RNA INTERFERENCE

Nuclear Dicer makes the cut

Dicer processes dsRNAs ... to promote the formation of heterochromatin

Post-transcriptional gene silencing, which has a central role in regulating gene expression in eukaryotes, is mediated through several types of small RNAs, such as siRNAs and microRNAs (miRNAs). Their biogenesis requires the cleavage of precursor RNAs by the processing enzyme Dicer (encoded by *DICER1*) before they are loaded onto Argonaute (AGO) to guide it to target mRNAs in the cytoplasm. In mammalian cells, it was thought that Dicer only had cytoplasmic functions; however, the presence of a non-canonical nuclear localization signal in Dicer suggests that this endonuclease might have a nuclear role. White *et al.* now show that, in mammalian cells, Dicer localizes to the nucleus and is recruited to loci of endogenously overlapping transcripts to promote the formation of heterochromatin.

To investigate the cellular distribution of Dicer, the authors used immunofluorescence and cellular fractionation analysis and found that

the endogenous protein localized to both the cytoplasm and the nucleus. By contrast, overexpressed GFP-tagged Dicer was only detected in the nucleus of human *DICER1*-knockout cells, which suggests that the nuclear levels of this enzyme are tightly controlled. Dicer co-immunoprecipitated with RNA polymerase II (Pol II), but this interaction was decreased in nuclear extracts that had been treated with the dsRNA-specific RNase V1; this suggests that the Dicer–Pol II association is dependent on the presence of dsRNA. Furthermore, using chromatin immunoprecipitation followed by sequencing the authors identified multiple genomic loci that were enriched in Dicer, and the addition of the transcription inhibitor α -amanitin (which is an amatoxin) reduced Pol II and Dicer levels at four selected genes. These findings suggest that Dicer and Pol II co-associate at transcriptionally active genes.

The levels of sense and antisense transcripts of these four genes were augmented in *DICER1*-knockdown cells, but this increase was reduced in the presence of the RNase V1, which suggests that Dicer decreases the formation of dsRNA. The authors investigated the possibility that nuclear Dicer induces transcriptional gene silencing in mammalian cells, a process that has been described in other eukaryotic cells and involves the siRNA–AGO-mediated recruitment of methyltransferases to target chromatin to promote the generation of repressive chromatin structures. They found that Dicer-positive loci had increased levels of AGO1 and the

repressive histone mark dimethylated Lys9 of histone H3 (H3K9me2), which suggests that Dicer induces the formation of H3K9me2 through the processing of dsRNAs into siRNAs and loading of AGO1. In agreement with this, the authors found that the levels of dsRNAs and small RNAs were increased in *DICER1*-knockout cells and, moreover, that dsRNAs occurred at chromatin-binding sites of Dicer.

Invading viral dsRNA is eliminated through the host interferon-response pathway, which ultimately kills the infected cell; so, the authors examined whether the accumulated dsRNA in Dicer-depleted cells induces this defence mechanism. Indeed, genes encoding key proteins of the interferon-response pathway were upregulated in *DICER1*-knockdown cells, which displayed increased levels of apoptosis, as evidenced by morphological abnormalities and increased annexin V labelling.

Together, the results of this study reveal a biological role for nuclear Dicer in mammalian cells. Dicer processes dsRNAs that arise during convergent transcription to promote the formation of heterochromatin possibly through AGO1-mediated recruitment of methyltransferases and the establishment of repressive histone marks.

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ORIGINAL RESEARCH PAPER White, E. *et al.* Human nuclear Dicer restricts the deleterious accumulation of endogenous double-stranded RNA. *Nature Struct. Mol. Biol.* <http://dx.doi.org/10.1038/nsmb.2827> (2014)

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