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RNA silencing and genome regulation

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Closely related RNA silencing phenomena such as posttranscriptional and transcriptional gene silencing (PTGS and TGS), *quelling* and RNA interference (RNAi) represent different forms of a conserved ancestral process. The biological relevance of these RNA-directed mechanisms of silencing in gene regulation, genome defence and chromosomal structure is rapidly being unravelled. Here, we review the recent developments in the field of RNA silencing in relation to other epigenetic phenomena and discuss the significance of this process and its targets in the regulation of modern eukaryotic genomes.

Introduction

RNA silencing is a general term for a particular collection of phenomena in which short RNA molecules trigger repression of homologous sequences. It is a highly conserved pathway, found in a large variety of eukaryotic organisms, and its main characteristic is the use of small RNA molecules of 21–28 nucleotides that confer high specificity to the target sequence. Originally, it was described as part of a 'co-suppression' phenomenon in plants [1–3] or 'quelling' in *Neurospora crassa* [4] and was later attributed to a posttranscriptional gene silencing process (PTGS; see Glossary) occurring in the presence of complementary RNA molecules that would bind and form double-stranded RNA [5]. A closely related effect described in *Caenorhabditis elegans* as 'RNA interference' (RNAi) [6,7] also requires long double-stranded precursor RNAs to induce and sustain efficient posttranscriptional repression of homologous sequences.

In RNA silencing, double-stranded RNA (produced by various mechanisms) enters the 'canonical pathway' after cleavage into small (21–28 nt) RNA duplexes by the helicase/RNase-like III *Dicer* [8]. Following unwinding, a single-stranded small RNA (small interfering RNA: siRNA) becomes part of protein complexes in which PAZ/PIWI domain proteins (PPD or *Argonaute*) are central players [9,10] (Figure 1a,b). These RNA-induced silencing complexes (RISC) then target homologous mRNAs and exert silencing either by inducing cleavage ('slicing') or, as in the case of micro-RNA-loaded RISC (see below), by also eliciting a block to translation (Figure 1c,e). RNA-dependent RNA polymerase (RdRP) also plays a role in nematodes [11], plants [12,13] and fungi [14,15] but is apparently not required or detectable in the genomes of flies and vertebrates. RdRP amplifies the RNAi/PTGS response by generating more double-stranded RNA from single-stranded targets that can then enter and continue to stimulate the RNA silencing pathway (Figure 1a). This positive-feedback system is crucial in plants and worms to amplify the siRNA signal transmitted from cell to cell and to mount a systemic form of silencing [16,17].

It is now evident that the core machinery required for RNA silencing plays crucial roles in cellular processes as diverse as regulation of gene expression, protection against the proliferation of transposable elements and viruses and modifying chromatin structure. While it appears that the basic pathway has been conserved, specialization has adapted the common RNA silencing machinery for these different purposes. This is implied both by the diversity of *Argonaute* proteins found in different species, such as *C. elegans* (more than 20), *Arabidopsis thaliana* (10) [18] and humans (8) [19] and also by the distinct phenotypic effects that arise from disrupting different *Argonaute* genes [20,21]. This specialization is most obvious in plants, which also encode multiple RdRP and Dicer-like proteins that are relevant for distinct small RNA pathways [22]. Here, we discuss these different pathways and the various levels through which small RNAs can influence the activity of the genome.

Regulation of gene expression – microRNAs

MicroRNA regulation is a clearly specialized branch of the RNA silencing pathway that evolved towards gene regulation, diverging from conventional RNAi/PTGS. MicroRNAs are a specific class of small RNAs that are encoded in gene-like elements organized in a characteristic inverted repeat. When transcribed, microRNA genes give rise to stem–looped precursor RNAs from which the

Glossary

5-Me-C 5-Methylcytosine
DNMT DNA *de novo* methyltransferase
dsRNA Double-stranded RNA
HDAC Histone deacetylase
HMT Histone methyltransferase
H3K9ac Histone H3 acetylated on lysine 9
H3K9me2/3 Histone H3 di/tri-methylated on lysine 9
LTR Long terminal repeat
PEV Position effect variegation
PPD PAZ/PIWI domain
PTGS Posttranscriptional gene silencing
RdRP RNA-dependent RNA polymerase
RISC RNA-induced silencing complex
RITS RNA-induced transcriptional silencing complex
RNAi RNA interference
siRNA Small interfering RNA
TE Transposable element
TGS Transcriptional gene silencing
TIR Terminal inverted repeat

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microRNAs are subsequently processed [23–25]. The released miRNAs are incorporated into RISC-like complexes containing a particular subset of Argonaute proteins that exert sequence-specific gene repression. The presence of these small RNAs was originally found to govern the expression timing of specific sets of developmental genes in *C. elegans* [26]. In the past few years, the number of genes encoding miRNAs identified in various systems has grown enormously, and it is now clear that hundreds of miRNAs regulate the expression timing of a large, but still underestimated, pool of genes [27,28]. A major challenge that remains is the accurate and comprehensive identification of all genes regulated by microRNAs. To date, miRNAs have not been described in simpler unicellular eukaryotes, suggesting that their evolution might be intimately linked to gene regulation in multicellular organisms. However, RNA-mediated silencing is present in both multi- and unicellular eukaryotes and performs a variety of other key functions.

