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RNAi mediates post-transcriptional repression of gene expression in fission yeast *Schizosaccharomyces pombe*

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ABSTRACT

RNA interference (RNAi) is a gene silencing mechanism conserved from fungi to mammals. Small interfering RNAs are products and mediators of the RNAi pathway and act as specificity factors in recruiting effector complexes. The *Schizosaccharomyces pombe* genome encodes one of each of the core RNAi proteins, Dicer, Argonaute and RNA-dependent RNA polymerase (*dcr1*, *ago1*, *rdp1*). Even though the function of RNAi in heterochromatin assembly in *S. pombe* is established, its role in controlling gene expression is elusive. Here, we report the identification of small RNAs mapped anti-sense to protein coding genes in fission yeast. We demonstrate that these genes are up-regulated at the protein level in RNAi mutants, while their mRNA levels are not significantly changed. We show that the repression by RNAi is not a result of heterochromatin formation. Thus, we conclude that RNAi is involved in post-transcriptional gene silencing in *S. pombe*.

1. Introduction

RNA-based repression mechanisms control gene expression and preserve genomic integrity in all kingdoms of life. They control general cellular processes, such as DNA methylation in plants, X chromosome inactivation in mammals, and heterochromatin formation in fission yeast and Drosophila, and locus-specific mechanisms such as repression of individual genes in animals and plants [1,2]. RNAi is one such gene silencing mechanism, acting at the post-transcriptional level [2].

Fission yeast *Schizosaccharomyces pombe* is the organism where the contribution of RNAi to the heterochromatin formation was first characterized. *S. pombe* is particularly suited for studies of RNA-mediated silencing because its genome encodes one of each of the core RNAi proteins: Dicer, Argonaute and RNA-dependent RNA polymerase (*dcr1*, *ago1*, *rdp1*). The current model for RNAi function in heterochromatin assembly postulates that nascent transcripts from centromeric repeats form dsRNA either by folding onto themselves or as a result of Rdp1 activity. dsRNA is a substrate for cleavage by Dcr1, and resulting sRNAs are incorporated into Ago1 effector complex RITS, which acts as a bridge between RNAi and chromatin and recruits histone modifying activities [1].

Even though the function of RNAi in heterochromatin assembly in *S. pombe* is well documented, its role in gene regulation is elusive. Early reports have described that *S. pombe* RNAi machinery can silence a reporter gene upon expression of dsRNA as an anti-sense transcript [3] and a long hairpin [4]. Limited evidence suggests that dsRNA-mediated silencing requires functional RNAi machinery and acts at a post-transcriptional level [4]. Previous studies aimed to characterize the role of RNAi failed to detect a substantial effect of RNAi mutants on gene expression [5–7]. However, these studies were designed to detect large differences in mRNA and protein levels, and may not have addressed the possible subtle changes caused by RNAi. In contrast to RNAi induced by exogenous nucleic acids, RNA silencing mediated by endogenous sRNAs may be modulatory. For example, microRNA-mediated mRNA downregulation was until recently thought not to affect levels of targeted mRNAs, only their translational output [8]. Hence we hypothesized that the detection of RNAi-mediated gene silencing may require more sensitive approaches than those used so far.

Abbreviations: dsRNA, double-stranded RNA; GO, gene ontology; H3K9, lysine 9 histone 3; RDRC, RNA-dependent RNA polymerase complex; RITS, RNAi-induced transcriptional silencing; RNAi, RNA interference; sRNA, small RNA; wt, wild type.

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2. Materials and methods

2.1. Strains and culture conditions

The list of *S. pombe* strains used in this study is presented in Supplementary Table S7. Strains Hu2380, Hu2389, Hu2391 were constructed by PCR tagging the HA:GFP sequence from plasmid pAH90 [9]. For transcription profiling and sRNA library, yeast was cultured in YES at 30 °C. For live imaging, Western blot and Northern blot, yeast was cultured in PMG with appropriate supplements at 30 °C. For spotting assays, yeast was cultured at the permissive temperature 25 °C and 5-fold dilutions were spotted on YES plates.

