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Minireview

RNAi-directed assembly of heterochromatin in fission yeast

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Abstract Heterochromatin is an epigenetically heritable and conserved feature of eukaryotic chromosomes with important roles in chromosome segregation, genome stability, and gene regulation. The formation of heterochromatin involves an ordered array of chromatin changes, including histone deacetylation, histone H3-lysine 9 methylation, and recruitment of histone binding proteins such as Swi6/HP1. Recent discoveries have uncovered a role for the RNA interference (RNAi) pathway in heterochromatin assembly in the fission yeast *Schizosaccharomyces pombe* and other eukaryotes. Purification of two RNAi complexes, RITS and RDRC, from fission yeast has provided further insight into the mechanism of RNAi-mediated heterochromatin assembly. These discoveries have given rise to a model in which small interfering RNA molecules act as specificity factors that initiate epigenetic chromatin modifications and double strand RNA synthesis at specific chromosome regions.

Keywords: Heterochromatin; RNAi; RITS; Clr4; Histone methylation; Epigenetics; Transcriptional gene silencing

1. Introduction

RNAi was initially defined by Fire et al. [1] as a process that is triggered by double strand RNA (dsRNA) and silences the expression of genes complementary to the dsRNA in *Caenorhabditis elegans*. We now know that RNAi is conserved from *Fungi* to mammals and is mechanistically related to other RNA silencing processes previously described in *Fungi* and plants, like quelling, co-suppression, and post-transcriptional gene silencing (PTGS) [2,3]. The RNAi pathway plays important roles in protecting the cell against viruses and repetitive DNA elements [4] (also see Ding and Li in this issue). Furthermore, a wide variety of cellular processes such as the regulation of development, growth, differentiation, apoptosis, and cancer require the RNAi pathway [5,6] (Plasterk in this issue).

The initiation of RNAi-mediated silencing involves the processing of double stranded RNA to small ~22 nucleotide interfering RNA (siRNA) by the ribonuclease III, Dicer [7] (reviewed by Hammond in this issue). Dicer also processes cellular hairpin RNA, transcribed from the genome by RNA polymerase II, into micro RNAs (miRNAs), which are similar in size to siRNAs [5,8]. Both types of small RNAs then act as specificity factors that guide effector complexes to complementary sequences. The RNA-induced silencing complex (RISC) uses siRNAs and miRNAs to target corresponding mRNAs for inactivation through either degradation or translational repression [9]. In addition to this type of post-transcriptional gene silencing, the RNAi pathway acts at the transcriptional level (transcriptional gene silencing, TGS) by promoting DNA or histone modifications leading to the assembly of a repressive chromatin structure called heterochromatin [10–12]. RNAi-mediated chromatin and DNA modifications play important roles in genome stability by inhibiting undesired recombination and mobilization of repetitive DNA elements such as retrotransposons [4]. RNAi-mediated heterochromatin formation is also required for proper chromosome segregation as Swi6, a structural component of heterochromatin, recruits the cohesin complex and promotes sister chromatid cohesion at pericentromeric regions [13,14]. The role of RNAi in directing repressive chromatin or DNA modifications appears to be conserved in *Drosophila*, plants, and mammals [15]. In addition, RNAi is involved in the massive and highly specific DNA elimination that occurs in the somatic macronucleus of the protozoa, *Tetrahymena* [16,17].

Studies in fission yeast have revealed a crucial role for components of the RNAi pathway in heterochromatin assembly at centromeres and the silent mating type loci [18,19]. Biochemical studies have identified the RNA-induced transcriptional silencing (RITS) complex that uses siRNAs to target specific chromosome regions for inactivation [20]. RITS also regulates dsRNA and siRNA synthesis by recruiting an RNA-directed RNA polymerase complex (RDRC) to sites of heterochromatin assembly [21]. This review is primarily focused on how the RNAi machinery mediates heterochromatin assembly in fission yeast. Other aspects of RNAi or links between RNAi and heterochromatin in other organisms have been covered in several excellent and recent reviews as well as in this issue of *FEBS* (see [10,17,22,23]).

2. Chromosomal targets of RNAi

Schizosaccharomyces pombe heterochromatin is known to be present at centromeres, telomeres and the mating type region. *S. pombe* centromeres span over 35–110 kb of DNA. The central core region (*cnt*) of the centromere has a unique

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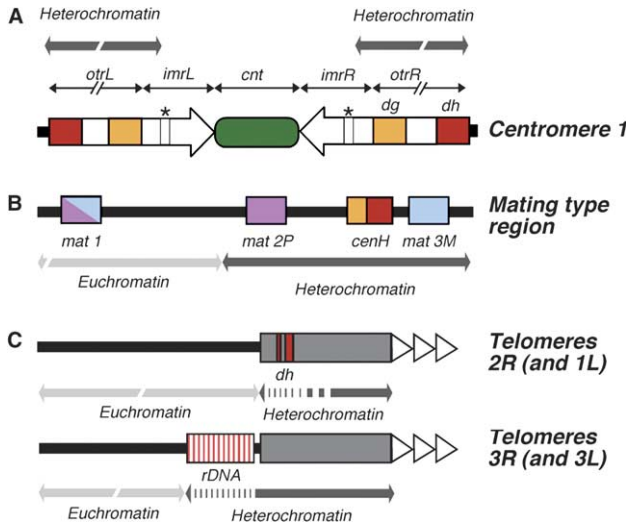