Defence – transposable elements and viruses

RNA silencing was first recognized by its effect on the expression of multicopy transgenes. This curious phenomenon was then interpreted as a process of genome defence against foreign 'invading' sequences. In fact, it was observed in the early 1990s that, in plants, co-suppression or PTGS could play a role in defending against viral invasion [29]. Known core components of the RNAi pathway were found to be required for repressing transposable elements (TEs) in several eukaryotes:

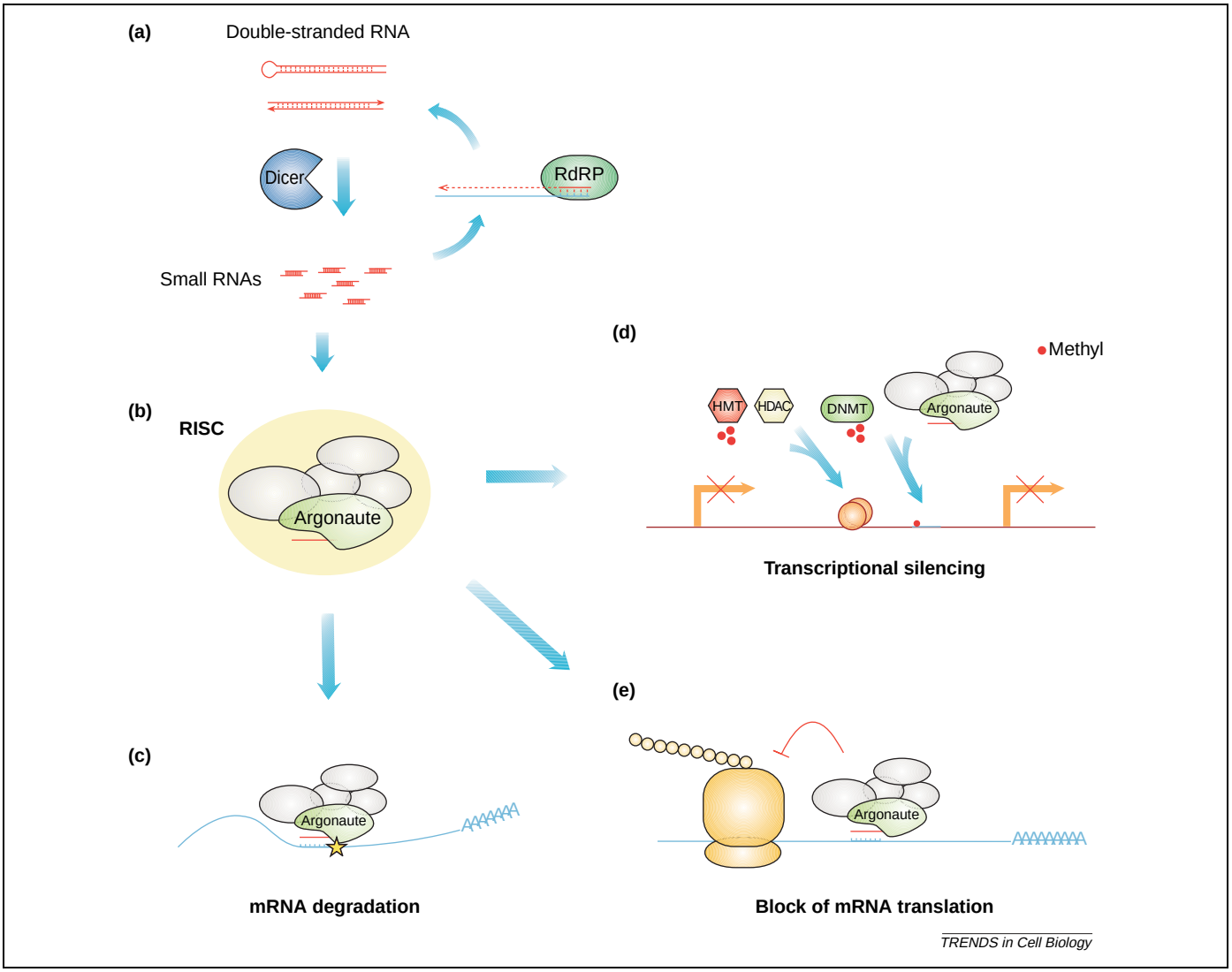

Figure 1. The different forms of RNA silencing. **(a)** Double-stranded RNA molecules derived from complementary transcripts or from a stem–loop structure are recognized by Dicer (in blue) and cleaved into small RNAs. The RdRP protein (in green) acts in a positive-feedback loop for the siRNA signal by producing complementary strands of the target RNA molecule, either by recognition of its 'aberrant' nature or by using small RNAs as primers [61], thus generating more homologous double-stranded RNA for Dicer processing. **(b)** The RISC complex, primed with a small RNA, can exert silencing in a variety of forms. In all cases, the small RNA confers target specificity, whereas the protein components within the RISC complex effect, or recruit mediators of, repression. **(c)** The conventional RNAi, PTGS or quelling pathway is depicted on the left, where the RISC complex associates with the target mRNA and employs the RISC 'slicing' activity of *Argonaute* protein to cleave the transcript [82,83] **(d)**. RISC can also induce transcriptional gene silencing (TGS) by using the siRNA specificity to direct silent chromatin modifications over homologous DNA loci. Target DNA (magenta line) and overlapping histones become methylated through the recruitment of DNA *de novo* methyltransferase (DNMT), histone deacetylase (HDAC) and histone methyltransferase (HMT) activities by a variant of the RISC complex, which can result in the shutdown of transcription. **(e)** A typical miRNA-loaded RISC does not affect mRNA turnover but binds to the 3′-UTR of the target transcript (blue line) and effectively blocks its translation by an unknown manner. It has been found recently that specific miRNAs can direct target mRNA cleavage and that an siRNA-loaded RISC can also block mRNA translation (see above), which suggests that it is the nature of the small RNA sequence, rather than the composition of RISC, that defines which process occurs [84,85].

C. elegans [30,31], *Chlamydomonas reinhardtii*. [32], *Drosophila melanogaster* [33] and now *Mus musculus* [34]. Since then, reports of small RNAs homologous to TE sequences have expanded to a larger variety of organisms [35–37], clearly implicating RNA silencing as both a conserved and widespread form of regulating transposon activity.

