Regulation of Heterochromatic Silencing and Histone H3 Lysine-9 Methylation by RNAi

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Eukaryotic heterochromatin is characterized by a high density of repeats and transposons, as well as by modified histones, and influences both gene expression and chromosome segregation. In the fission yeast *Schizosaccharomyces pombe*, we deleted the argonaute, dicer, and RNA-dependent RNA polymerase gene homologs, which encode part of the machinery responsible for RNA interference (RNAi). Deletion results in the aberrant accumulation of complementary transcripts from centromeric heterochromatic repeats. This is accompanied by transcriptional de-repression of transgenes integrated at the centromere, loss of histone H3 lysine-9 methylation, and impairment of centromere function. We propose that double-stranded RNA arising from centromeric repeats targets formation and maintenance of heterochromatin through RNAi.

Eukaryotic chromosomes are characterized by the presence of condensed tracts of constitutive heterochromatin that stain differentially and have a low density of expressed genes but a high density of transposons and repeats. The juxtaposition of heterochromatic regions with euchromatic genes results in gene silencing, often in a variegated fashion in which founder cells pass on alternate epigenetic states to their descendants (*1*–*3*). Transposable elements can also regulate neighboring genes by conferring epigenetic expression states, and it has been proposed that many of the properties of heterochromatin stem from these elements (*4*).

RNAi is the process by which double-stranded RNA (dsRNA) inhibits the accumulation of homologous transcripts from cognate genes (*5*). It is thought to be responsible for posttranscriptional gene silencing (PTGS), or co-suppression, a mechanism by which endogenous genes are silenced in the presence of a homologous transgene (*6*). Several of the genes required for RNAi have been isolated from *Arabidopsis*, *Caenorhabditis elegans*, and *Drosophila*. These include an RNAseIII helicase, an RNA-dependent RNA polymerase (RdRP), and several members of the *ARGONAUTE* gene family. The *Drosophila* RNAseIII helicase *Dicer* has been shown to specifically cleave dsRNA into sense and antisense RNA oligonucleotides 21 to 24 nucleotides (nt) in length (*7*). These small interfering RNAs (siRNA) were first observed in plants (*8*) and are believed to guide the RNA interference silencing complex (RISC) to messenger RNA transcribed from homologous genes (*9*, *10*). One component of RISC is *Argonaute 2* (*11*), a gene highly conserved in animals and plants (*12*). The dsRNA is thought to be re-generated, or amplified, by a RdRP that uses siRNA to prime dsRNA synthesis (*13*, *14*). Although the primary sequence of RdRP is not related to viral replicases, RdRP mutants can be complemented by ssRNA viruses in plants (*15*), which are important targets of RNAi (*16*).

Many of the genes required for RNAi are redundant in plants and animals, although some RNAi mutants can be lethal or sterile, suggesting that endogenous genes are direct or indirect targets. For example, in *Arabidopsis*, the related genes *ARGONAUTE* and *PINHEAD/ZWILLE* have synergistic effects on stem cell maintenance and organogenesis (*17*), and double mutants result in embryo lethality (*18*). Unlike higher eukaryotes, fission yeast has only a single homolog of the *argonaute* gene (*19*), and for this reason we have extended our studies from plants to *Schizosaccharomyces pombe*, by deleting the homolog, *ago1*$^+$. With the discovery that *argonaute* homologs in *C. elegans* and *Neurospora* are required for RNAi, we also deleted the helicase/RNAseIII and RdRP genes (*20*) that we have designated *dcr1*$^+$ and *rdp1*$^+$, respectively.

Dependence of centromeric silencing depends on the RNAi machinery. *ago1*$^+$, *dcr1*$^+$, and *rdp1*$^+$ were deleted by homologous gene replacement in diploid strains (*21*), and the mutants were viable as haploids. In *S. pombe*, mating type loci undergo gene silencing (*22*), but mating was not obviously impaired in *ago1*$^-$ strains. However, other mutants are known that affect centromeric silencing without affecting the mating type locus (*23*). Therefore, we tested whether centromeric silencing was affected in the three mutants.