Fig. 1. DNA and chromatin organization of fission yeast centromeres, the mating type locus and telomeres. (A) Centromeres: All three *S. pombe* centromeres share a common organization, containing a central core domain (*cnt*, green), juxtaposed by innermost (*imr*, open arrows) and outermost (*otr*) repeats; *imr* DNA sequence is quasi identical on both side of a centromere, but different from one centromere to another, and contains tRNA genes (asterisk) ,*otr* DNA contains tandem *dh* and *dg* repeats (dark and light brown, respectively). The number of *dh* and *dg* repeats and their orientation varies from one centromere to another; only one copy of *dh* and *dg* is present on both sides of centomere 1 (shown in A), whereas centromeres 2 and 3 (not shown) have from one to seven repeats on each side. (B) The mating type locus: This region contains a domain known as *cenH*, which is about 3 kb in size and shares over 96% identity with a *dh*/*dg* centromeric repeat. Mating type heterochromatin spans over a 10 kb DNA region that encompasses Mat2P and Mat3M. (C) Telomeres: Sequencing of telomeric DNA regions has not yet being completed, triangles denote telomeric DNA repeats, DNA fragments with high identity (97%) to centromeric *dh* repeats are present in two identical copies of a gene present in subtelomeric region (dark grey rectangular box) on the left arm of chromosome 1 and the right arm of chromosome 2. The rDNAs repeats, which are a target of RNAi, are found next to the subtelomeric regions of chromosome 3.

DNA sequence for each centromere and is flanked by two types of repeated DNA, the *innermost* (*imr*) and *outermost* (*otr*) repeats (Fig. 1). The *otr* region itself is composed of two types of repeat sequences called the *dg* and *dh* repeats. Mutations in components of the RNAi pathway lead to loss of heterochromatin at centromeres, and accumulation of sense and anti-sense transcripts that originate from the *dg*/*dh* regions [18].

S. pombe has only one gene for each of the key RNAi proteins: Dicer (Dcr1), a ribonuclease III-like enzyme that cleaves long dsRNAs into siRNAs; Argonaute (Ago1), which binds directly to siRNA; and an RNA-directed RNA polymerase (Rdp1), which synthesizes dsRNA from an RNA template (Table 1). Deletion of $dcr1^+$, $ago1^+$ or $rdp1^+$ leads to loss of silencing of a reporter gene integrated at a centromere, as well as absence of H3-K9 methylation and recruitment of Swi6 [18]. Furthermore, Rdp1 localizes to centromeric DNA and RNA in immunoprecipitation experiments [18,21,24]. These results suggest that transcription of sense and anti-sense RNA from centromeres produces dsRNA, which is then processed into siRNA. Consistent with these observations, siRNAs complementary to the fission yeast centromeric DNA were identified by sequencing of a library of small RNAs [25].

RNAi also contributes to the formation of heterochromatin at other genomic regions. The *mat2/3* mating type locus contains a DNA region, called *cenHomology* (*cenH*), that shares more than 96% identity with a portion of *dh* and *dg* centromeric repeats (Fig. 1). Unlike centromeres, disruption of RNAi only affects mating type heterochromatin when combined with deletions of other genes [19,26]. This difference is explained by the fact that two stress-response transcription factors, Atf1 and Pcr1, work in parallel to RNAi to initiate heterochromatin formation at the *mat2/3* locus [26,27]. Therefore, to completely block heterochromatin assembly at this locus, both pathways need to be disrupted. These results highlight the fact that heterochromatin formation can occur both in an RNAi-dependent and -independent manner. This might also be the case at telomeres. During normal meiosis telomeres cluster, but in RNAi mutants this clustering is impaired suggesting that RNAi plays a role in this process [28]. The subtelomeric regions of the left arm of chromosome 1 and the right arm of chromosome 2 also contain fragments of the *dh* centromeric repeat inserted in an open reading frame [29] (Fig. 1). When RNAi is disrupted the mRNA from this gene accumulates further supporting the idea that RNAi plays a role at subtelomeric DNA regions [29]. On the other hand, as for the mating type locus, disruption of RNAi does not prevent the localization of hallmarks of

Table 1
Conservation of fission yeast RNAi components in other organisms. Proteins homologous to the characterized *S. pombe* RNAi components are presented for *C. elegans*, *A. thaliana*, *Drosophila* and human

S. pombe	*C. elegans*	*A. thaliana*	*Drosophila*	Human
Dcr1	Dcr-1	Dcl-1 to -4	Dcr-1 and -2	Dcr-1
Chp1	(–)	(–)	(–)	(–)
Tas3[a]	(–)	(–)	(–)	(–)
Ago1	Rde1, Alg-1 and -2, Prg-1 and -2[b]	Ago-1 to -10	Ago-1 to -3 Piwi, Aubergine/sting	Ago-1 to -4, hPiwil -1 to -4
Rdp1	Ego1, Rrf-1 to -3	Rdr-1 to -6	(–)	(–)
Hrr1	ZK1067.2	[c]	GH20028p	KIAA1404
Cid12	Rde3[d]	[d]	[d]	[d]

