

 SMALL RNAS

An epigenetic silencing influence

MicroRNAs (miRNAs) regulate gene expression by base pairing with target RNAs, leading to their cleavage in plants or translational inhibition in animals. Now evidence has emerged that in moss, miRNAs can also silence gene expression at the transcriptional level by interacting with DNA, leading to methylation. This discovery broadens the regulatory influence of miRNAs, and the mechanism may also be applicable to other organisms.

Khraiwesh *et al.* examined the role of Dicer proteins in the moss *Physcomitrella patens*, which has four Dicers (DICER-LIKE 1a (DCL1a), DCL1b, DCL3 and DCL4). They chose *P. patens* because the dual functions of Dicers in miRNA biogenesis and target cleavage are separable in

this species. Using targeted knock-out-mutants of *P. patens*, the authors showed that DCL1a is required for miRNA biogenesis and DCL1b is required for miRNA-induced target RNA cleavage.

Intriguingly, *DCL1b*-null mutants had reduced levels of miRNA target transcripts despite the absence of miRNA-guided cleavage. How do miRNAs regulate their targets in *DCL1b* knockouts? The authors found that the genes that encode miRNA targets were methylated in *DCL1b* knockouts, but were not methylated in wild-type controls. They then showed that there was a reduced rate of transcription of miRNA target genes compared with unmethylated non-miRNA-target genes in *DCL1b* knockouts, indicating that the observed methylation leads to transcriptional silencing of genes that encode miRNA targets.

The authors suggested that in *DCL1b*-null mutants, miRNAs form stable duplexes with mRNAs within an RNA-induced transcriptional silencing complex. Consistent with this model, the miRNA targets primed cDNA synthesis without the addition of exogenous primers, supporting the existence of miRNA–mRNA duplexes. Do the levels of miRNAs, and therefore the levels of these duplexes, influence DNA methylation? Khraiwesh *et al.* created transgenic *P. patens* lines that expressed different levels of an artificial miRNA (amiRNA) and found that with increased expression

of the amiRNA, there was increased silencing of the amiRNA target. Moreover, methylation and silencing were not restricted to *DCL1b* knockouts — they also occurred when high levels of amiRNA were expressed in wild-type *P. patens* lines.

As transcriptional silencing was also observed in non-transgenic *P. patens*, the authors investigated whether this pathway could also operate in wild-type *P. patens* under physiological conditions. Treatment of *P. patens* with the plant hormone abscisic acid (ABA) led to reduced levels of an miRNA target transcript (*bHLH*) that encodes a basic helix–loop–helix transcription factor, which in turn led to increased levels of the cognate miRNA (miR1026) and methylation of the *bHLH* gene. As ABA is a mediator of stress signaling, these results suggest that miRNAs might epigenetically regulate stress-responsive genes.

The physiological regulation of this epigenetic miRNA-induced silencing pathway and the conservation of miRNA pathway components among species suggest that this mechanism might be generally applicable — a topic for future investigation.

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ORIGINAL RESEARCH PAPER Khraiwesh, B. *et al.* Transcriptional control of gene expression by microRNAs. *Cell* **140**, 111–122 (2010)
FURTHER READING Ghildiyal, M. & Zamore, P.D. Small silencing RNAs: an expanding universe. *Nature Rev. Genet.* **10**, 94–108 (2009)