The central region of each centromere is flanked by large inverted repeats containing transfer RNA (tRNA) genes in the innermost region (*imr*), and then by the outermost region (*otr*), which is composed of tandem alternating copies of *dg* and *dh* repeats, also known as *K* repeats (Fig. 1A) (*24*, *25*). A series of *ura4*$^+$ transgenes integrated in each of these regions on chromosome 1 are silenced in wild-type strains (*26*, *27*), whereas a *ura4* minigene (*ura4*$^-$ *DS/E*) located on the chromosome arm is unaffected (*28*). Two transgenes located centromere distal to the tRNA genes were de-repressed in *ago1*$^-$, *dcr1*$^-$, and *rdp1*$^-$, but a transgene located within the central region remained silent. Similar results were obtained in all three mutant strains, as assayed by growth on medium lacking uracil and by Northern blots (Fig. 1, B) (*21*).

In *Drosophila*, the *ARGONAUTE* homolog *sting/aubergine* is responsible for processing a heterochromatic RNA, *Stellate*, from the Y chromosome (*29*, *30*). We therefore tested whether the centromeric repeats themselves were transcribed in fission yeast RNAi mutants. Although centromeric repeats of *S. pombe* resemble transposons in some respects, transcripts derived from *cen3* repeats were not observed in wild-type strains, in agreement with previous reports (*31*). However, three major transcripts that hybridized to the repeats were found to accumulate at high levels in each of the RNAi mutants (Fig. 1C). These transcripts were also found in *swi6*$^-$ (Fig. 1D) but at a much lower level. *ago1*$^-$, *swi6*$^-$ double mutants had higher levels of transcript than *swi6*$^-$ alone (Fig. 1D), indicating Ago1 could function in the absence of Swi6 and likely acted upstream (*21*).

The centromeric transcripts were derived from both strands in each of the RNAi mutants, but only from one strand (which we designated as the forward transcript) in *swi6*$^-$ (Fig. 2, A to C). In wild-type cells the forward transcript did not accumulate, although very low levels of reverse transcripts were detected when the number of polymerase chain reaction (PCR) cycles was increased (*32*). In order to test whether these transcripts were transcriptionally or posttranscriptionally regulated, we performed run-on transcription experiments (*21*). RNA was purified from nuclei that had been permeabilized with detergents to inhibit transcriptional initiation while allowing elongation and incorporation of radioactive uridine triphosphate (UTP). This RNA was hybridized to slot-blots of strand-specific probes for both the actin

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gene and the centromeric repeats (Fig. 2D).

Nascent forward transcripts were detected in mutant but not in wild-type strains. This indicates that they are primarily under transcriptional control, presumably through Swi6. In contrast, nascent reverse transcripts were detected in both mutant and wild-type nuclei but only accumulated in the RNAi mutants and not in *swi6*$^-$. Thus the reverse strand is always transcribed in wild-type cells but is rapidly turned over by RNAi. The forward strand is not transcribed in wild-type cells but is transcribed in the mutants, indicating the RNAi machinery must repress forward transcription indirectly.

RNAi machinery and histone H3 lysine-9 methylation. In plants, RNAi is thought to guide DNA methylation of integrated transgenes, which can repress transcription (*33*–*35*). *S. pombe* has no DNA methylation, but heterochromatin is marked by methylation of histone H3 at lysine-9 (K9) whereas methylation of lysine-4 (K4) is preferentially associated with expressed genes (*36*–*38*). We therefore tested whether K9 and K4 methylation of H3 were affected by using antibodies specific for each modification in chromatin immunoprecipitation experiments (Fig. 3A) (*21*). Specific primers were chosen that exploited small differences in sequence allowing "real-time" quantitative PCR (*21*) to be used to determine the level of centromeric repeats found in the chromatin immunoprecipitations. We found that both K9 and K4 methylation was associated with centromeric repeats in wild-type cells, but *dcr1*$^-$, *rdp1*$^-$, and *ago1*$^-$ cells had increased levels of K4 in the centromeric region in comparison to actin controls (Fig. 3B). In contrast, levels of K9 were greatly reduced. As controls for K9, we used primers specific for the related K repeat found at the mating type locus (*39*), which was relatively unaffected (*40*).