(–) no apparent homolog.
[a]Tas3 shares potentially significant homology with a mouse ovary testis transcribed specific protein (NP_035152).
[b]*C. elegans* has a total of 24 Argonaute genes.
[c]*S. pombe* Hrr1 is closely related to another *pombe* gene SPAC16C9.06c, encoding a protein involved in non-sense-mediated mRNA decay. *C. elegans*, *Drosophila* and human each have one gene related to both the *S. pombe* Hrr1 and SPAC16C9.06c, whereas the *A. thaliana* genome appears to possess only genes that are more closely related to SPAC16C9.06c.
[d]Cid12 belongs to a large family of protein, which are conserved from yeast to mammals and include the classical polyA polymerases, 2′–5′ oligoadenylate synthetases, and Trf4/5 like nucleotidyltransferases.

heterochromatin like Chp1, Tas3, H3-K9 methylation, or Swi6 to telomeres, suggesting that an alternative pathway can initiate heterochromatin assembly at telomeres [19,30]. Therefore, the three main heterochromatic regions in *S. pombe* are under the control of RNAi, but unlike centromeres, the mating type region and telomeres can maintain heterochromatin in the absence of RNAi. In addition, the highly repetitive ribosomal RNA genes, which are located adjacent to telomeres of chromosome 3, are a target of RNAi-directed histone H3-K9 methylation [31].

RNAi-dependent chromatin modifications seem to also take place at genomic regions other than centromeres, telomeres, and the mating type locus. In a recent comprehensive analysis of RNAi targets in *S. pombe*, several genes that are not associated with the known heterochromatic loci were identified as possible targets for RNAi-mediated chromatin silencing [31]. Therefore, as is the case with RNAi-mediated transcriptional silencing of transgenes and some endogenous loci in *Arabidopsis* [32], RNAi-mediated heterochromatin assembly may regulate individual genes in *S. pombe*. Finally, RNAi-mediated heterochromatin modifications can also be artificially induced at normally euchromatic chromosome regions by the introduction of short dsRNA molecules into plant or animal cells [33–36]. However, in *S. pombe* the expression of a hairpin RNA, complementary to a GFP reporter gene, leads to the production of siRNAs from the hairpin and silences GFP expression at the post-transcriptional level, without any detectable effects at the chromatin level [37]. These results suggest that in *S. pombe* siRNAs may be unable to efficiently initiate de novo chromatin modifications in *trans*.

3. RITS: an RNAi effector complex that uses siRNAs to initiate heterochromatin assembly

The RITS complex was purified using an affinity tag on the chromodomain protein Chp1 [20], which had previously been shown to be a structural component of heterochromatin [38,39]. In addition to Chp1, RITS contains the sole *S. pombe* Argonaute protein, Ago1, a protein of unknown function, Tas3, and siRNA molecules [20]. Tas3 and other components of RITS are required for heterochromatic gene silencing, histone H3-K9 methylation, and recruitment of Swi6 to centromeres [20]. This complex therefore provides a direct physical link between RNAi and heterochromatin assembly. Furthermore, RITS-associated siRNAs are complementary to centromeric DNA repeats, where heterochromatin assembly is initiated [20]. In contrast to wild type cells, in dcr1Δ cells, in which siRNAs are not produced, RITS no longer localizes to centromeric repeats, and centromeric heterochromatin does not form [20]. These results demonstrate that siRNAs are required for association of RITS with chromatin and suggest that RITS uses siRNAs to target specific chromosome regions (Figs. 2 and 3). Therefore, like RISC, RITS may use siRNAs for target recognition, but unlike RISC, RITS acts in the nucleus and initiates chromatin modifications. Whether RITS has siRNA-dependent RNA cleavage activity and also mediates nuclear PTGS remain to be determined.

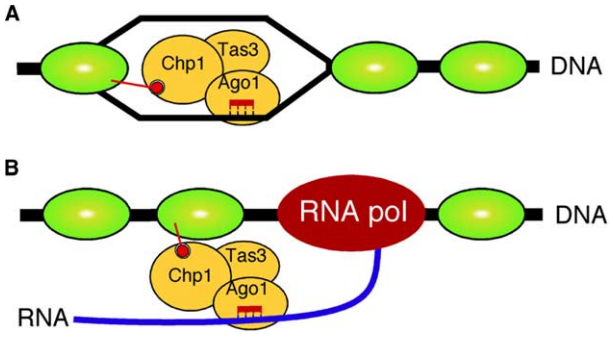

Fig. 2. Models for targeting of specific chromosome regions by an siRNA-programmed RITS. (A) RNA–DNA recognition model, (B) RNA–RNA recognition model. DNA is in black, RNA in blue, Nucleosomes in green, Methylated lysine 9 of histone H3 in red, RITS complex in brown and Chp1 chromodomain is represented as a white circle. See text for more details.