2.2. RNA profiling

Total RNA was labeled using Whole Transcripts Sense Target Labeling Assay (Affymetrix) with actinomycin D treatment [10] and hybridized to GeneChip *S. pombe* Tiling 1.0FR Array (Affymetrix). Three biological replicates were made for each strain. Data was analysed with Tiling Analysis Software (Affymetrix), with scaling and quantile normalization. Strand-specific gene expression values were calculated using Podbat [11].

2.3. sRNA library cloning

sRNA libraries were cloned from 40 μg of total, size-selected RNA. RNAs of 10–40 nt were enriched using flashPAGE (Ambion). The adapters (Small RNA Oligo Only, Illumina) were ligated sequentially using T4 RNA Ligase (Promega). Adapter ligation required the presence of 5′-monophosphate and 3′-OH. The library was reverse-transcribed using Superscript-II (Invitrogen) and amplified using Phusion Hot Start DNA Polymerase (Finnzymes). PCR products were purified by PAGE. The library was sequenced on Illumina Genome Analyser. Reads were selected based on the presence of adapter sequences and mapped to *S. pombe* genomic and mRNA sequences (Sanger, release 23-8-2007) using WUblast (Gish, W. 1996–2003). The data were analysed using Perl scripts; a detailed description is given in the Supplementary methods.

2.4. Live cell imaging

Cells were resuspended in PMG and immobilized on poly-L-lysine-coated slides. Photomicrographs were obtained using Axioplan2 (Zeiss), FITC and YFP filters and CCD camera (Hamamatsu) at room temperature. Images of the reporters in wt and mutant backgrounds were acquired during the same session, with identical microscope and camera settings. GFP fluorescence intensity was quantified using Fiji by drawing a line through the subcellular localization area of each reporter, and recording average pixel intensity. Background, measured outside of cells, was subtracted from each intensity measurement. The average intensity for each strain was calculated from at least 60 cells and intensities of one reporter in different genetic backgrounds were tested against each other using two-tailed Student's *t*-test. All statistical analyses were performed using *R* (r-project.org) and Excel (Microsoft).

2.5. Western blot

Cells were spheroblasted using 0.4 mg/ml zymolase100T (USBiological) in 10 mM DTT, 50 mM Tris pH 7.5, 0.8 M sorbitol. Spheroblasts were resuspended in RIPA with protease inhibitors and briefly sonicated. Extracts were cleared by centrifugation. Protein concentration was determined using Coomasie Protein Assay Reagent (Thermo). Equal amount of total protein per lane was electrophoresed in 10% bis-tris polyacrylamide gels in 1× MOPS buffer (Invitrogen). The proteins were wet-blotted onto PVDF membranes in 10 mM CAPS pH 10.5, 10% methanol. The antibodies used were: anti-HA (16B12, Covance), anti-actin (ab8224, Abcam) and ECL-anti-mouse-HRP (NA931V, GE Healthcare). The signal was detected using SuperSignal Femto Substrate (Thermo). Images were collected using ChemiDocXRS and band intensities were determined using QuantityOne (BioRad).

2.6. Northern blot

10 μg of total RNA was electrophoresed in MOPS-acetate-formaldehyde agarose gel, and transferred to Hybond N+(GE Healthcare) in 20× SSC. The RNA was UV crosslinked. 32P-labeled riboprobes were prepared using MaxiScript Transcription Kit (Ambion). GFP and *atb2* ORFs were used as templates for probe synthesis. Probes were hybridized in 5× Denhardt's solution, 6× SSC, 10 mM EDTA, 0.5% SDS, 0.1 mg/ml salmon sperm DNA at 68 °C for 16 h. The blots were imaged using Molecular Imager FX (BioRad). Band intensities were quantified using QuantityOne (BioRad).

2.7. Data availability

The sRNA sequencing and RNA profiling data are deposited in GEO: GSE54195.

3. Results

3.1. A subset of genes produces anti-sense sRNAs in S. pombe

To address effects of the RNAi pathway on gene expression we analysed the RNA profiles from wt, *dcr1Δ*, and *rdp1Δ S. pombe* strains using a tiling microarray. The RNA profiles confirmed earlier observations of no gross changes of mRNA levels for most of the genes [5,6]. Eighty genes were up-regulated above the 1.3-fold threshold in *dcr1Δ* and *rdp1Δ* compared to wt (Fig. S1).