Among the three mutants, *ago1*$^-$ retained more K9 methylation than *dcr1*$^-$. We investigated this further by repeating the chromatin immunoprecipitation using antibodies raised against branched histone H3 peptide tails methylated on K9. These antibodies are thought to detect closely packed, condensed modified nucleosomes in mammalian cells (*41*). We found that all three mutants had similarly low levels of modification as detected by these antibodies (Fig. 3B).

We next examined the pattern of histone H3 modification associated with *ura4*$^+$ transgenes integrated in the outer region of *cen1* (Fig. 3C). These transgenes were associated with K9 methylation in wild-type cells, but this was lost in each of the three mutants. As expected, Swi6, which depends on histone modification for chromatin binding, was delocalized from the *ura4*$^+$ transgenes (Fig. 3C). Silencing of the transgene was lost in each of the three mutants, consistent with these changes (Fig. 1B). However, silencing of the transgene was largely retained when it was integrated in the central portion of *cen1* (Fig. 1B). This may reflect the replacement of histone H3 with the histone variant cenpA in this region (*42*), as cenpA lacks both lysine-4 and lysine-9.

We conclude that all three mutants lead to a loss of K9-modified nucleosomes from the centromeric repeats relative to the mating type region. *ago1*$^-$ and rdp1$^-$ retain somewhat more K9 overall than *dcr1*$^-$ (Fig. 3B). This difference between the three mutants is not observed at the *ura4*$^+$ transgenes integrated nearby on *cen1* (Fig. 3C). One possibility is that spreading of heterochromatin is blocked in *ago1*$^-$ and *rdp1*$^-$ and initiation is blocked in *dcr1*$^-$.

Targeting of heterochromatin. If RNAi is responsible for chromatin modification at the centromere, then components of the

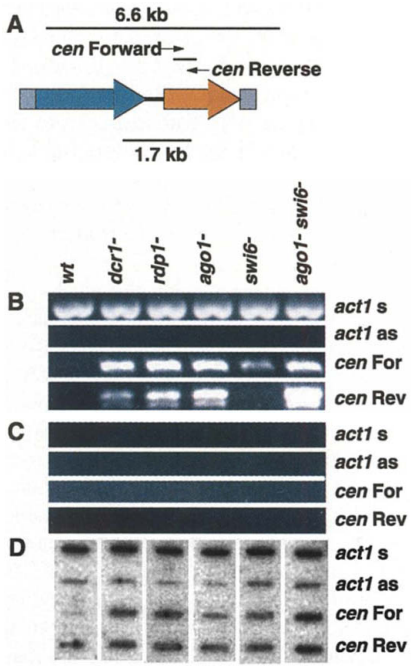

Fig. 2. Centromeric *otr* transcripts are both transcriptionally and posttranscriptionally regulated. (**A**) A schematic diagram showing the direction of transcription of forward and reverse transcripts corresponding to the *dh* repeat. Strand-specific RT-PCR analysis was performed in the presence (**B**) or absence (**C**) of reverse transcriptase. Samples were incubated with primers from the *dh* repeat complementary to either the forward (*cen* For) or reverse (*cen* Rev) centromeric transcripts in first strand cDNA synthesis reaction (primer locations are summarized in Fig. 5). Both primers were present in subsequent cycles of PCR amplification after heat inactivation of the reverse transcriptase. Treatment of control reactions lacking reverse transcriptase (C) was identical except that these samples were not subjected to first strand synthesis. Strand-specific control reactions were also conducted using primers specific for *act1* sense (*act1* s) or *act1* antisense (*act1* as) transcripts. Strand-specific analysis of nascent RNA transcripts was performed by nuclear run-on assay (**D**). Radiolabeled nascent RNA purified from mutant and wild-type strains was hybridized to nylon membranes containing strand-specific probes made using the same primer pair as in (A). These probes recognized either forward (*cen* For) or reverse (*cen* Rev) centromeric transcripts. Control probes recognized either sense (*act1* s) or antisense (*act1* as) actin transcripts.