4. siRNA-mediated recognition of specific chromosome regions

Two models have been proposed to explain how RITS targets specific chromosome regions [11,12,20] (Fig. 2). In the first model, RITS-associated siRNAs directly base pair with DNA. This mechanism would require the unwinding of the DNA double helix to allow siRNA–DNA base pairing. In the second model, RITS is recruited indirectly to chromatin by base pairing interactions between siRNAs and nascent RNA transcripts at the target locus. Recent results, discussed below, provide evidence in favor of the nascent transcript model.

A direct prediction of the nascent transcript model is that RITS should associate with transcripts at the target locus in an siRNA-dependent fashion. RNA immunoprecipitation (RIP) experiments show that RITS specifically associates with non-coding centromeric RNAs but not with actin mRNA, transcribed from a euchromatin gene [21]. And indeed, as for RITS-chromatin interactions monitored by ChIP experiments, the association of RITS with non-coding centromeric RNA is Dicer- and therefore siRNA-dependent. RITS localization to chromatin requires methylation of H3-K9 by Clr4 [40]. To address if RITS-RNA interaction takes place on chromatin, Motamedi et al. asked if Clr4, the H3-K9 histone methyltransferase, is also required for this RNA association. They found that the association of RITS with centromeric RNAs does require Clr4, suggesting that these RNAs are chromatin-associated and very likely nascent transcripts [21].

5. RNA-directed RNA polymerase, dsRNA synthesis, and heterochromatin

The generation of siRNA, which plays a central role in RNAi-mediated heterochromatin formation, requires a source of dsRNA. In principle, centromeric dsRNA could originate from two different sources: (i) transcription of sense and anti-sense RNA from both DNA strands and (ii) the synthesis of a complementary RNA from an RNA template by an RNA-directed RNA polymerase (RDR). Recently, the contribution of each pathway to dsRNA synthesis in plants and fission yeast has been addressed. In plants, an RDR is required for RNAi-mediated histone and DNA modifications, and is thought to amplify siRNAs by an autoregulatory loop [3]. Fission yeast has one RDR, Rdp1, which associates with

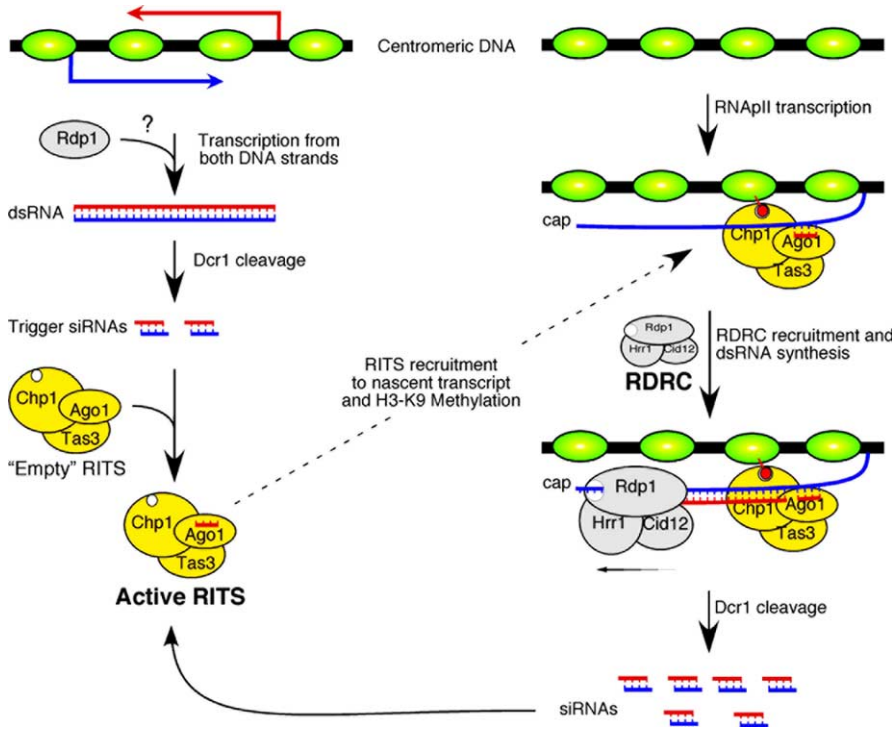

Fig. 3. Model for chromosome-associated amplification of dsRNA. In this model, the RITS complex recruits RDRC to nascent transcripts in an siRNA- and H3-K9 methylation-dependent manner, resulting in *cis* amplification of dsRNA. Trigger siRNA, presumed to be generated from the processing of the dsRNA product of sense and anti-sense centromeric transcripts, cannot be detected in *rdp*1Δ cells, raising the possibility that Rdp1 is also involved in an early step in dsRNA synthesis (left side). Once RITS is loaded with siRNA it can target complementary nascent transcripts and recruit RDRC to promote the synthesis of dsRNA in *cis* (right side). See text for details.