To analyze putative products of the RNAi pathway, we sequenced an sRNA library from wt yeast. We found that 25% of sequence tags normalized for multiple genomic matches mapped to protein coding genes (Fig. S1, Table S1). Ten percent of the genic sequence tags were mapped in anti-sense orientation to protein coding sequences. Moreover, 235 and 2335 genes contained 5 or more normalized sRNA reads in anti-sense and sense orientation, respectively (Fig. 1A). The number of mapped sRNA reads did not correlate with the levels of transcripts when compared on a gene-to-gene basis (Fig. 1B, Fig. S1C). Finally, we detected the presence of low numbers of intron–intron and exon–intron boundary-derived sRNAs (Fig. 1C).

Surprisingly, genes enriched in anti-sense sRNAs were not up-regulated at the mRNA level in *dcr1Δ* nor reported amongst genes associated with Dcr1 at the chromatin level [12] (Fig. 1D). Moreover, GO terms over-represented among genes with anti-sense sRNAs were distinct from terms enriched among genes up-regulated at the mRNA level in *dcr1Δ* and genes with Dcr1-chromatin association (Tables S2–S4). This divergence in functional annotation of anti-sense sRNA-enriched genes and genes regulated by Dcr1 at the mRNA level suggests that they represent independent processes.

This apparent disjuncture of genes with Dcr1-chromatin binding, genes up-regulated at the mRNA level in *dcr1Δ* and genes enriched in anti-sense sRNAs, prompted us to seek an alternative model of Dcr1 effect on gene expression.

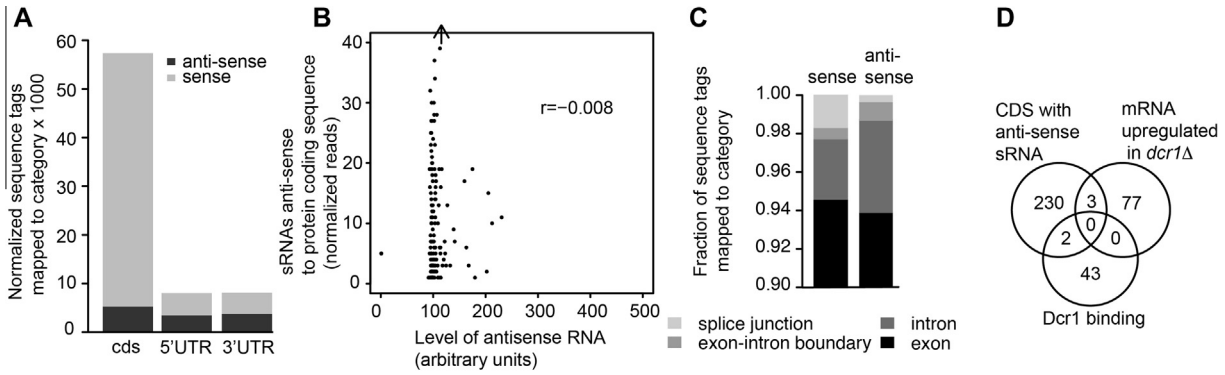

Fig. 1. sRNAs map to protein coding genes in *S. pombe*. (A) Number of sequence tags, mapped to protein coding genes; CDS – coding sequence, 5′UTR – 5′ untranslated region, 3′UTR – 3′ untranslated region. (B) Number of anti-sense sRNAs mapped to genes does not correlate with levels of anti-sense transcripts. Number of anti-sense normalized sRNA reads mapped to a given gene plotted against level of anti-sense transcript from the same locus; outliers are removed. *r* – the Pearson Product-Moment correlation coefficient. (C) Genic sRNA sequence tags predominantly map to exons. (D) Mapping of sRNAs anti-sense to protein coding genes does not correlate with increase of mRNA level in *dcr1Δ* and interaction of Dcr1 with chromatin [12]. mRNA up-regulated in *dcr1Δ* – genes identified by RNA profiling by applying 1.3-fold threshold to *dcr1Δ*-to-wt ratio; CDS with anti-sense sRNA – genes with 5 or more mapped normalized sRNA reads.