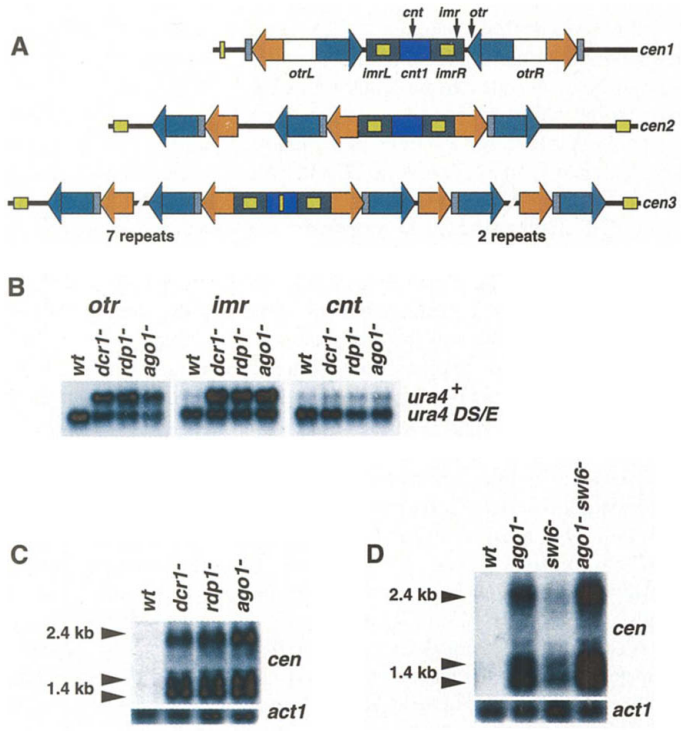

Fig. 1. Centromeric silencing is relieved in *ago1*$^-$, *dcr1*$^-$, and *rdp1*$^-$ mutant strains compared to wild type. Diagram of the three *S. pombe* centromeres (**A**) including locations of *ura4*$^+$ transgenes as well as outermost (*otr*), innermost (*imr*), and central (*cnt*) centromeric regions (*24*, *25*). Conserved *dg* (green) and *dh* (red) repeats are indicated as arrows. Regions containing one or more tRNA genes are indicated by yellow boxes. Northern analysis (**B**) of RNA transcripts transcribed from centromeric *ura4*$^+$ transgenes and a *ura4*$^+$ (*DS/E*) mini-gene located on the chromosome arm. Transcripts derived from centromeric repeats were detected by Northern blotting (**C** and **D**) using probes specific for *dg* centromeric repeats (Fig. 5).

RNAi machinery might be expected to interact with centromeric sequences. To test this possibility, triple hemagglutinin (HA)–tagged Dcr1 and Rdp1 proteins were immunoprecipitated from chromatin extracts (*21*). Centromeric repeats could be selectively amplified from HA-tagged Rdp1 immunoprecipitates but not from Dcr1-tagged immunoprecipitates (Fig. 4). Strains carrying tagged Ago1 did not silence centromeric repeats, suggesting that the tagged protein was no longer functional (*32*). Therefore, immunoprecipitation was not attempted in this case.

Independently of this study, small RNA (between 20 and 25 nt) thought to be Dicer cleavage products were cloned and sequenced from wild-type strains of *S. pombe* (*43*). Although only 12 sequences were obtained that were neither tRNA nor rRNA, all 12 came from the outermost region of the centromeric repeats and matched all three centromeres. In *cen1*, for example, they clustered in two locations separated by 8 kilobases (kb). Nine heterochromatic siRNAs were found in a 1.7-kb portion of the *dh* repeat that included the reverse transcript detected in wild-type cells by nuclear run-on in Fig. 2. This portion of *dh* was conserved on all three centromeres, and partially reiterated on *cen2* and *cen3*. The other three siRNAs matched a centromere proximal region of 562 bp, which lies between the integration sites of the two *ura4*$^+$ transgenes whose silencing depended on RNAi. The portion of *dg* assayed for association with K9 (Fig. 3) and Rdp1 (Fig. 4) lies only 500 bp from this region (Fig. 5), although it is likely that K9 and Rdp1 coat additional sequences as well (*32*). The colocalization of siRNA with the sites of dsRNA transcription, transgene silencing, and chromatin modification suggests that siRNAs guide heterochromatic silencing.

