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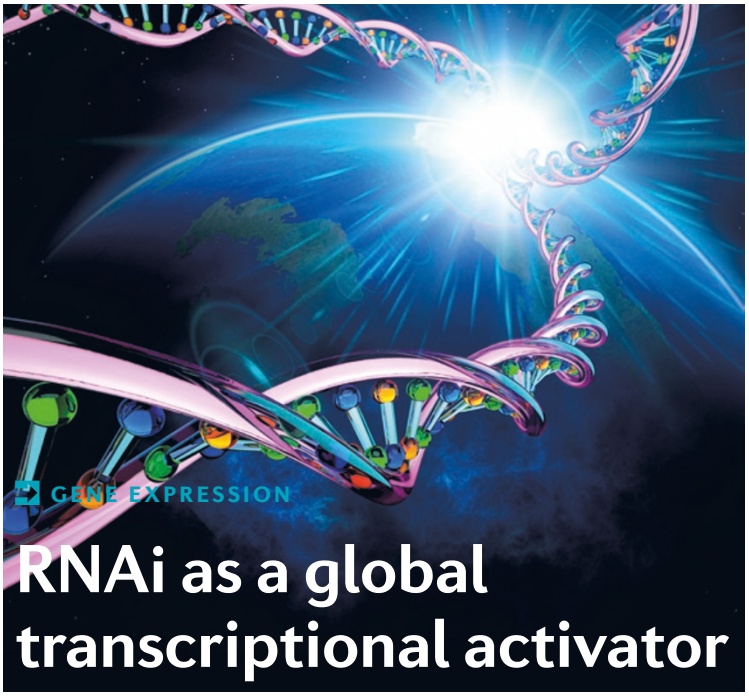

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RNAi as a global transcriptional activator

> the CSR-1 RNAi pathway promotes sense-oriented transcription by Pol II and concomitantly reduces antisense transcription

In *Caenorhabditis elegans*, the Argonaute protein CSR-1 binds to thousands of germline endogenous siRNAs (known as 22G-RNAs) with sequences complementary to germline transcripts. However, the role of the CSR-1 pathway in gene expression regulation remains controversial. Grishok and colleagues now report that this RNAi pathway promotes sense-oriented RNA polymerase II (Pol II)-mediated transcription.

The best-characterized function of endogenous siRNAs is the inhibition of gene expression at the post-transcriptional level. However, the CSR-1 pathway has been shown to activate the expression of some genes. To evaluate the genome-wide effects of the CSR-1 pathway on Pol II transcription, the authors used a global run-on sequencing (GRO–seq) method that enables mapping of the position, and determination of the amount and orientation of transcriptionally engaged Pol II. In *csr-1* mutants, and in mutants of Dicer-related helicase *drh-3* (which are depleted of 22G-RNAs), there was a global reduction of transcription at CSR-1 target genes but also a global increase in transcription of non-CSR-1 target genes. This suggests that CSR-1 specifically promotes transcription of its target genes in a 22G-RNA-dependent manner.

These experiments also provided insights into which step of Pol II transcription is regulated by CSR-1–22G-RNAs. The authors observed that Pol II transcription in CSR-1 pathway mutants was reduced along the entire length of genes, including the promoters. Moreover, they found that CSR-1 interacted with the Pol II complex and that this interaction was dependent on RNA. Thus, CSR-1 seems to have a direct effect on the Pol II complex at target genes by associating, through 22G-RNAs, with nascent transcripts.

Another important finding was a global increase in antisense Pol II transcription in *csr-1* and *drh-3* mutants. This suggests that by interacting with nascent sense RNAs, CSR-1-associated 22G-RNAs stabilize the sense-oriented Pol II machinery. In turn, this may reduce the probability of Pol II initiation in antisense orientation.

Further analysis of the genome-wide distribution of Pol II revealed that in wild-type animals Pol II was predominantly located at genes that are highly expressed in the germ line, whereas in CSR-1 pathway mutants Pol II was depleted at these genes and instead became enriched at genes that should be weakly expressed or silenced. Consistent with this, mutants displayed increased transcription in domains that normally contain the centromeric histone H3 variant CENPA and the H3K27me3 (trimethylation at Lys27 on histone H3) repressive mark.

Together, these results indicate that the CSR-1 RNAi pathway promotes sense-oriented transcription by Pol II and concomitantly reduces antisense transcription. In this way, it contributes to the propagation of silent and active chromatin and to the maintenance of genome organization. As Argonaute proteins have been associated with euchromatin in other animal systems, positive regulation of transcription may be a conserved function of endogenous RNAi.

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