3.2. sRNA-enriched genes are up-regulated in dcr1Δ at the protein level

Given the overall small effect of *dcr1* deletion on mRNA levels (Fig. S1A) and the enrichment of many genes in anti-sense sRNAs (Fig. 1D), we hypothesized that the effect of RNAi on gene expression might be at the post-transcriptional level. To test this, we selected a group of candidate genes with many anti-sense sRNAs, no increase of mRNA levels in *dcr1Δ* in our and previous studies [5,6] and no enrichment in heterochromatin mark, methylated H3K9 [13]. We used reporter strains with a HA:GFP tag integrated into each locus, which produced C-terminally tagged fusion proteins [9]. We examined the reporter strains for GFP levels by live cell imaging of exponentially growing cultures. In 5 out of 6 candidates, GFP levels were increased in *dcr1Δ* compared to wt (Fig. 2). The HA:GFP fusion with Tpr1, a protein encoded by a gene with no mapped sRNAs, served as a negative control, and was not up-regulated in *dcr1Δ*. The positive control Hsp16:HA:GFP is strongly up-regulated at the mRNA and protein level in RNAi mutants [5–7] (Fig. 2A). The increase in protein levels of HA:GFP reporter fusions of three candidates in *dcr1Δ* was confirmed by Western blot using anti-HA antibody. Consistently with GFP imaging analyses, selected reporter HA:GFP fusions showed protein level increase in *dcr1Δ* compared to wt (Fig. 2B).

To exclude possible effects related to the chromatin context of each HA:GFP reporter, we analysed the expression of the same candidate genes fused to the YFP reporter and expressed under the control of the *nmt1* promoter integrated into the *leu1-32* locus [14]. Similarly to the GFP-tagged reporters, the YFP reporters exhibited a higher YFP signal in *dcr1Δ* compared to wt (Fig. S2B and C). The increase of the reporter protein levels in *dcr1Δ* in both experimental setups, using both the native (Fig. 2A) and the heterologous promoter (Fig. S2B and C) suggests that the genes enriched in anti-sense sRNAs are under post-transcriptional control by Dcr1.

To demonstrate that the protein level increase has physiological consequences in the absence of RNAi, we examined the effect of *dcr1Δ* on the expression of Cdc1. *cdc1* is an essential gene and encodes a subunit of DNA polymerase δ [15]. The lethality of *cdc1-M78* allele at the restrictive temperature 36 °C can be rescued by overexpression of the mutant variant of Cdc1-M78 [15]. We selected 34 °C as the assay temperature, because RNAi is inhibited at 36 °C [16]. The elevated expression of Cdc1-M78 in *cdc1-M78 dcr1Δ* double mutant was sufficient to partially suppress the growth retardation of *cdc1-M78* at the semi-restrictive temperature 34 °C (Fig. 2C). This finding corroborates protein up-regulation in the absence of RNAi.

3.3. Control of protein coding genes by RNAi is post-transcriptional

To further address the post-transcriptional mode of RNAi-mediated gene regulation, we evaluated mRNA levels and transcriptional output of selected *candidate:HA:GFP* fusions. First, we assayed mRNA levels of *HA:GFP* fusions by Northern blot using a GFP-specific probe. The mRNA levels of each tested reporter were unchanged or only slightly elevated in *dcr1Δ*. The RNA level of *tpr1:HA:GFP* was also subtly increased in *dcr1Δ*. mRNA of *hsp16:HA:GFP* was strongly up-regulated in *dcr1Δ* (Fig. 3A).

Furthermore, we quantified the GFP signal from a transcriptional reporter of *SPAC824.09c* in wt and *dcr1Δ* background. The HA:GFP sequence was fused directly to the *SPAC824.09c* promoter. Consistent with the post-transcriptional mode of gene regulation, the GFP signal of the promoter HA:GFP reporter for *SPAC824.09c* was not increased in *dcr1Δ* compared to wt. This contrasts with the marked increase in GFP signal of the full-length *SPAC824.09c+* reporter (Fig. 3B). The *hsp16* transcriptional reporter, similarly to the full-length reporter, produced an increase in GFP intensity in *dcr1Δ* (Fig. 3B). Thus, repression of *SPAC824.09c* by Dcr1 required the presence of the protein coding sequence.

