been reported for exosome mutants (Ohkura et al. 1988; Kinoshita et al. 1991).

So how could ncRNAs pose a threat to the cell at all? Aberrant ncRNAs may be deleterious because they accidentally induce epigenetic changes by recruiting chromatin modifiers (Fig. 2). Therefore, it is important for a cell to efficiently remove such RNAs. Alternatively, unwanted

ncRNAs could sequester factors that would be used elsewhere, as recently exemplified in *S. pombe*. Although deletion of Cid14 decreases centromeric siRNA production, recent data suggest that Cid14 is only indirectly involved in the RNAi pathway (Buhler et al. 2007, 2008). High-throughput sequencing of siRNAs has shown that the levels of centromeric siRNAs are reduced in *cid14*-deficient cells, while the levels of other small RNAs increase dramatically. Moreover, RNAs usually processed by TRAMP now enter the RNAi pathway suggesting that the RNA surveillance machinery prevents abundant, nonspecific RNAs from becoming substrates of siRNA generation. Thus, aberrant RNAs may have deleterious effects by interfering with the generation of endogenous siRNAs or serving as templates to generate new siRNAs with the potential to silence genetic information. One prominent new class of siRNAs in *cid14*-deficient cells includes those that match ribosomal RNA sequences. Interestingly, recent analyses in *S. pombe* have revealed a role for Cid14 in maintaining the integrity of the rDNA repeats (Wang et al. 2008), raising the possibility that ribosomal siRNAs produced in *cid14* deficient cells have negative effects at the rDNA locus. However, a function for Trf4 in rDNA copy number control has also been demonstrated in *S. cerevisiae* (Houseley et al. 2007), suggesting that the genomic instability at the rDNA locus is more likely to be a direct consequence of the lack of Cid14/Trf4 and not induced by siRNAs.

Concluding remarks

In this review, I have summarized the emerging evidence showing that RNA turnover mechanisms are also relevant to chromatin-dependent regulation of gene expression and genome stability. Chromatin architecture as well as epigenetic memory can be regulated by RNA-directed processes at many levels by a wide array of mechanisms. Although the details are not known, abundant evidence suggests that RNA turnover mechanisms can regulate gene expression at the level of chromatin. Although the mechanistic details differ, RNA "talks" to chromatin in a large number of biological systems, only a few of which have been discussed here more thoroughly. No matter whether biologically meaningful or not, the overwhelmingly widespread transcription of eukaryotic genomes is subject to thorough nuclear surveillance ensuring genome integrity. Dissecting mechanisms linking RNA turnover to chromatin and distinguishing functional ncRNAs from transcriptional noise are demanding but exciting tasks, likely to keep researchers captivated for many years to come.

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