centromeric heterochromatin [13]. Surprisingly, in *rdp*1Δ cells, where dsRNA is still presumably produced by transcription of both centromeric DNA strands, no siRNAs are detected in RITS [21], or in total RNA Northern blots [41,42] (M. Bühler, personal communication). The dsRNA synthesis activity of Rdp1 therefore plays a primary role in producing siRNAs [21]. One explanation for these results is that two distinct mechanisms contribute to dsRNA generation in *S. pombe*. Initially, transcription of both centromeric DNA strands results in the production of a low amount of dsRNA and siRNA. These initial or "trigger" siRNA then recruit Rdp1 to homologous RNA transcripts, resulting in the amplification of dsRNA and the production of larger amounts of siRNA. Remarkably, this amplification step appears to occur in *cis* (that is at the site where heterochromatin forms) and involves the recruitment of Rdp1 by the RITS complex [21] (see Fig. 3 and below).

Additional insights into the mechanisms of Rdp1 recruitment to chromatin and dsRNA synthesis come from the biochemical purification and analysis of the Rdp1 complex [21]. The fission yeast Rdp1 is associated with two other proteins, Hrr1, a putative RNA helicase, and Cid12, a putative polyA polymerase, in a complex termed the RDRC [21]. In vitro, RDRC has an RNA-directed RNA-polymerase activity, which is required for siRNA generation and silencing in vivo [21,24]. Similar to what has been previously described for the *Neurospora crassa* Qde1 [43], the in vitro RNA polymerase activity of RDRC is independent of an RNA primer [21]. How then is specific dsRNA synthesis achieved in vivo? RDRC physically interacts with the RITS complex in an siRNA-dependent fashion [21]. This siRNA-dependent association of RDRC with RITS and their co-dependent localization to chromatin [20,21,24] suggest that in vivo RITS may directly recruit RDRC to the appropriate RNA template, providing specificity (Fig. 3). However, whether or not the siRNA is directly involved in priming Rdp1-dependent RNA polymerization is unknown. dsRNA synthesis may involve direct extension from the 3′ end of the siRNA. Alternatively, the primary role of siRNA-programmed RITS may be to recruit RDRC to the target RNA.

Although RDR enzymes are conserved in plants and *C. elegans*, they appear to be absent in *Drosophila* and mammals. It remains to be seen whether other polymerases, perhaps RNA polymerase II, can in some instances use RNA as a template and produce dsRNA. We note that the two *S. pombe* Rdp1-associated proteins, Hrr1 and Cid12, belong to conserved protein families that have members in larger eukaryotes, including *Drosophila* and human (Table 1). It would be interesting to know if Hrr1- and Cid12-like proteins in other organisms are also associated with RNA or DNA-dependent polymerases.

6. The recruitment of the histone H3-K9 methyltransferase Clr4 to chromatin and its role in siRNA production

Clr4 is a homolog of the human and *Drosophila* Suv39h methyltransferases and is required for histone H3-K9 methylation during heterochromatin assembly in *S. pombe*. Furthermore, Clr4 plays an important role in the regulation of

siRNA synthesis. In *clr4Δ* cells, purified RITS contains little or no siRNAs [21], and the level of centromeric siRNAs in total RNA Northerns are similarly diminished [41](M. Bühler, personal communication). The biochemical purifications described above suggest that Clr4 regulates siRNA generation by promoting the association of RITS and RDRC with target chromatin regions. By methylating H3-K9, Clr4 allows RITS to efficiently bind to chromatin through interactions with both the nascent transcript via siRNAs, and methylated nucleosomes via the chromodomain of Chp1 (Fig. 3). We have proposed that this stable binding is required for RDRC recruitment and dsRNA synthesis. In support of this hypothesis, in *clr4*Δ cells, RITS and RDRC no longer interact [21], and Rdp1 fails to associate with centromeric DNA or RNA [21,24]. Furthermore, mutations in the chromodomain of Chp1 that disrupt its interaction with methylated lysine K9 of histone H3 also dramatically reduce the amount of siRNA in RITS [40]. These observations suggest that dsRNA amplification occurs on the chromosome, in *cis*, and is regulated by Clr4-dependent H3-K9 methylation [21] (Fig. 3).

Although RITS and the RNAi pathway act upstream of histone H3-K9 methylation, it remains unclear how RNAi recruits Clr4 to chromatin. Some clues into this problem come from the identification of Clr4-associated proteins. Clr4 interacts with the heterochromatin protein Rik1 [44], which, like Clr4, is required for H3-K9 methylation and heterochromatin formation [45]. Rik1 shares sequence similarity with, CPSF-A and Ddb1, two nucleic acid-binding proteins. The C-terminal 245 amino acids of Rik1 are 30% identical and 47% similar to the C-terminus of CPSF-A, a subunit of the cleavage polyadenylation specificity factor that is thought to recognize the polyadenylation signal. Rik1 shares sequence similarity throughout its entire length with Ddb1 (21% identity and 43% similarity), which is involved in recognition of UV-damaged DNA. These sequence similarities raise the possibility that Rik1 directly links the RNAi machinery to histone H3-K9 methylation through the recognition of a specific nucleic acid substrate generated during siRNA targeting of specific chromosome domains. Possible targets in this speculative model include the siRNA–nascent RNA hybrid, dsRNA generated by RDRC and polyadenylated by Cid12, or even an siRNA–DNA duplex (Fig. 4).