A summary of the measurements of mRNA and protein levels for genes with antisense sRNAs in *dcr1Δ* vs. wt is presented in Table S5. We have compared mRNA levels measured with three independent microarrays ([5,6], this study) and Northern blots. We also compared protein levels of HA:GFP fusions in live cells and Western blots. The protein levels are up-regulated for Cdc1, Cul3, Rsd1, Ulp2 and SPAC824.09c whereas the corresponding mRNA levels are relatively unchanged. Thus, we conclude that Dcr1 mediates post-transcriptional control of at least these five protein coding genes in fission yeast.

3.4. Genetic requirements for post-transcriptional gene silencing in S. pombe

In all RNAi systems described, silencing is elicited by an Argonaute-containing complex [2]. We tested whether Ago1 is required for silencing of the post-transcriptional silencing candidates in *S. pombe* by analysing the intensity of selected HA:GFP fusions in *ago1Δ*. Consequently with the model where Ago1 plays an effector role in sRNA-mediated silencing, the HA:GFP reporters displayed an increase in GFP intensity in *ago1Δ* compared to wt (Fig. 4A). Efficient RNAi acting on *S. pombe* centromeres requires the activity of the RDRC [1]. We asked whether Rdp1 is required for RNAi on protein coding genes. Similarly to *dcr1Δ* and *ago1Δ* backgrounds,