Using RNA from mutant strains, we sequenced reverse transcriptase–PCR (RT-PCR) and rapid amplification of cDNA ends (RACE)–PCR products unique to each centromere and transcribed in both directions, including at least 1.7 kb of contiguous sequence spanning *dg* and *dh* at *cen3* (Fig. 5). However, because of the inherent complexity of the repeats, we do not know whether the siRNAs detected in wild-type cells are derived from one or more transcripts. For example, the proximal region of *cen1* into which the *ura4*$^+$ transgenes are integrated is duplicated at the distal margins of *cen2* and *cen3* and could potentially be transcribed at these locations (Fig. 5).

Overall, we can summarize our findings in the model presented in Fig. 6. In wild-type cells, reverse strand transcripts are initiated in *dh* repeats on each chromosome arm and are transcribed toward the centromere (Fig. 6). However, they are rapidly turned over by RNAi. Secondary structure predictions failed to detect hairpin structures that might trigger cleavage. Instead, occasional transcription from the forward strand of *cen1* could generate dsRNA, triggering processing into the observed heterochromatic siRNAs. dsRNA could then be continually regenerated by priming of the reverse transcript by Rdp1 bound to the chromatin. The resulting amplified heterochromatic siRNA would then guide histone modification, which would in turn recruit *swi6*$^+$, thus silencing the forward strand (Fig. 6). This order of events is consistent with transcript levels in the *swi6*$^-$, *ago1*$^-$ double mutant (Fig. 2). In *Neurospora*, siRNA from transgenes accumulates in *qde-2* (argonaute), but not in *qde-1* (RdRP) mutant strains (*44*). If siRNA were the targeting signal, this could account for the relatively mild effect of *ago1*$^-$ on histone H3 methylation (Fig. 3).

clr4$^+$ is responsible for histone H3 lysine-9 methylation and acts upstream of *swi6*$^-$ (*36*, *38*). Both genes encode a chromodomain, which has been implicated in binding RNA (*45*) as well as histone H3 methylated on lysine-9 (*45–47*). Thus RNAi and histone modification could be intimately related. However, it is clear that not all forms of silencing and chromatin modification depend on RNAi. *Saccharomyces cerevisiae* has a complex and well-studied silencing machinery, but, unlike *S. pombe*, it lacks enzymes responsible for methylation of histone H3 lysine-9. It also lacks each of the genes required for RNAi, as well as centromeric repeats (*19*, *20*).

Transposons and gene silencing. The centromeric repeats resemble transposons in many respects (*31*). First, they bind Abp1 and Cpb1, highly conserved Cenp-b proteins that are required for centromere function and the associated histone H3 K9 methylation (*48–50*). Cenp-b is related to the transposase encoded by Mariner/Tc1 transposable elements, which are targets of RNAi in *C. elegans* (*51–53*). Second, sequences related to centromeric repeats are located between the *mat2* and *mat3* loci and are required for silencing of the mating type region (*39*). These sequences may have arisen by transposition and have been shown to silence neighboring genes when placed elsewhere in the genome, an effect that can be mimicked by the *dh* portion alone (*54*). This portion matches some heterochromatic siRNAs, in-

