Biochemical and Biophysical Research Communications 444 (2014) 254–259

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

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

Agata Smialowska [a,b,*], Ingela Djupedal [a], Jingwen Wang [a], Per Kylsten [b,1], Peter Swoboda [a], Karl Ekwall [a,b,*]

[a] Center for Biosciences, Department of Biosciences and Nutrition, Karolinska Institute, Huddinge 141-83, Sweden
[b] School of Life Sciences, Södertörn Högskola, Huddinge 141-89, Sweden

ARTICLE INFO

Article history:
Received 27 December 2013
Available online 23 January 2014

Keywords:
RNAi
PTGS
Fission yeast

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.

* Corresponding authors at: Center for Biosciences, Department of Biosciences and Nutrition, Karolinska Institute, Huddinge 141-83, Sweden.
E-mail addresses: smialowskaa@gmail.com (A. Smialowska), Karl.Ekwall@ki.se (K. Ekwall).
[1] Present address: Europaskolan, 645-30 Strängnäs, Sweden.

0006-291X/$ - see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2014.01.057

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.