Additional parallels between the mechanism by which Rik1 and Ddb1 are recruited to their substrates come from the biochemical purification of a Rik1/Clr4 complex. In addition to Rik1 and Clr4, this complex contains the Cullin4 (Cul4) E3 ubiquitin ligase and two previously uncharacterized proteins, named Cmc1 and Cmc2 (also called Raf1/Dos1 and Raf2/Dos2), which are all required for H3-K9 methylation and heterochromatin formation [41,42,46]. These results suggest that ubiquitination of an unknown substrate is required for heterochromatin assembly in *S. pombe*. Like Rik1, the Ddb1 protein also associates with the Cul4 ubiquitin ligase [47,48]. It has therefore been speculated that the ubiquitin ligase activity of the Ddb1–Cul4 and Rik1–Cul4 complexes may be similarly activated by recruitment to a specific nucleic acid substrate, such as damaged DNA and an siRNA-dependent structure (see above), respectively [41]. Clearly, our knowledge of the interface between RNAi and chromatin modifications is at a rudimentary stage, and many surprises still await us.

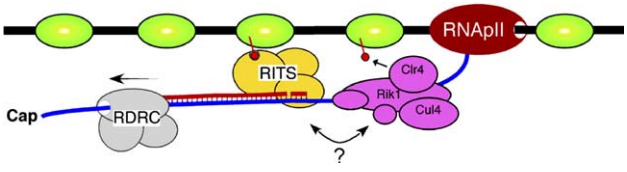

Fig. 4. A speculative model for recruitment of the dsRNA amplification machinery (RDRC) and the Rik1–Clr4–Cul4 complex to the nascent transcript by the RITS complex. See text for details.

7. RNA polymerase II and heterochromatin assembly

Recent studies suggest that the structural integrity of RNA polymerase II (RNApII) is required for RNAi-mediated heterochromatin formation in *S. pombe*. A point mutation in Rpb2, the second largest subunit of RNApII, disrupts siRNA generation and histone H3-K9 methylation in centromeric chromatin regions [49]. This *rpb2* mutant has no major effect on growth or general transcription profile, suggesting that RNApII may play a specific role in RNAi-mediated heterochromatin assembly [49]. Several RNA processing events, such as proper capping and splicing, are coupled to RNApII transcription [50]. One intriguing possibility is that a specific RNA processing event is required for siRNA-mediated binding of RITS or recruitment of other components of the heterochromatin assembly machinery. In addition to pre-mRNA processing, several posttranslational histone modifications, such as H3-K4 methylation by the Set1 complex, are mediated by enzymes that are physically associated with an elongating RNApII [51]. Therefore, another possibility is that histone H3-K9 methylation may be coupled to RNApII transcription elongation. A role for a putative RNA polymerase in RNAi has also been demonstrated in *Arabidopsis*, where RNA polymerase IV (RNApIV) is required for RNAi-mediated methylation of repetitive DNA elements [52–54]. RNApIV, which is specific to plants, is most closely related to DNA-dependent RNA polymerases. Although no activity for this enzyme has yet been detected, RNApIV may be part of a system that couples transcription to RNAi-mediated DNA methylation in plants.

8. Concluding remarks

Studies in *S. pombe* have shown that the RNAi pathway is directly involved in assembly of heterochromatin at specific chromosome regions. Remarkably, nascent RNA transcripts at the target locus are physically associated with RNAi complexes and may play a central role in the process by acting as the recognition platforms for RITS as well as templates for dsRNA synthesis by RDRC. It remains to be determined whether the processing of dsRNA to siRNA by Dicer also occurs on the chromosome, how RNAi complexes recruit histone modifying activities such as Clr4 to chromatin, and whether the targeting mechanism also involves base pairing between siRNA and DNA. Finally, the role of RNAi in directing chromatin modifications is broadly conserved (see reviews by Herr, Chen, Plasterk, Zhu, Voinnet, Birchler,

Grishok, Ding, and Aravin in this issue), suggesting that similar complexes and mechanisms may direct chromatin modifications in other organisms.