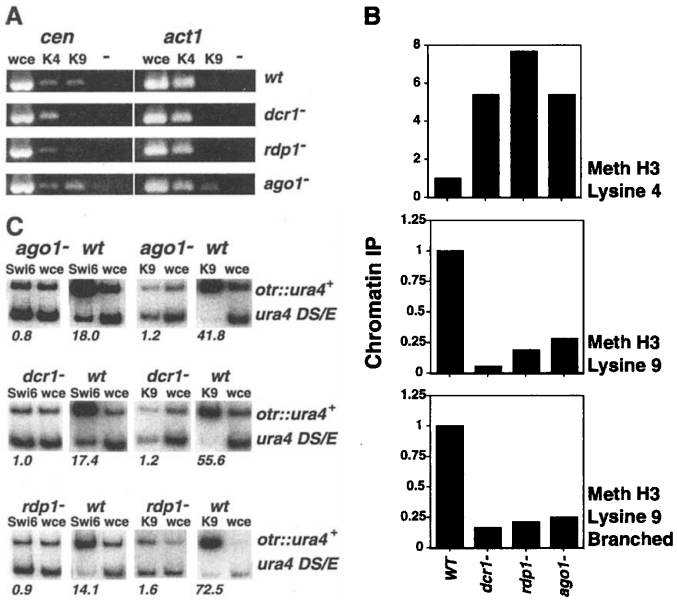

Fig. 3. Chromatin structure at centromeric repeats is altered in *ago1*$^-$, *dcr1*$^-$, and *rdp1*$^-$ mutants. Chromatin immunoprecipitation (ChIP) was performed on extracts from formaldehyde fixed mutant or wild-type cells. DNA fragments purified from whole-cell extracts (wce) or co-precipitated with antibodies to K4 or K9 histone H3 were amplified by PCR using primers specific for centromeric *dg* repeats (Fig. 5) or *act1* (**A**). Quantitation was performed using quantitative PCR (**B**) and normalized to actin (K4) and mating type region (K9) controls, which were amplified from the same ChIPs in each genotype (*21*). DNA fragments from wce or co-precipitated with antibodies to Swi6 or K9 histone H3 were amplified by multiplex PCR using *ura4*-specific primers (**C**). These primers amplify both a *ura4*$^+$ transgene located in the *otr* of *cen1* (*otr::ura4*$^+$) and the *ura4 DS/E* minigene (*ura4 DS/E*) located on the chromosome arm. Relative levels were estimated using a FUJI phosphoimager and are indicated below each lane.

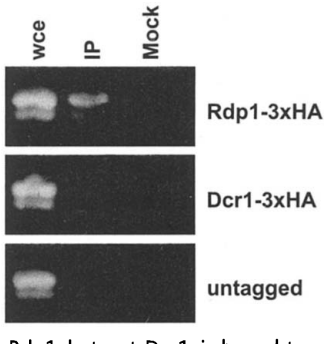

Fig. 4. Rdp1, but not Dcr1, is bound to centromeric chromatin. Chromatin immunoprecipitation (ChIP) was performed on extracts derived from triple-HA tagged Dcr1 or Rdp1 strains. DNA fragments from wce or co-precipitated with antibodies raised against the triple-HA tag were amplified by PCR using centromere-specific primers and compared to untagged strains. Mock reactions were identical except without the addition of primary antibody.

dicating they may mediate silencing (*43*).

Silencing of transgenes integrated near heterochromatic repeats resembles the silencing of genes by neighboring transposons, a phenomenon that led B. McClintock to refer to transposons as "controlling elements" (*4*). Furthermore, at least in *C. elegans*, transposons are a target of RNAi. It is tempting to speculate that RNAi, through its ability to silence transposons, targets heterochromatin to neighboring genes. In an accompanying paper, we demonstrate that histone H3 lysine-9 methylation is conserved in plant heterochromatin and is preferentially associated with transposable elements (*55*). Maintenance requires the *swi2/snf2* remodeling factor DDM1. In *ddm1* mutants, transposons are strongly activated, potentially triggering RNAi, which might eventually restore histone modification to the region. Small RNAs (20 to 25 nt) in *Arabidopsis* have been found that match Mutator (MULE), Suppressor-Mutator (CACTA), and Athila transposons (*56*), which are found in heterochromatin and activated in *ddm1* mutants (*55*). However, unlike *S. pombe*, *Arabidopsis* has dozens of euchromatic microRNAs (*57*), indicating that RNAi may have evolved a more elaborate function in multicellular organisms. Targeting of heterochromatin to centromeric transposons may be the primitive role of RNAi.

RNAi machinery and centromere function. *csp7* to *-13* define 6 loci that are required in trans for centromeric but not mating type silencing (*23*). This could be because mating type silencing depends on cis-acting sequences at the mating type locus in addition to the *dg* and *dh* repeats (*54*). Alternatively, these mutants could represent weak alleles of genes that regulate both types of silencing, such as *swi6*$^-$ (*23*). Similar to *swi6*$^-$, centromere function is impaired in *csp* mutants, which have a high frequency of lagging chromosomes, especially at reduced temperatures. We have also observed mis-segregation of green fluorescent protein marked *cen1* (*58*) in preliminary experiments with *ago1*$^-$. Though this observation is difficult to interpret unequivocally, the effect of RNAi mutants on centromere function has subsequently been confirmed by allelism tests, in which *csp9* has proven to be allelic with *ago1* (*59*).

