

 GENE REGULATION

# RNAi gets stuck into transcription

RNAi is best known for its various roles in post-transcriptional gene silencing in the cytoplasm. It is also known to have nuclear functions associated with repressive chromatin or gene silencing, such as the formation of heterochromatin in some species. Researchers have now uncovered a rather different nuclear role for some key RNAi proteins: interaction with the core transcriptional machinery at transcriptionally active loci.

Through a series of cellular and chromatin fractionation experiments in *Drosophila melanogaster* cells, Cernilogar *et al.* showed that the RNAi components Dicer 2 (DCR2) and Argonaute 2 (AGO2) are mainly nuclear and associated with chromatin. *In vivo* staining with antibodies against these proteins revealed that they localize to euchromatic, transcriptionally active regions of *D. melanogaster* polytene chromosomes, including loci containing copies of the heat-shock gene *Hsp70*.

The authors found that depletion of AGO2 or DCR2 resulted in increased expression of heat-shock genes under non-heat-shock

conditions. A key mode of transcriptional regulation of the heat-shock genes is RNA polymerase II (RNAPII) pausing; prior to heat shock, RNAPII stops a short distance downstream of the transcriptional start site and heat shock triggers transcriptional elongation. Through further experiments, including chromatin immunoprecipitation to look at the distribution of RNAPII, Cernilogar *et al.* showed that these RNAi proteins are involved in maintaining RNAPII pausing. This influence on pausing also occurs at loci other than heat-shock genes: in particular, AGO2 and DCR2 seem to be involved in the global transcriptional repression of non-heat-shock genes that occurs after heat shock, and their influence on RNAPII dynamics requires their enzymatic activity.

The authors examined the relationship between these RNAi proteins and the core transcriptional machinery in more detail. They found that DCR2 and AGO2 interact with RNAPII and negative elongation factor E (NELFE) and that they also influence the association of

RNAPII with NELFE; this might be a mechanism by which the RNAi proteins influence RNAPII dynamics. However, small RNAs also seem to be involved in the activity of the RNAi proteins at active loci: high-throughput sequencing of RNAs that are associated with AGO2 produced small RNA tags that map across the transcription unit of active genes. This distribution suggests a possible influence of AGO2 on RNAPII processivity as well as pausing. The AGO2-associated RNAs were derived from the antisense strand, and so they might target AGO2 to sense transcripts.

This work reveals a novel sphere of influence of RNAi. It will be interesting to explore whether this involvement in RNAPII activity occurs in other species and also to further dissect the mechanisms and the types of genes that it acts on.

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**ORIGINAL RESEARCH PAPER**

Cernilogar, F. M. *et al.* Chromatin-associated RNA interference components contribute to transcriptional regulation in *Drosophila*. *Nature* 6 Nov 2011 (doi: 10.1038/nature10492)



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