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References

[1] Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E. and Mello, C.C. (1998) Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature 391, 806–811.
[2] Mello, C.C. and Conte Jr., D. (2004) Revealing the world of RNA interference. Nature 431, 338–342.
[3] Baulcombe, D. (2004) RNA silencing in plants. Nature 431, 356–363.
[4] Plasterk, R.H. (2002) RNA silencing: the genome's immune system. Science 296, 1263–1265.
[5] Bartel, D.P. (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116, 281–297.
[6] Croce, C.M. and Calin, G.A. (2005) miRNAs, cancer, and stem cell division. Cell 122, 6–7.
[7] Bernstein, E., Caudy, A.A., Hammond, S.M. and Hannon, G.J. (2001) Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature 409, 363–366.
[8] He, L. and Hannon, G.J. (2004) MicroRNAs: small RNAs with a big role in gene regulation. Nat. Rev. Genet. 5, 522–531.
[9] Hannon, G.J. (2002) RNA interference. Nature 418, 244–251.
[10] Matzke, M.A. and Birchler, J.A. (2005) RNAi-mediated pathways in the nucleus. Nat. Rev. Genet. 6, 24–35.
[11] Grewal, S.I. and Moazed, D. (2003) Heterochromatin and epigenetic control of gene expression. Science 301, 798–802.
[12] Lippman, Z. and Martienssen, R. (2004) The role of RNA interference in heterochromatic silencing. Nature 431, 364–370.
[13] Volpe, T., Schramke, V., Hamilton, G.L., White, S.A., Teng, G., Martienssen, R.A. and Allshire, R.C. (2003) RNA interference is required for normal centromere function in fission yeast. Chromosome Res. 11, 137–146.
[14] Bernard, P., Maure, J.F., Partridge, J.F., Genier, S., Javerzat, J.P. and Allshire, R.C. (2001) Requirement of heterochromatin for cohesion at centromeres. Science 294, 2539–2542.
[15] Wassenegger, M. (2005) The role of the RNAi machinery in heterochromatin formation. Cell 122, 13–16.
[16] Mochizuki, K., Fine, N.A., Fujisawa, T. and Gorovsky, M.A. (2002) Analysis of a piwi-related gene implicates small RNAs in genome rearrangement in tetrahymena. Cell 110, 689–699.
[17] Bernstein, E. and Allis, C.D. (2005) RNA meets chromatin. Genes Dev. 19, 1635–1655.
[18] Volpe, T.A., Kidner, C., Hall, I.M., Teng, G., Grewal, S.I. and Martienssen, R.A. (2002) Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. Science 297, 1833–1837.
[19] Hall, I.M., Shankaranarayana, G.D., Noma, K., Ayoub, N., Cohen, A. and Grewal, S.I. (2002) Establishment and maintenance of a heterochromatin domain. Science 297, 2232–2237.
[20] Verdel, A., Jia, S., Gerber, S., Sugiyama, T., Gygi, S., Grewal, S.I. and Moazed, D. (2004) RNAi-mediated targeting of heterochromatin by the RITS complex. Science 303, 672–676.
[21] Motamedi, M.R., Verdel, A., Colmenares, S.U., Gerber, S.A., Gygi, S.P. and Moazed, D. (2004) Two RNAi complexes, RITS and RDRC, physically interact and localize to noncoding centromeric RNAs. Cell 119, 789–802.
[22] Meister, G. and Tuschl, T. (2004) Mechanisms of gene silencing by double-stranded RNA. Nature 431, 343–349.
[23] Hannon, G.J. and Rossi, J.J. (2004) Unlocking the potential of the human genome with RNA interference. Nature 431, 371–378.
[24] Sugiyama, T., Cam, H., Verdel, A., Moazed, D. and Grewal, S.I. (2005) RNA-dependent RNA polymerase is an essential component of a self-enforcing loop coupling heterochromatin assembly to siRNA production. Proc. Natl. Acad. Sci. USA 102, 152–157.
[25] Reinhart, B.J. and Bartel, D.P. (2002) Small RNAs correspond to centromere heterochromatic repeats. Science 297, 1831.
[26] Kim, H.S., Choi, E.S., Shin, J.A., Jang, Y.K. and Park, S.D. (2004) Regulation of Swi6/HP1-dependent heterochromatin assembly by cooperation of components of the mitogen-activated protein kinase pathway and a histone deacetylase Clr6. J. Biol. Chem. 279, 42850–42859.
[27] Jia, S., Noma, K. and Grewal, S.I. (2004) RNAi-independent heterochromatin nucleation by the stress-activated ATF/CREB family proteins. Science 304, 1971–1976.
[28] Hall, I.M., Noma, K. and Grewal, S.I. (2003) RNA interference machinery regulates chromosome dynamics during mitosis and meiosis in fission yeast. Proc. Natl. Acad. Sci. USA 100, 193–198.
[29] Mandell, J.G., Bahler, J., Volpe, T.A., Martienssen, R.A. and Cech, T.R. (2005) Global expression changes resulting from loss of telomeric DNA in fission yeast. Genome Biol. 6, R1.
[30] Petrie, V.J., Wuitschick, J.D., Givens, C.D., Kosinski, A.M. and Partridge, J.F. (2005) RNA interference (RNAi)-dependent and RNAi-independent association of the Chp1 chromodomain protein with distinct heterochromatic loci in fission yeast. Mol. Cell Biol. 25, 2331–2346.
[31] Cam, H., Sugiyama, T., Chen, E.S., Chen, X., Fitzgerald, P. and Grewal, S.I. (2005) Comprehensive analysis of heterochromatin- and RNAi-mediated epigenetic control of the fission yeast genome. Nat. Genet.
[32] Zilberman, D., Cao, X. and Jacobsen, S.E. (2003) ARGONAUTE4 control of locus-specific siRNA accumulation and DNA and histone methylation. Science 299, 716–719.
[33] Mette, M.F., Aufsatz, W., van der Winden, J., Matzke, M.A. and Matzke, A.J. (2000) Transcriptional silencing and promoter methylation triggered by double-stranded RNA. EMBO J. 19, 5194–5201.
[34] Morris, K.V., Chan, S.W., Jacobsen, S.E. and Looney, D.J. (2004) Small Interfering RNA-induced transcriptional gene silencing in human cells. Science 305, 1289–1292.
[35] Kawasaki, H. and Taira, K. (2004) Induction of DNA methylation and gene silencing by short interfering RNAs in human cells. Nature 431, 211–217.
[36] Ting, A.H., Schuebel, K.E., Herman, J.G. and Baylin, S.B. (2005) Short double-stranded RNA induces transcriptional gene silencing in human cancer cells in the absence of DNA methylation. Nat. Genet. 37, 906–910.
[37] Sigova, A., Rhind, N. and Zamore, P.D. (2004) A single Argonaute protein mediates both transcriptional and posttranscriptional silencing in *Schizosaccharomyces pombe*. Genes Dev 18, 2359–2367.
[38] Partridge, J.F., Borgstrom, B. and Allshire, R.C. (2000) Distinct protein interaction domains and protein spreading in a complex centromere. Genes Dev 14, 783–791.
[39] Thon, G. and Verhein-Hansen, J. (2000) Four chromo-domain proteins of *Schizosaccharomyces pombe* differentially repress transcription at various chromosomal locations. Genetics 155, 551–568.
[40] Noma, K., Sugiyama, T., Cam, H., Verdel, A., Zofall, M., Jia, S., Moazed, D. and Grewal, S.I. (2004) RITS acts in cis to promote RNA interference-mediated transcriptional and post-transcriptional silencing. Nat. Genet. 36, 1174–1180.
[41] Hong, E.E., Villen, J., Gerace, E.L., Gygi, S.P. and Moazed, D. (2005) A Cullin E3 ubiquitin ligase complex associates with Rik1 and the Clr4 histone H3-K9 methyltransferase and is required for RNAi-mediated heterochromatin formation. RNA Biol. (in press).
[42] Li, F., Goto, D.B., Zaratiegui, M., Tang, X., Martienssen, R. and Cande, W.Z. (2005) Two novel proteins, dos1 and dos2, interact with rik1 to regulate heterochromatic RNA interference and histone modification. Curr. Biol. 15, 1448–1457.
[43] Makeyev, E.V. and Bamford, D.H. (2002) Cellular RNA-dependent RNA polymerase involved in posttranscriptional gene silencing has two distinct activity modes. Mol. Cell 10, 1417–1427.