The term 'transposon' or 'transposable element' (TE) defines a selfish DNA entity capable of using a genome as an ecosystem where it can survive and proliferate. This definition can also be applied to a viral DNA sequence integrated in the host genome. TEs are powerful genome-destabilizing factors for a variety of reasons. Transposition events frequently induce positional mutations at the insertion and excision sites, and extensive TE activity favors recombination events that can lead to dramatic chromosomal rearrangements [38]. Although TEs are believed to contribute significantly to genome evolution, uncontrolled TE activity can be potentially detrimental to the fitness of the host [39,40]. Therefore, mechanisms that silence TEs have evolved to stabilize the genome.

Transposable elements and heterochromatin

In general, TEs and related DNA sequences are often found in chromatin domains that are transcriptionally silent and structurally distinct from the open euchromatic regions [41]. These heterochromatic regions have conspicuous features, which can include dense methylation of DNA (5-methylcytosine; 5-Me-C), hypo-acetylation of lysine residues in the N-terminal tails of histones H3 and H4 and methylation of specific lysine residues such as lysine 9 on histone H3 (H3K9me2/3). Some of these modifications create binding sites for particular proteins that, in general, promote transcriptional repression and the formation of silent chromatin or heterochromatin [42,43]. The packaging of TEs into heterochromatin represses their expression and blocks their ability to transpose. Hence, the assembly of TEs into this 'silent' chromatin is an effective way of inhibiting TE proliferation that has been employed by many eukaryotes. Because this form of regulation based on chromatin structure is independent of the primary DNA sequence, specialized mechanisms for recognizing these parasitic elements must be required to selectively trap them in heterochromatin. It is now evident that the formation of this heterochromatin is linked to the process of RNA silencing.

RNA silencing reaches chromatin

The same pathway that acts to repress genes posttranscriptionally can enforce modification of homologous chromatin in a way that alters its structure and consequently its function. Transcriptional gene silencing (TGS) (Figure 1d) was initially observed in plants and was associated with repression of exogenously introduced transgenes and viral suppression [44]. Remarkably, the presence of dsRNAs homologous to the promoter or the coding region in the DNA result in robust silencing that persists even after the trigger has been removed [45,46]. The TGS response triggered by double-stranded RNAs results in the complete transcriptional shutdown of a gene and is associated with *de novo* DNA methylation on the homologous DNA sequences.

TGS indeed appears to be employed to silence/inhibit the activity of several classes of TEs in plant genomes. Apart from the characteristic Dicer-like, *Argonaute* and small RNAs, the persistence of TE DNA methylation in *Arabidopsis thaliana* requires chromatin-modifying factors such as histone deacetylases, methyltransferases, DNA methyltransferases and *SWI2/SNF2*-related chromatin remodeling components – some of which are also required for the persistence of TE siRNAs [37] and for PTGS [47]. This underscores the intimate relationship between RNA silencing and chromatin regulation in plants and their role in repression of TEs [37,48,49].