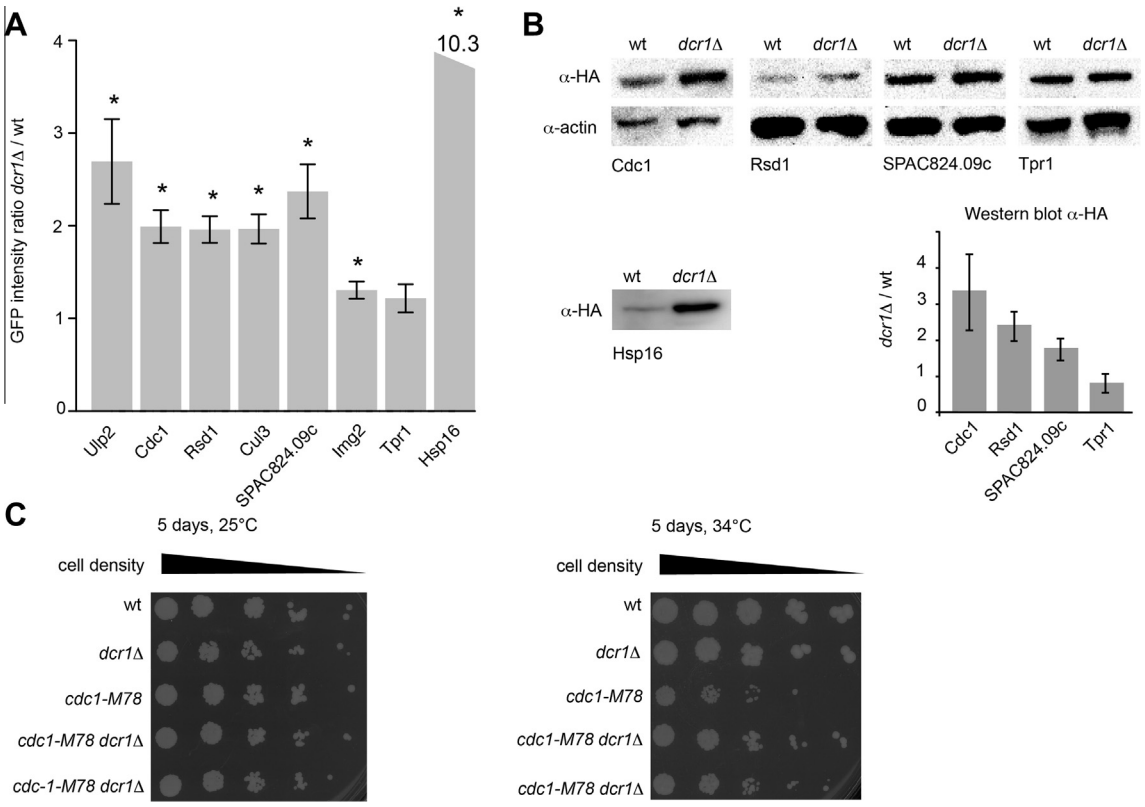

Fig. 2. Genes with mapped anti-sense sRNAs are repressed by RNAi. (A) GFP fluorescence intensity of reporter HA:GFP fusions of candidate genes with mapped anti-sense sRNAs in wt and *dcr1Δ*. Ratios of GFP intensity in *dcr1Δ*:wt are presented. Results from one representative experiment are shown ($n > 80$). Error bars represent 95% confidence interval for the mean. Asterisks denote statistically significant increase of the GFP signal intensity in mutant compared to wt ($p < 0.001$ by Student's *t*-test). Tpr – a negative control, encoded by a gene with no sRNAs mapped; Hsp16 – a positive control, encoded by a gene upregulated in *dcr1Δ* at the mRNA and protein level [5,7]. (B) Western blot images and quantitation of HA:GFP reporters in *dcr1Δ* and wt ± SD ($n = 3$ for Cdc1 and Tpr1, $n = 2$ for Rsd1 and SPAC824.09c). (C) Increase of sRNA-target protein level in *dcr1Δ* has physiological consequences. Spotting assay demonstrating partial suppression of *cdc1-M78* allele in *dcr1Δ* in the semi-restrictive temperature 34 °C (right panel), compared to the permissive temperature 25 °C (left panel).

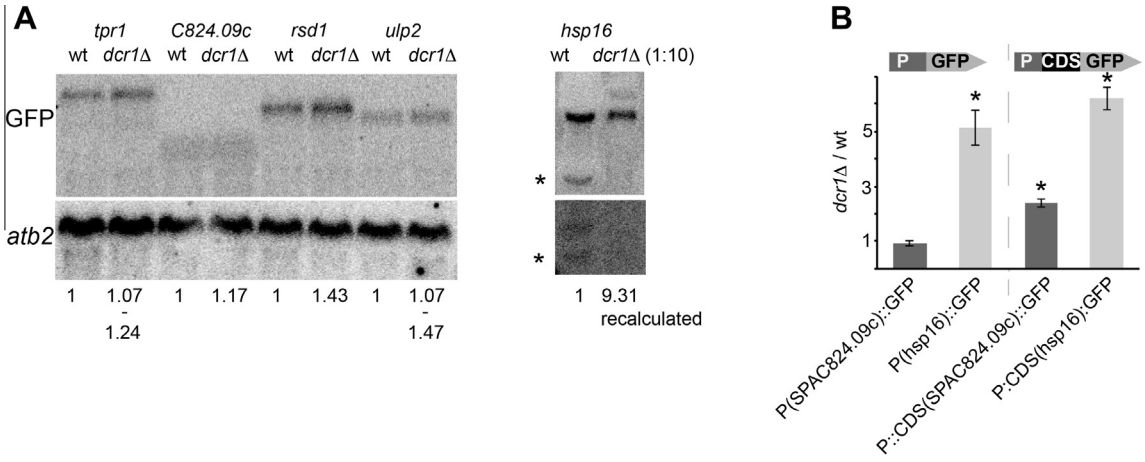

Fig. 3. Control of genes with anti-sense sRNAs is post-transcriptional. (A) Northern blot of total RNA from HA:GFP reporter strains using riboprobes specific to GFP (upper panel) and tubulin *atb2+* (lower panel). The numbers denote ratio of normalized signal in *dcr1Δ*:wt. Two numbers represent two independent experiments. The asterisk denotes a band of unknown specificity in *hsp16:HA:GFP* strains. Note that the RNA of *dcr1Δ hsp16:HA:GFP* is diluted 10-fold. (B) Quantitation of GFP signal from endogenous *SPAC824.09c* promoter-driven GFP with or without the protein coding sequence (CDS). Ratios of GFP intensity in *dcr1Δ*:wt are presented. Results from one representative experiment are shown ($n > 80$). Error bars represent 95% confidence interval for the mean. Asterisks denote statistically significant increase of the GFP signal intensity in mutant compared to wt ($p < 0.001$ by Student's *t*-test).

the GFP signal of the reporter:HA:GFP fusions was increased in *rdp1Δ* (Fig. 4A).