[44] Sadaie, M., Iida, T., Urano, T. and Nakayama, J. (2004) A chromodomain protein, Chp1, is required for the establishment of heterochromatin in fission yeast. EMBO J. 23, 3825–3835.
[45] Nakayama, J., Rice, J.C., Strahl, B.D., Allis, C.D. and Grewal, S.I. (2001) Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. Science 292, 110–113.
[46] Horn, P.J., Bastie, J.N. and Peterson, C.L. (2005) A Rik1-associated, cullin-dependent E3 ubiquitin ligase is essential for heterochromatin formation. Genes Dev. 19, 1705–1714.
[47] Groisman, R., Polanowska, J., Kuraoka, I., Sawada, J., Saijo, M., Drapkin, R., Kisselev, A.F., Tanaka, K. and Nakatani, Y. (2003) The ubiquitin ligase activity in the DDB2 and CSA complexes is differentially regulated by the COP9 signalosome in response to DNA damage. Cell 113, 357–367.
[48] Holmberg, C., Fleck, O., Hansen, H.A., Liu, C., Slaaby, R., Carr, A.M. and Nielsen, O. (2005) Ddb1 controls genome stability and meiosis in fission yeast. Genes Dev. 19, 853–862.
[49] Kato, H., Goto, D.B., Martienssen, R.A., Urano, T., Furukawa, K. and Murakami, Y. (2005) RNA polymerase II is required for RNAi-dependent heterochromatin assembly. Science 309, 467–469.
[50] Maniatis, T. and Reed, R. (2002) An extensive network of coupling among gene expression machines. Nature 416, 499–506.
[51] Hampsey, M. and Reinberg, D. (2003) Tails of intrigue: phosphorylation of RNA polymerase II mediates histone methylation. Cell 113, 429–432.
[52] Herr, A.J., Jensen, M.B., Dalmay, T. and Baulcombe, D.C. (2005) RNA polymerase IV directs silencing of endogenous DNA. Science 308, 118–120.
[53] Mette, M.F., Aufsatz, W., Kanno, T., Daxinger, L., Rovina, P., Matzke, M. and Matzke, A.J. (2005) Analysis of double-stranded RNA and small RNAs involved in RNA-mediated transcriptional gene silencing. Methods Mol. Biol. 309, 61–82.
[54] Onodera, Y., Haag, J.R., Ream, T., Nunes, P.C., Pontes, O. and Pikaard, C.S. (2005) Plant nuclear RNA polymerase IV mediates siRNA and DNA methylation-dependent heterochromatin formation. Cell 120, 613–622.