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Small RNAs Correspond to Centromere Heterochromatic Repeats

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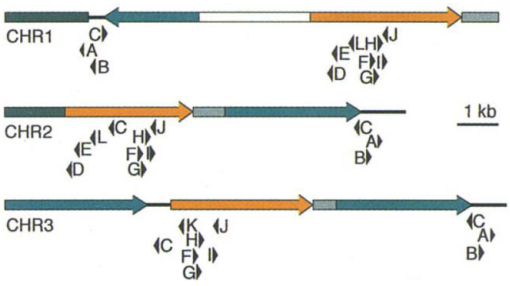
Small interfering RNAs (siRNAs) and microRNAs (miRNAs) are two types of ~22–nucleotide (nt) noncoding RNAs that play important roles as regulators of gene expression in eukaryotes (*1*). siRNAs derive from the successive cleavage of long double-stranded RNA (dsRNA). They direct the destruction of corresponding mRNA targets during RNA interference in animals and probably during other RNA-silencing phenomena, including posttranscriptional gene silencing of plants and quelling of *Neurospora*. miRNAs are processed from endogenous hairpin transcripts such that a single miRNA is produced from one arm of each hairpin molecule. Certain *Caenorhabditis elegans* miRNAs are known to direct translational repression of mRNA targets needed for proper larval development, and numerous plant and animal miRNAs are thought to play similar roles in other contexts by targeting mRNAs for translation attenuation or destruction (*1*–*3*). The ribonuclease III protein Dicer is required for the processing of both siRNAs and miRNAs from their respective precursor RNAs, and Argonaute (PAZ/PIWI domain) proteins, whose biochemical functions are unclear, are also necessary for production or function of both miRNAs and siRNAs (*1*).

A Dicer and an Argonaute homolog are found in the genome of *Schizosaccharomyces pombe*, implying that siRNAs, miRNAs, or another class of small RNAs might play an important role in fission yeast. To investigate this possibility, we cloned endogenous RNAs from exponentially growing *S. pombe* using a method designed to clone RNAs with the features of Dicer cleavage products, i.e., ~22-nt RNAs with 5′-phosphate and 3′-hydroxyl groups (*4*). Of 61 sequenced clones, 49 were fragments of degraded rRNA or tRNA, which are typically seen as background in such cloning efforts. Each of the remaining 12 sequences matched the *S. pombe* centromeric repeats (Fig. 1).

The majority of the centromeric RNAs are from the dh repeat, an element that can confer heterochromatic silencing on another locus and is sufficient for centromere function along with the centromeric central core (*5*, *6*). None of the RNAs match other heterochromatic regions, such as the centromeric dg repeat, the centromeric core sequences, or the mating type locus region homologous to the dh repeat, although our sampling of small RNAs is far from complete. Because *S. pombe* centromeres are large regions (40 to 100 kb) with homologous repeating units, we do not know if these small RNAs arose from a single domain of one centromere or from multiple sites on different chromosomes. These small RNAs do not appear to be miRNAs, in that transcription of adjacent genomic sequence would not produce foldback structures akin to those of the miRNA precursors. Instead, the small RNAs are reminiscent of siRNAs, corresponding to transcripts generated from both DNA strands of the repeat region (Fig. 1). Indeed, Volpe *et al.* report in this issue that transcripts are produced from both strands of the dh repeat, and these transcripts markedly increase in abundance in *S. pombe* mutants of *dcr1*, the Dicer homolog, and *ago1*, the Argonaute homolog (*7*). Most of our centromeric small RNAs cluster within or near these transcripts, suggesting that RNAs produced from each strand of the repeat anneal to form dsRNA that is cleaved by Dicer into the small RNAs (*7*). Mutations in *dcr1* and *ago1* in *S. pombe* reduce centromeric repeat H3 K9 methylation, which is necessary for centromere function (*7*). Accordingly, we refer to these small RNAs as heterochromatic siRNAs and suggest that they specify the epigenetic modifications.



A) GAGGCUUUCG GUUUAGUCGC
B) AAUGCGGAGU AAGGCUAAUC ACGGUA
C) UCUAGCUUCG CCAUCAAUAA GUA
D) UGGAUUAAGG AGAAGCGGUA
E) ACAAGUGAUA AGAGUAGGUG U
F) UGCGCAACUC CUGCUUAUCG UC
G) UACAAGAUAU AGCGCCACAC U
H) UGAGCAUAUC CUAAUGACAG UA
I) UGCCUAUUUA UACAUUUCCC
J) UCUACCUCAG CAGUCCUUGG GAAA
K) UGUGUCCAUA UCCAUGCUGU GUCCA
L) UAAACAACUU GCAAUAUCUG CCA

Fig. 1. Heterochromatic siRNAs (sequences A through L) from *S. pombe*. The loci and orientation of matches to representative repeats are indicated below each centromere fragment. Sequence K only matches ChrIII, and sequences D, E, and L match other repeats on ChrIII. Centromeric repeats (*10*) are in green (dg) and orange (dh). Although the innermost centromeric repeats contain tRNA sequences, all tRNA fragments that were cloned map elsewhere.

Reports of endogenous small RNAs in other species have not described heterochromatic siRNAs. Much of the focus has been on miRNAs, many of which appear to regulate expression levels of cell fate determinants and thus are likely to be far more abundant in plants and animals than in unicellular yeast (*1*, *3*). Endogenous siRNAs that could cleave complementary mRNAs are also described in plants and animals. However, other cloned RNAs do not fall into these two classes and might be heterochromatic siRNAs. In fact, epigenetic modifications have been correlated with small RNAs in multicellular eukaryotes, such as methylation of promoter DNA during transcriptional silencing of *Arabidopsis* transgenes (*8*). DNA methylation, which is downstream of H3 K9 methylation in *Neurospora* (*9*), might be a consequence of heterochromatic siRNA-mediated H3 K9 methylation. Therefore, sequence-specific targeting of histone modifications could be another broadly conserved mode of gene silencing by small RNAs.

References and Notes

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