To determine whether sRNAs mapped anti-sense to the reporter genes are incorporated into Ago1 complexes, we compared the read counts in libraries of Ago1-bound sRNAs [17,18]. We found significant numbers of sRNA reads anti-sense to the reporter genes in one of two libraries prepared from wt yeast (Table S6).

Next, we tested whether heterochromatin is involved in RNAi-mediated gene repression. Clr4 methylates H3K9, creating a binding site for the heterochromatin protein Swi6 [1]. Absence of

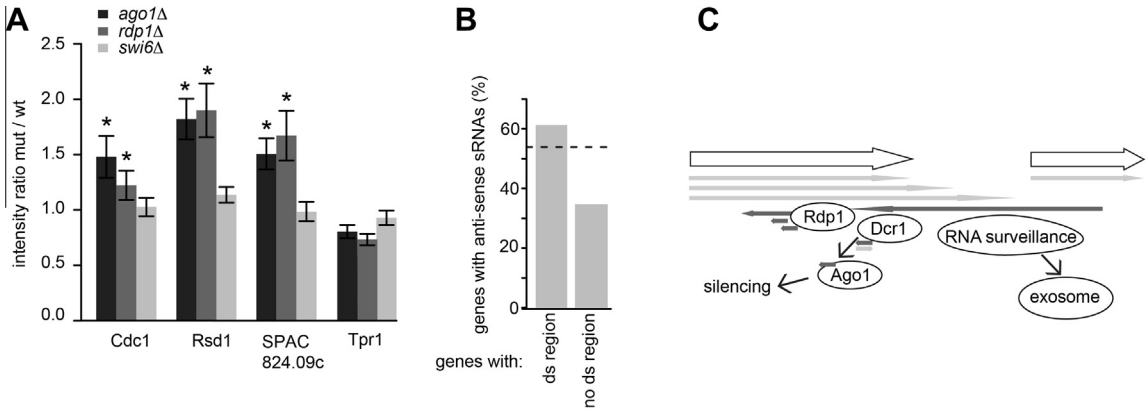

Fig. 4. Genetic requirements for RNAi-mediated gene silencing in fission yeast. (A) Quantitation of GFP signal as in Fig. 2A. Ratios of GFP intensity in mutant:wt are presented. Results from one representative experiment are shown ($n > 80$). Error bars represent 95% confidence interval for the mean. Asterisks denote statistically significant increase of the GFP signal intensity in mutant compared to wt ($p < 0.001$ by Student's t-test). (B) Genes containing anti-sense sRNA reads within their coding sequence and UTRs are over-represented among genes associated with dsRNA regions; the dashed line depicts genome-wide average ($p < 0.0001$, Fisher's exact test). (C) A putative model for RNAi-mediated gene repression of protein coding genes in *S. pombe*. Antisense transcripts may arise as products of read-through transcription or Rdp1 activity, and by base pairing with mRNA form double-stranded RNA, which can serve as a substrate for Dcr1. Gene-specific sRNAs are incorporated by Ago1, which directs silencing.

methylated H3K9 within the gene was one of our criteria for reporter selection. Additionally, we did not detect statistically significant overlap of genes with anti-sense sRNAs, accumulating methylated H3K9 and up-regulated at the mRNA level in *clr4Δ* [6,13] (Fig. S3). Live cell imaging of selected HA:GFP reporter fusions in *swi6Δ* revealed no increase in the GFP level compared to wt (Fig. 4A).

To address the mechanism of sRNA generation, we asked whether dsRNA can serve as a source of gene-specific sRNAs. We identified regions of detectable RNA profiling signal on both strands within the protein coding genes. We found that in many instances sRNAs mapped to these regions (Fig. S4A). Moreover we observed a statistically significant overlap between dsRNA-associated genes and genes containing anti-sense sRNAs (Fig. S4B).

