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Review

miRNA Biogenesis: A Dynamic Pathway

Natalia P. Achkar,[1,2] Damián A. Cambiagno,[1,2] and Pablo A. Manavella[1,*]

MicroRNAs (miRNAs) modulate plant homeostasis through the inactivation of specific mRNAs, especially those encoding transcription factors. A delicate spatial/temporal balance between a miRNA and its targets is central to achieving the appropriate biological outcomes. In this review we discuss our growing understanding of the dynamic regulation of miRNA biogenesis. We put special emphasis on crosstalk between miRNA biogenesis and other cellular processes such as transcription and splicing. We also discuss how the pathway is regulated in specific tissues to achieve harmonious plant development through a subtle balance between gene expression and silencing.

Trends

The assembly of the miRNA biogenesis machinery starts during miRNA gene transcription.

There is crosstalk between components of the miRNA biogenesis and mRNA splicing machineries, which mutually regulate each other.

miRNA production is regulated tissue-specifically and varies depending on each pri-miRNA.

The miRNA pathway is a fluid, dynamic, and interconnected process.

Plant miRNA Biogenesis

MicroRNAs (miRNAs) (see Glossary) are essential components of the gene silencing machinery in most eukaryotic organisms. The plant miRNA pathway, both at the biogenesis and silencing levels, differs substantially from its counterpart in mammals. In plants, miRNAs are produced from a primary miRNA transcript (pri-miRNA), which includes a foldback structure, by the nuclear RNase **DICER-LIKE 1** (DCL1) and its accessory proteins SERRATE (SE) and HYPONASTIC LEAVES1 (HYL1). The central components of the plant miRNA biogenesis pathway, such as DCL1, HYL1, and SE, were identified long ago and served as the foundation of the earliest models of miRNA biogenesis [1]. Only recently has the true complexity of miRNA biogenesis pathway, especially its plasticity, regulation, and crosstalk with other biological processes, become apparent. We now know that the production of miRNAs is a tissue-specific process, is tightly associated with transcription and splicing, and even varies between miRNA precursors. Many aspects of the miRNA pathway, such as biogenesis, turnover, subcellular compartmentalization, and response to the environment, have been reviewed in recent years [1–4]. Instead, in this review we explore the pathway from a different perspective. We center our discussion on the dynamics of miRNA production, its crosstalk with other cellular processes, and how it is regulated in a very subtle and specific manner. We ultimately aim to help the reader construct a fresh view of miRNA biogenesis as a fluid and adaptable process.

Early Transcriptional Events

As for any coding mRNA, the pri-miRNAs are transcribed by the RNA polymerase II (RNAPII) in a process regulated by the MEDIATOR complex and by phosphorylation of the **C-terminal domain of RNA polymerase II** (RNAPII-CTD) largest subunit by CYCLIN-DEPENDENT KINASES (CDKF1 and CDKDs) [5,6]. Similarly to the transcriptional regulation of coding genes, the expression of each individual miRNA gene is controlled by specific transcription factors. However, some transcription factors have been described to affect the miRNA pathway as a whole. NEGATIVE ON TATA LESS 2 (NOT2) interacts with the RNAPII and regulates the transcription of miRNA genes [7]. The MYB-related protein CELL DIVISION CYCLE 5 (CDC5) associates with miRNA gene promoters to increase RNAPII occupancy and transcription [8]. PLEIOTROPIC REGULATORY LOCUS 1 (PRL1) interacts with DCL1 and pri-miRNAs [9]. Such interactions

[1]Instituto de Agrobiotecnología del Litoral, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Facultad de Bioquímica y Ciencias Biológicas (FBCB), Universidad Nacional del Litoral (UNL), Paraje El Pozo, 3000 Santa Fe, Argentina
[2]These authors contributed equally.

*Correspondence: pablomanavella@ial.santafe-conicet.gov.ar (P.A. Manavella).

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enhance pri-miRNA processing potentially by stabilizing the **processing complex**. Because PRL1 also interacts with RNAPII and CDC5, it is possible that this cofactor is also recruited to the nascent pri-miRNA co-transcriptionally [9,10]. In a less well understood process, the cycling DOF transcription factor (CDF2) directly interacts with a subset of miRNA gene promoters where it can act as either a positive or negative regulator [11]. Transcription factors regulating the expression of core components of the biogenesis machinery have a general impact on the production of miRNAs. This is the case of XAP5 CIRCADIAN TIMEKEEPER (XCT), a nuclear protein that modulates **small RNA** production by regulating the transcription of DCL coding genes [12]. HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 1 (HOS1) is an ubiquitin E3 ligase that promotes RNAPII occupancy at the miRNA168b (miR168b) promoter and enhances its transcription. Even when HOS1 only regulates miR168b, it ultimately affects the accumulation of AGO1 (target of miR168b) and thus the activity of most miRNAs [13]. Similarly, any transcription factor that regulates the expression of miR162 and miR863, which target DCL1 and SE respectively, will affect miRNA production. In another layer of regulation, it was recently discovered that pri-miRNA can encode self-regulating small peptides (miPEP) [14]. At the chromatin level it has been shown that the ATP-dependent SWR1 chromatin-remodeling complex (SWR1-C) regulates the transcription of miRNA genes by altering nucleosome dynamics [15]. Another example of epigenetic regulation is the indirect repression of DCL1, SE, HYL1, and **ARGONAUTE1** (AGO1) by the histone acetyltransferase GENERAL CONTROL NON-REPRESSED PROTEIN 5 (GCN5) [16].