Furthermore, it is becoming increasingly clear that TGS is a common form of general RNA silencing rather than a particular feature of RNA-mediated silencing in plants. Small RNAs are also known to direct chromatin modifications in other organisms. For instance, in the ciliate *Tetrahymena thermophila*, small RNAs are used to mark particular DNA sequences for elimination from the transcriptionally active macronucleus, most of which are of a repetitive nature [86,87]. In the fission yeast *Schizosaccharomyces pombe*, it has been clearly demonstrated that RNA silencing acts to facilitate chromatin modifications over repetitive sequences for the purpose of TE silencing, as in plants, but also impacts upon basic chromosomal functions [15,50,51].

Chromosomal function – the fission yeast centromere

In fission yeast, silent chromatin assembled over the outer repeat arrays at the centromeres is required for proper chromosome segregation during mitosis. The high density of cohesin complexes associated with this silent chromatin ensures that sister chromatids are held tightly together at centromeres after DNA replication and up until the onset of anaphase [52,53]. RNA silencing must play a direct role in this process in fission yeast as deletion of any gene encoding key RNAi components leads to defects in chromosome segregation. In fact, RNAi effector proteins are required to establish and maintain this pericentromeric heterochromatin and thus prevent premature sister-chromatid separation [15]. In addition, RNAi also acts to initiate a similar form of silencing at the mating-type locus in *S. pombe* [54]. It is thought that transcription from both strands of the outer repeats at the centromeres (*dg-dh/K-L*) and the related *cenH* element from the mating-type locus results in homologous dsRNA that then enters the RNA silencing pathway, resulting in the production of complementary small RNAs. Incorporation of these small RNAs into a variant of the RISC complex called RITS (RNA-induced transcriptional silencing complex), containing Ago1 (*Argonaute*), Chp1 (chromodomain protein) and Tas3, directs H3K9me2 methylation over homologous chromatin [55] (Figure 2). This requires an RdRP, the action of histone deacetylases and the histone methyltransferase Clr4 (SET domain protein, related to the mammalian *Suv39*) that forms a binding site for the HP1 (heterochromatin protein 1) ortholog Swi6 and Chp1 [42]. In turn, binding to H3K9me2 of Swi6 and Chp1 promotes spreading of the silenced chromatin state as well