4. Discussion

Small RNAs mapped to protein coding genes in *S. pombe* have been observed in earlier studies of Ago1-bound sRNA populations [17,18]. However, the biogenesis and function of gene-specific sRNAs have been elusive. A recent report proposed that gene-specific sRNAs are products of RNA surveillance of aberrant mRNAs, and arise in a Dcr1-independent manner [18]. In this study we show that the presence of sRNAs anti-sense to protein coding genes does not correlate with the level of anti-sense transcripts. In our model of gene-specific sRNA generation, sRNAs could be products of endonucleolytic activity of Dcr1 directed against locally formed dsRNA. The presence of splice junction-mapped anti-sense sRNAs suggests that in some cases dsRNA can be a product of RNA-dependent RNA polymerase activity. However, this activity does not lead to signal amplification, as is the case in the co-transcriptional RNAi at the centromere, as levels of gene-specific sRNAs are much lower than the centromeric ones. Additionally, dsRNA could arise from read-through transcription of adjacent genes [19] or pervasive anti-sense transcription [20]. We note that the sRNAs may also arise in a Dcr1-independent manner, possibly as products of exosome-mediated RNA degradation, which has been proposed to act in parallel to RNAi and compete for aberrant RNA substrates [18,21].

Our findings extend the current model in which the RNAi and the methylation of H3K9 are interdependent processes in *S. pombe* [1]. Centromeric transcripts as well as transcripts of stress-induced genes are subject to co-transcriptional degradation by RNAi [1,22]. In addition, RNAi is implicated in transient heterochromatin assembly and co-transcriptional regulation of convergent gene pairs in the G1-S phase of the cell cycle [19]. It is unlikely that the gene repression described here represents the co-transcriptional or heterochromatin-mediated silencing.

Firstly, heterochromatin did not play a role in the RNAi-mediated repression described here. Moreover, sRNA libraries and downstream analyses of protein and mRNA abundance were performed using unsynchronized cultures, which were predominantly in G2, so the effect we described was not limited to G1-S. Furthermore, gene orientation did not have a decisive effect on RNAi-mediated repression. Our findings are mechanistically similar to post-transcriptional silencing of a reporter GFP upon overexpression of a GFP hairpin [4]. In contrast to the hairpin-reporter system, we did not observe large differences in the steady state mRNA levels. A possible reason could be that an overexpression system produces much more abundant sRNAs than endogenous RNAi, resulting in a larger response.

In our model of RNAi-mediated gene regulation, sRNAs are products of dsRNA cleavage by Dcr1, and are incorporated to an Ago1-containing effector complex, which targets mRNAs via base pairing with anti-sense sRNA. In line with this model, we found numerous sRNA reads mapped anti-sense to protein coding genes, including reporters used in this study, in Ago1-bound sRNA library [17]. sRNAs may target nascent transcripts in the nucleus, consistently with intron–intron and exon–intron boundary-derived anti-sense sRNAs. Given that we observe a very small increase in mRNA levels in few cases of genes with anti-sense sRNAs, we propose that the general mechanism of gene-specific RNAi in *S. pombe* does not involve gross target destabilization. Instead, mRNAs could be sequestered in non-translatable form and their decay could take place at a later stage, as a consequence of silencing rather than being its cause. Such scenario is in agreement with small changes in mRNA levels of target genes. A similar mechanism is utilised by miRNAs [8].

RNAi has been implicated in gene regulation in other eukaryotes in post- and co-transcriptional manner [2]. This dual functionality of the RNAi pathway is present already in the unicellular eukaryote fission yeast. The co-transcriptional mode of RNAi is well characterized. In this study, we present evidence that protein coding genes are controlled by RNAi in a post-transcriptional manner, seemingly without alterations of their chromatin state. These mechanistically different modes of RNAi are all executed using a non-redundant set of core proteins (Dcr1, Ago1, Rdp1), pointing

to a versatility of the RNAi machinery in output selection. An intriguing question is how these different modes of RNAi are selected and which factors dictate the resulting mode of RNA silencing. For example, it is possible that in the absence of certain factors and in the nucleosome composition context of protein coding genes, H3K9 cannot be efficiently methylated, preventing heterochromatin formation, thus redirecting the silencing effects to post-transcriptional repression.

Our results provide the first evidence in fission yeast that protein coding genes are under post-transcriptional control by endogenous RNAi. Given the growing evidence that highlights the importance of RNAi at the interface of gene regulation and chromatin state control, our system may serve as a non-redundant and a genetically tractable model to dissect the differences between different modes of RNAi.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.01.057.

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