Cotranscriptional Assembly of the Processing Complex

In plants, pri-miRNA transcription and processing were long believed to be separated processes taking place one after the other. Recent evidence suggests that both processes are tightly associated, with at least the assembly of the processing complex starting during transcription. One of the strongest lines of evidence for coupling between miRNA gene transcription and processing was the discovery of the Elongator complex as a key component in the miRNA biogenesis [17]. This complex is required for RNAPII occupancy at miRNA loci and interacts with DCL1 and SE. More strikingly, DCL1 was found to associate, in an Elongator-dependent manner, with the chromatin of miRNA loci [17]. This suggests that DCL1, and probably SE, are recruited to the nascent pri-miRNAs co-transcriptionally, likely during transcript elongation. Supporting this idea, C-TERMINAL DOMAIN PHOSPHATASE-LIKE 1 and 2 (CPL1 and CPL2) are able to interact both with the RNAPII-CTD and the miRNA processing machinery, specifically with SE. CPL1 and CPL2 modulate RNAPII activity by dephosphorylating its CTD and regulating miRNA biogenesis by modulating HYL1 activity [18,19]. Owing to its dual interaction with the RNAPII-CTD and SE, we speculate that CPL proteins are also recruited into the processing complex during miRNA gene transcription. NOT2, CDC5, PRL1, and CDF2, that associate with RNAPII and miRNA gene promoters as described above, also interact with components of the miRNA processing machinery [7,8,11]. CDC5, PRL1 and NOT2 interact with DCL1 and SE to promote pri-miRNA processing. It would not be surprising if these proteins acted by assisting the recruitment of processing factors to the nascent pri-miRNAs [7,8]. Similarly, TOUGH (TGH), which was originally described to interact with TATA binding proteins, also interacts with pri-miRNAs, DCL1, HYL1, and SE [20,21]. Interestingly, TGH may modulate both the interaction of pri-miRNAs with the DCL1-containing complex as well as the activity of DCL proteins [21].

The **nuclear cap-binding complex** (CBC) binds to the 5′ cap of nascent pri-miRNAs leading to the proper production of miRNAs [22–24]. CBP20 and CBP80 were shown to interact with SE, CBP20 also with NOT2b, linking the CBC and the processing machinery [7,25]. However, it is still unclear if the CBC acts as a scaffold to recruit SE and NOT2b to the nascent pri-miRNAs or if such interactions take place after complex formation to stabilize the pri-miRNAs.

Glossary

ARGONAUTE (AGO): the AGO proteins, loaded with small RNAs (sRNAs), catalyze the transcriptional or post-transcriptional silencing of target genes. There are 10 members of the AGO family of proteins in *Arabidopsis*.
C-terminal domain of RNA polymerase II (RNAPII-CTD): highly conserved tandem heptapeptide repeats (Y–S–P–T–S–P–S) located within the C-terminal end of the largest subunit of RNAPII. Post-translational modifications of the RNAPII-CTD regulate RNAPII activity, the recruitment of transcription factors, and RNA processing.
DICER-LIKE (DCL): homologs of the human DICER protein. DCLs are RNase III endonucleases that process long double-stranded RNA (dsRNA) into sRNA.
Dicing body (D-body): subnuclear speckles that contain pri-miRNAs and several components of the miRNA biogenesis machinery.
HUA ENHANCER1 (HEN1): a methyltransferase that methylates the 3′ nucleotides of sRNA duplexes, protecting them from uridylation-triggered degradation.
MicroRNAs (miRNAs): DCL1-dependent ~21 nt sRNAs that guide the RISC complex to target genes, inducing their cleavage or translational inhibition.
Nuclear cap-binding complex (CBC): a protein complex formed by two subunits, CBP20 and CBP80, that binds to the mRNA 5′-cap. The CBC acts in many aspects of mRNA metabolism including mRNA splicing, export, and decapping protection.
Processing complex: defined as the set of proteins required for the processing of a pri-miRNA into a mature miRNA duplex.
RNA-induced silencing complex (RISC): protein complex responsible for triggering sRNA-mediated gene silencing. It contains an Argonaute protein, a loaded sRNA, and accessory proteins.
Small RNA: group of small (20–25 nt) single-stranded RNAs derived from dsRNA precursors that induce transcriptional (TGS) and post-transcriptional (PTGS) gene silencing.