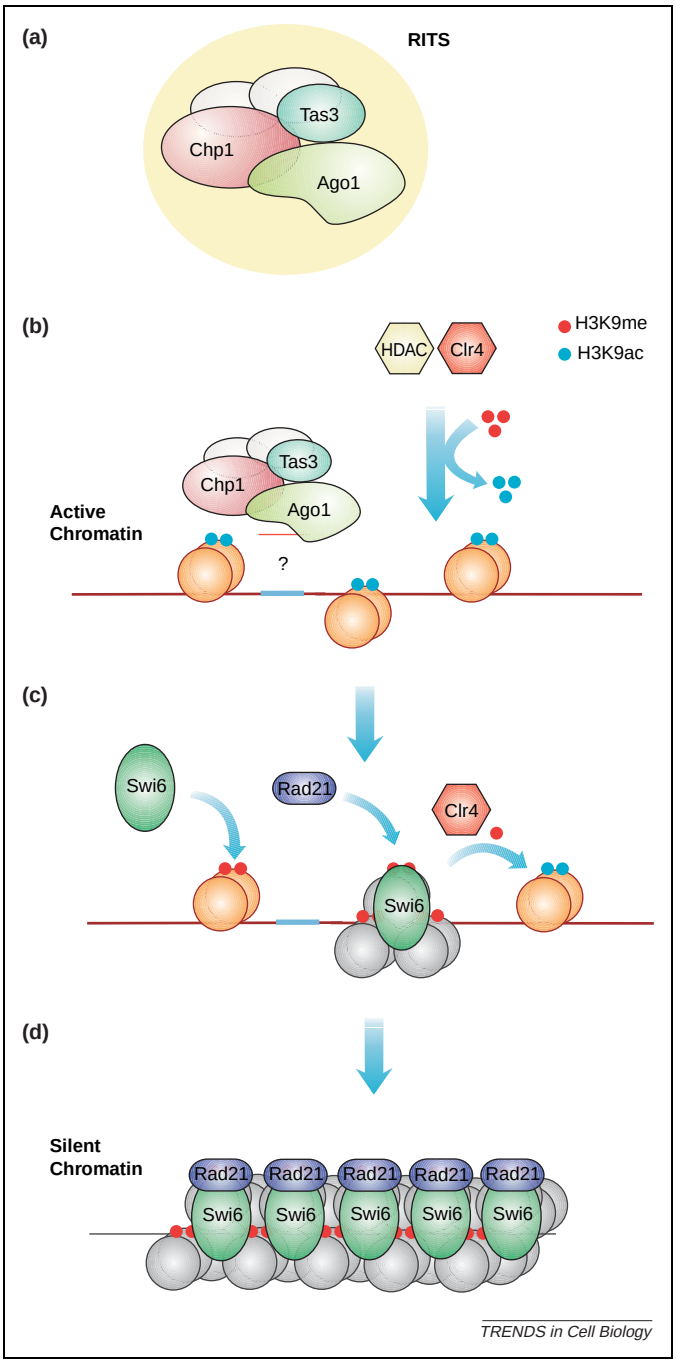

Figure 2. RNA-mediated heterochromatin formation in fission yeast. **(a)** The RISC-variant RITS complex with its known components: the *Argonaute*/PPD protein Ago1, the chromodomain protein Chp1 and Tas3. **(b)** RITS attracts Clr4 and an unknown HDAC to deacetylate and methylate histone H3-K9 over target DNA (blue line). It is still unclear whether the target recognition process involves RNA–DNA interactions between the small RNA and the target DNA or RNA–RNA interactions with a nascent transcript. **(c)** Nucleosomes bearing H3K9me are preferentially bound by Swi6/HP1, which promotes the recruitment of heterochromatin proteins, such as the cohesin subunit Rad21, and allows spreading of the heterochromatin domain to upstream and downstream regions. **(d)** This results in the assembly of a patch of heterochromatin that is rich in Swi6/HP1 and Rad21, as well as being transcriptionally silent.

as allowing the recruitment of the Rad21 cohesin and physical cohesion [52].

Expression of a synthetic hairpin RNA producing dsRNA (a conventional RNAi inducer in many systems) taps into this mechanism to promote silencing by directing histone H3 K9 methylation and recruitment of Swi6 and cohesin over a normally expressed euchromatic locus [50]. This demonstrates that the generation of siRNAs from a dsRNA precursor is sufficient to target chromatin modification to a homologous locus and also indicates that the primary DNA sequence does not play a role in specificity. Thus, the process of RNA-directed transcriptional gene silencing provides DNA targeting properties that facilitate the placement of histone modifications at specific loci for the purpose of TE repression in plants and fungi.

Repeats attract RNA silencing

To grasp the biological relevance of RNA-directed chromatin modifications, it is important to investigate the nature of the DNA sequences that generate the endogenous siRNAs that influence chromatin structure. To date, all natural targets for RNAi-mediated heterochromatin formation appear to involve TEs or repetitive DNA. This suggests that RNA silencing recognizes an intrinsic property common to these sequences in the context of centromeric function or transposon/viral control. But what could this defining characteristic be?

It has been suggested that *S. pombe* outer centromeric repeats, as well as the satellite sequences found around metazoan centromeres, resemble or are derived from TE sequences. Some centromeric repeats are bound by CENP-B proteins, which bear close resemblance to transposases encoded by the pogo superfamily of TEs [56]. Moreover, regions in the terminal inverted repeat (TIR) of the *Tigger* TE match almost perfectly the DNA binding motif recognized by CENP-B in human centromeric α-satellite repeats [56]. The implication is that perhaps all the currently known targets for RNAi-mediated heterochromatin formation are derived from TEs. Thus, in *S. pombe*, RNA silencing might be directed towards TE-derived repetitive DNA sequences by default. In this case, it appears that the cell has exploited a natural form of repeat silencing based on genome defence mechanisms (inhibition of transposition) to promote gene silencing. This now acts to ensure that specific chromatin structures are assembled over the outer repeat regions (flanking the kinetochore) at centromeres and the related sequences at the mating-type locus, which are now important for centromere-specific cohesion and the regulation of cell mating type.

But what triggers an RNA silencing response against such sequences? As dsRNA is the general substrate for the canonical RNAi pathway, it seems likely that a dsRNA is responsible for triggering RNA-mediated heterochromatin formation. Invariably, transcriptional activity is coupled to the transposition cycle of most TEs. Even isolated TE-derived repeats, such as solo LTRs, can remain transcriptionally active [57]. Since transcription alone is not sufficient to render such elements as targets, some process must generate a dsRNA substrate. Intuitively, two transcription events on opposite strands converging on any given sequence could generate complementary transcripts that would combine and form dsRNA (as used in various organisms to direct knockdown of gene expression: Figure 3a). Alternatively, complementary strands could be transcribed from different copies residing at distinct locations in the genome and subsequent