In *csp9*$^-$, as in *swi6*$^-$, up to half of all anaphase spindles have one or more lagging chromosomes at mitosis when grown at reduced temperature (*23*). The mechanism by which centromere function is impaired is unknown, but it seems likely to involve the formation of heterochromatin because the flanking *otr* repeats play an important role in centromere function (*60*). Genome sequencing has revealed that a 1.78-kb portion of the *dg* repeat, which is required for centromere function, is highly conserved across all three centromeres (*19*). The mechanism by which this sequence has been conserved is unknown, but it overlaps the 1.7-kb region of *cen3* that is transcribed in RNAi mutants (Fig. 5), suggesting this may be under selection.

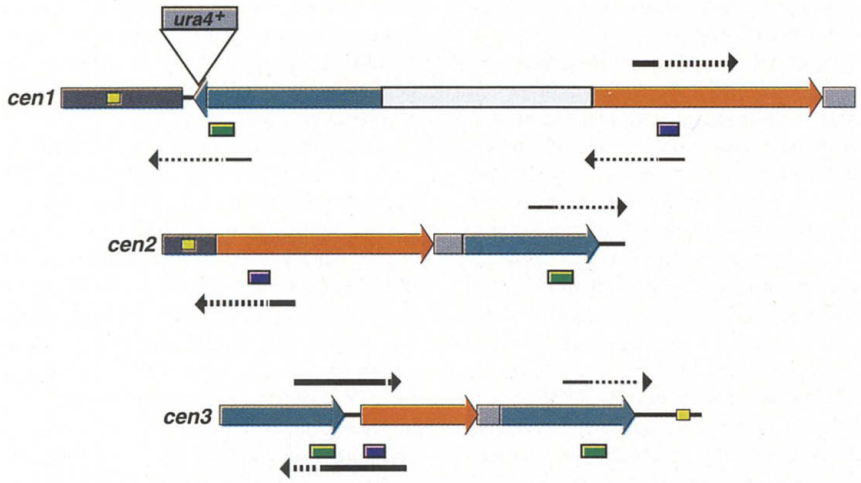

Fig. 5. One repeat unit from each of the three centromeres is shown (see Fig. 1). Transcripts from *ago1*$^-$ mutant cells were identified by sequencing RT-PCR and RACE-PCR products, and those unique to each centromere are shown as thick arrows. Transcripts whose origin was ambiguous due to sequence identity between the centromeres are shown as thin arrows. Transcript length, determined by Northern blots (Fig. 1), allowed us to estimate the approximate position of the transcribed region (indicated by dashed lines). The PCR product that detected run-on *dh* repeat transcription toward the centromere in wild-type cells (Fig. 3) is indicated as a blue bar. The PCR product amplified in ChIP experiments with K9 and K4 antibodies (Fig. 3A) as well as with rdp-HA antibodies (Fig. 4) is indicated as a green bar underneath each *dg* repeat. A similar PCR product was used as the probe for the Northern blot shown in Fig. 1.

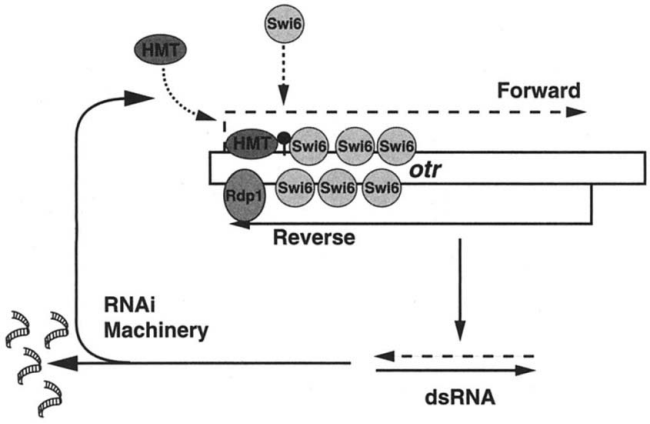

Fig. 6. The RNAi machinery is required for the initiation and maintenance of the heterochromatic state of centromeric repeats. Reverse strand centromeric transcription occurs in wild-type cells and is degraded posttranscriptionally by the RNAi machinery. Low-level transcription from the forward strand and/or amplification by Rdp1 results in generation of dsRNA, which is converted to siRNA by RNAi. Rdp1, bound to the chromatin, promotes targeting of histone modifications to specific sequences via siRNA, resulting in maintenance of the heterochromatic state (HMT, histone methyl transferase).