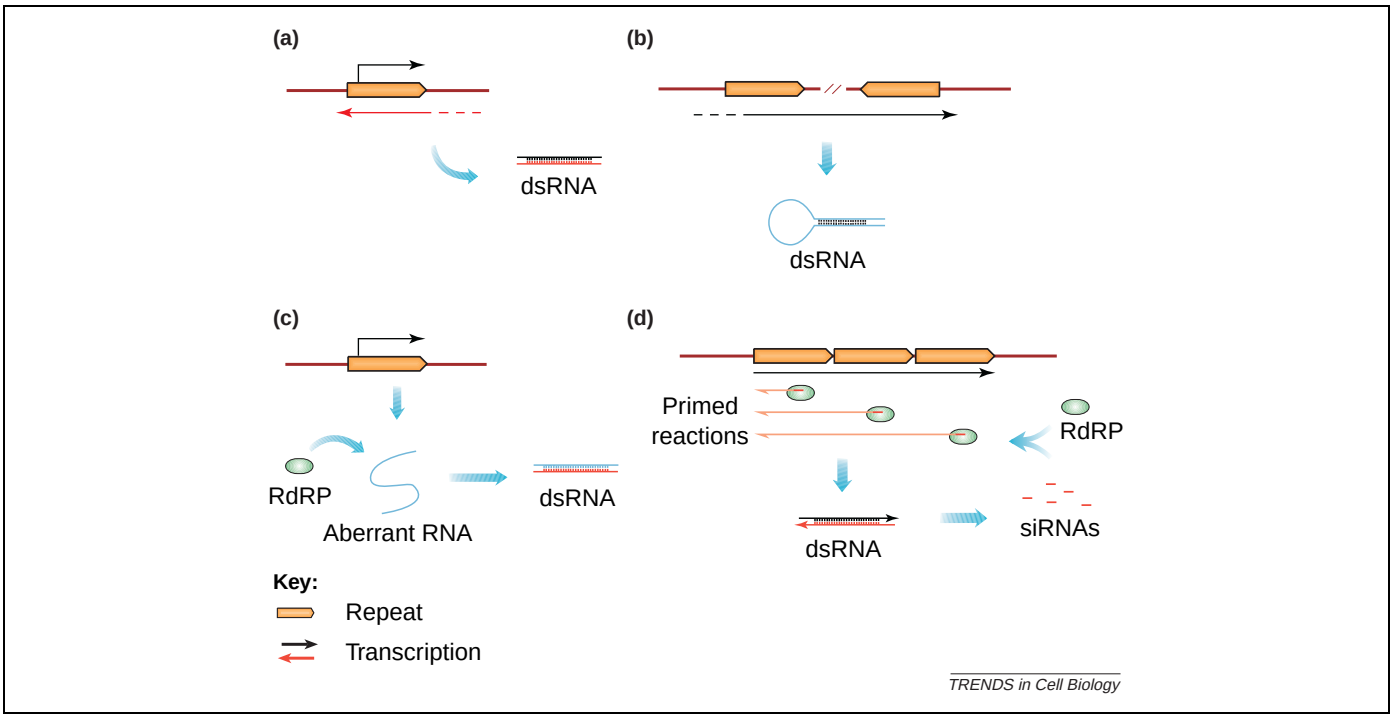

Figure 3. Possible ways by which transposable elements (TEs) and repeat dsRNA are generated. **(a)** Transcription of a repeat or TE (orange 'arrow box'), either from an internal promoter (black arrow) or leaking/originating from flanking sequences (red arrow), that occur in both sense and antisense strands can generate dsRNA. **(b)** Transcription through an inverted repeat disposition of repeats, such as the TIR repeats within DNA transposons, could give rise to an RNA molecule (in blue) that snaps back and adopts a stem–loop or hairpin structure, exposing segments of dsRNA. **(c)** RdRP recognizes the 'aberrant' nature of a TE/repeat transcript and uses it as a template to generate the complementary strand. **(d)** Transcription through a tandem array of repeats produces a transcript that bears multiple sites for production of complementary strands (red arrows) by RdRP (green) using repeat-specific siRNAs (in red) as primers. Extension of the complementary strand over multiple repeats generates a long dsRNA molecule that can be cleaved into higher numbers of repeat-specific siRNAs than in the case of an isolated repeat.

hybridization would allow the formation of a dsRNA substrate. Another simple way of obtaining dsRNA is by transcribing an inverted repeat, which produces a transcript that can snap back and form a stem–loop or hairpin structure (as with endogenous microRNA precursors: Figure 3b). This has been suggested as one source of dsRNA specific for *C. elegans* Tc1/Mariner TEs, which bear terminal inverted repeats in their structure [58]. Although siRNAs against Tc1/*Mariner* TIR repeats appear to be more abundant, transcripts from both strands of these TEs are produced, in the same way as has been observed for centromeric repeats and also interspersed LTRs in *S. pombe* [15,50,58]. The presence of siRNAs specific to most regions within these TEs in *C. elegans* suggests that full-length TE dsRNAs contribute to the induction of RNAi against these TEs. The origin of the convergent, read-through and/or complementary transcription events involved in TE dsRNA formation is obscure. They might arise from the activity of promoter sequences within the repeats, but transcriptional 'leakage' from flanking genes and from flanking cryptic promoters could contribute to the production of a TE homologous dsRNA pool.

An alternative explanation is that RdRP can in some way recognize transcripts coming from TEs or viral sources as 'aberrant' or 'foreign' transcripts and use them as templates to generate dsRNA [12,59,60] (Figure 3c). This idea is supported by the observation that the RdRP can produce dsRNA *in vitro* from a ssRNA template in a primer-independent manner [61]. More recent work suggests that transcripts lacking a 5′ cap are targeted by RdRP, although it is still possible that RdRP is attracted to other characteristics, such as premature termination or absence of polyadenylation, or a combination of features [62]. In the case of tandem repeat arrays, such as those commonly associated with pericentromeric regions, it has been suggested that this arrangement results in the production of transcripts that serve as more efficient RdRP substrates, thus ensuring the stability of the assembled heterochromatin over these regions [63] (Figure 3d). However, RdRP-independent strategies have presumably arisen in flies and vertebrates to maintain TEs and repeats under the influence of RNA silencing.

RNA-induced chromatin silencing in metazoans

SiRNAs act to target histone and/or DNA modifications to homologous sequences in plants, ciliates and fission yeast. But do noncoding RNAs play a pivotal role in gene silencing and chromatin modifications in metazoans? Clearly X-inactivation in female mammals requires expression of *Xist* RNA in *cis* to effect chromatin modifications that result in gene silencing [64]. In addition, imprinting of paternally derived *Igf2r* requires expression of the associated *Air* noncoding RNA [65]. Likewise, chromosomal rearrangements that result in antisense transcription of the gene encoding α-globin lead to DNA methylation of its promoter region and transcriptional silencing [66]. However, there is no evidence linking these phenomena to the process of RNAi. Nevertheless, several recent reports imply that the RNA pathway can mediate both chromatin modifications and gene silencing in metazoans.

As mentioned above, the placement of a gene close to domains of constitutive heterochromatin such as those residing at pericentromeric regions leads to variable expression (PEV). Unstable repression is thought to be due to the stochastic dynamics associated with heterochromatin assembly along chromatin fibres. This classic epigenetic effect can be imitated in euchromatic regions in fruit flies by arrays of a reporter gene such as *mini-white*, which also display variable expression. The RNAi/PTGS pathway affects the formation of silent chromatin over these arrays since the *piwi*, *aubergin*e (both *Argonaute* homologs) and *spindle-E* (*homeless*: an RNA helicase) mutations alleviate their silencing [67]. The most likely explanation is that siRNA derived from *mini-white* array-generated dsRNA directs the assembly of heterochromatin over the *mini-white* sequences. It is not known whether these same mutations can alleviate silencing of a marker embedded in centromeric heterochromatin, but they do result in loss of H3K9me2/3 and in the redistribution of HP1 from centromeric regions.

A link between RNAi and TE silencing is also evident in *Drosophila* as siRNAs homologous to TE, satellite and microsatellite DNA have been detected [35]. While it is not known if these small RNAs exert repression at a transcriptional level, it is clear that their cognate sequences are normally associated with heterochromatin and are subject to RNA silencing in *D. melanogaster*.

A recent study suggests that RNA silencing is also involved in sister-chromatid cohesion in vertebrates, similar to what is observed in *S. pombe*. A chicken DT40 cell line containing human chromosome 21 was engineered creating a conditional allele allowing Dicer (and thus the RNAi pathway) to be turned off [68]. Cells depleted of Dicer displayed a mitotic phenotype, with disrupted HP1 and Rad21 localization, premature sister-chromatid separation and chromosome mis-segregation [51]. This implies that RNA silencing is also involved in the formation of pericentric heterochromatin in vertebrate cells and that this acts as a platform to promote efficient cohesion at centromeres.

A more direct test of the link between RNAi and chromatin modification in metazoans has come from the application of siRNAs to human cell lines. One study demonstrated that siRNAs homologous to the promoter of an integrated GFP reporter construct can induce transcriptional silencing of the gene encoding GFP [69]. Cytosine methylation at one site within the EF1A promoter was shown to increase after transfection of the homologous siRNAs. The effect was reversed by treatment with inhibitors of DNA methylation and histone deacetylation. A more comprehensive study conducted by Kawasaki *et al.* [70] underscored the ability of siRNAs to induce DNA and chromatin modifications in human MCF7 and mammary epithelial cells. Both transfection of siRNAs or expression of hairpin precursor RNAs homologous to the promoters of either the *E-cadherin* or *erbB2* genes resulted in effective gene silencing accompanied by DNA methylation and histone H3 K9 methylation.