Noncoding RNA, RNAi, and DNA methylation. In the mouse, X inactivation and *Igf2r* imprinting are mediated in cis by specific noncoding antisense RNA (*61–63*). At least in the case of X inactivation, histone H3 lysine-9 methylation immediately follows the appearance of the noncoding *Xist* transcript. *Xist* is regulated by an antisense RNA, *Tsix*, and by promoter methylation (*64*). This configuration of transcripts and chromatin changes parallels the arrangement we describe in *S. pombe*. Our results raise the possibility that sequence-specific histone modification is targeted by the RNAi machinery in X inactivation and imprinting, which may in turn lead to DNA methylation and epigenetic silencing. In mammals, higher order structure in pericentromeric heterochromatin has recently been shown to involve histone H3 K9 modification and an RNAse-sensitive component found in total cellular RNA, indicating that such a mechanism may be highly conserved (*65*).

Unlike *S. pombe*, filamentous fungi such as *Ascobolus immersus* and *Neurospora crassa* have DNA methylation as well as histone modification. In *N. crassa*, cytosine methylation of ribosomal DNA and targets of RIP (repeat induced point mutation) were found to require the histone H3 lysine-9 methyltransferase *dim-5* (*66*). Homology-dependent silencing ("quelling") is also often

associated with DNA methylation in *Neurospora*, but it is unaffected in *dim-2* DNA methyltransferase mutants (*67*). Instead, genes encoding components of the RNAi machinery are required (*68*, *69*). Our results would be consistent with these observations, if histone H3 K9 methylation were the direct consequence of RNAi, but DNA methylation is only an indirect consequence.

Thus, our results provide a possible link between RNAi and DNA methylation. dsRNA derived from repeated sequences might trigger RNAi, which would then initiate histone H3 lysine-9 methylation. Histone modification would then signal DNA methylation. This mechanism could guide eukaryotic DNA methyltransferases to specific regions of the genome, such as transposable elements, even though they have little sequence specificity in themselves (*4*). Such an arrangement could be reinforced by maintenance methyltransferase activity, as well as by the deacetylation of histones guided by methyl DNA binding complexes (*70*).

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BRCA2 Function in DNA Binding and Recombination from a BRCA2-DSS1-ssDNA Structure

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Mutations in the *BRCA2* (breast cancer susceptibility gene 2) tumor suppressor lead to chromosomal instability due to defects in the repair of double-strand DNA breaks (DSBs) by homologous recombination, but BRCA2's role in this process has been unclear. Here, we present the 3.1 angstrom crystal structure of a ~90-kilodalton BRCA2 domain bound to DSS1, which reveals three oligonucleotide-binding (OB) folds and a helix-turn-helix (HTH) motif. We also (i) demonstrate that this BRCA2 domain binds single-stranded DNA, (ii) present its 3.5 angstrom structure bound to oligo(dT)$_9$, (iii) provide data that implicate the HTH motif in dsDNA binding, and (iv) show that BRCA2 stimulates RAD51-mediated recombination in vitro. These findings establish that BRCA2 functions directly in homologous recombination and provide a structural and biochemical basis for understanding the loss of recombination-mediated DSB repair in BRCA2-associated cancers.

Germline mutations in *BRCA2* are responsible for a highly penetrant, autosomal dominant predisposition to breast cancer (*1*–*3*), and they also confer increased risk of early-onset ovarian, prostate, pancreatic, and male breast cancers (*4*). In addition, hypomorphic mutations in *BRCA2* have recently been found in cells from patients in the FANC-B and FANC-D1 subgroups of Fanconi's anemia, an autosomal recessive cancer susceptibility syndrome (*5*).

A role for BRCA2 in the maintenance of