To recap, chromatin modifications can be directed by small RNAs in fungi, plants and metazoans. The process involves components of the RNAi machinery that appear to be utilized to provide sequence specificity by homing in on targets bearing homology to siRNAs carried by the RNAi effector complex. This is related to the process that acts on transcripts derived from outer centromeric repeats in fission yeast and appears to be a conserved mechanism that acts at centromeric regions in vertebrates to ensure tight physical cohesion and normal chromosome segregation.

Transposable elements and repeats can influence gene regulation

The action of RNA silencing on centromeric repeat transcripts is important in defining structures and functions associated with these chromosomal regions. However, a large proportion of repetitive sequences are not concentrated in pericentromeric regions but are scattered throughout the genome. TE insertions are known to have dramatic effects on expression levels of surrounding genes by disturbing the transcriptional activity of the affected regions. Moreover, it now seems likely that observed changes of gene expression associated with TEs could result from transposon silencing events involving the formation of silent chromatin on such elements [71]. In light of this, it is interesting to re-evaluate the action of RNA silencing and TEs in terms of their consequences for gene activity.

A clear demonstration of transposon silencing affecting gene expression comes from the analyses of retrotransposons containing long terminal repeats (LTRs) in *S. pombe*. Most of the ~300 Tf1/2 LTRs are dispersed along chromosome arms as solo elements in various states of decay. Only a few (26) remain associated with full-length retrotransposons [72]. In one case, repression of a few nearby meiotically regulated genes during vegetative growth was shown to be connected to RNA silencing by LTR sequences [50]. The mechanism of this repression has not been completely unravelled, but LTRs are subjected to RNA-mediated chromatin silencing, resulting in H3K9me2 methylation and Swi6 association. One possibility is that binding of Swi6/HP1 to H3K9me2 promotes recruitment of additional Swi6/HP1 and chromatin modification factors to surrounding histones, which stabilizes the silent chromatin domain and also allows it to expand laterally and engulf neighboring genes [73–75]. Indeed, Swi6 was found to be required for repression of these nearby meiotic genes, suggesting that the LTR acts as a nucleation site from which silent chromatin spreads out and represses nearby genes, in the same way that it can spread from a region of a gene targeted by artificially induced siRNAs [50].

In plants, H3K9me and DNA methylation seem to be largely confined to transposon sequences or promoters of silenced genes and do not in general engulf neighbouring genes [49,63]. However, repression of a few genes in *Arabidopsis* that harbor insertions of repetitive sequences was found to be dependent on *DDM1* (a *SWI2/SNF2* chromatin remodeling factor required for maintenance of DNA methylation and H3K9me over TE sequences) [76].

Repeats and silent chromatin modifications are intimately linked in mammalian somatic cells – tandemly repeated satellite DNA as well as mobile genetic elements

and their DNA remnants are characterized by extensive histone deacetylation, H3K9, H3K27 and H4K20 methylation as well as 5-Me-C DNA methylation [77,78]. RNA-mediated silencing is fully active during early stages of embryonic development and cellular differentiation but inactive during the later stages of development and in the soma. Thus, it remains active during the stages where epigenetic reprogramming processes occur, before the establishment of cell fate [79]. The presence of Dicer is crucial for mouse embryonic viability, but it is also involved in repressing LTR-retrotransposons in mouse pre-implantation embryos [34,80]. Recent investigations of chromatin status over repetitive elements in the mouse genome have revealed that the modifications associated with TEs and related interspersed LTRs display a dynamic behaviour throughout differentiation stages of embryonic stem cells [78]. This is in contrast to the relatively stable H3K9me3 and H4K20me3 modifications associated with pericentromeric repeats. Given that RNA-dependent heterochromatin assembly appears to occur in vertebrates, it is seems quite likely that RNA silencing plays a key role in establishing transcriptional repression of these sequences upon determination of cell fate [81]. However, it is not clear whether transcriptional repression of these sequences plays a role in the process of cellular differentiation.

Concluding remarks

Noncoding RNA is the central player of an ancient and conserved form of silencing. Although the different forms of RNA silencing were initially unearthed as seemingly distinct phenomena, basic machinery is held in common between PTGS, TGS, *quelling* and RNAi. In addition, these same components are conserved in a large variety of organisms and thus must have arisen early in eukaryotic evolution. Since its discovery several years ago, the biological relevance of RNA-directed silencing mechanisms is rapidly becoming clear, and it is already evident in distinct processes such as chromosomal structure, genome defence and gene regulation. Despite the large body of information available on RNA silencing pathways, important questions still remain unanswered. Issues such as the total number of endogenous targets of siRNAs and microRNAs in the genome or the amount of crosstalk between the different manifestations of RNA silencing are currently being addressed and might yet reveal further surprises.

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