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Plant microRNAs

Shaping Development and Environmental Responses

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Preface

Since they were first described in 1993, microRNAs have been recognized as central players in the regulation of gene expression. This important class of small non-coding RNA molecules is present in widely diverse groups of organisms, including plants and animals. The investigation of microRNA roles in cellular processes and their regulatory functions in response to the external environment and in disease is a highly active and prolific research field.

In plants, there are a number of relatively well-characterized microRNAs which are present across different taxonomic groups. The roles of some of these conserved microRNAs in diverse biological processes, either related to development or interaction with external factors, have been already characterized in some detail, especially in model plant species such as *Arabidopsis thaliana*. However, a wealth of new sequencing data is being produced at an increasing pace. The release of a growing number of plant genome sequences and the relatively low cost of coding and non-coding transcriptome sequencing represent both an opportunity and a challenge. On the one hand, the amount of available information allows the discovery of novel, non-conserved microRNAs, in a wide range of plant species, with as yet unknown but potentially relevant functions. On the other hand, the identification and unequivocal annotation of such sequences are still a major challenge, and the criteria used by different research groups are not homogeneous or consensual. Significant advances in this area are expected to occur in the near future taking advantage of more advanced technologies for investigating regulatory processes *in vivo* and with cellular resolution.

In this book, we provide a state-of-the-art overview of the functions of microRNAs in the regulation of plant development and their responses to the surrounding environment. This overview is presented in the form of review chapters which are organized around four main subjects, including microRNA investigation and annotation, their regulatory roles in diverse developmental processes, and in response to abiotic and biotic factors. Each of the 11 chapters, authored by experts in the field, details a specific aspect of microRNA investigation, including a systematized revision of the key findings in that area or topic. The book will be useful

to those interested in up-to-date knowledge about microRNAs in plants, including undergraduate and graduate students, teachers and researchers. Due to the promising applications of microRNAs in crop breeding and protection, the book will be valuable for scientists in academia and in the private sector as well.

Lisbon, Portugal — Célia Miguel
Norwich, UK — Tamas Dalmay
Oeiras, Portugal — Inês Chaves

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Editors and Contributors

About the Editors

Prof. Célia Miguel is Assistant Professor at the Faculty of Sciences, University of Lisbon, Portugal, and Senior Researcher at the BioISI (Biosystems Integrative Sciences Institute). She has been research group leader for more than 10 years, working on the molecular mechanisms that control key developmental processes in trees, and responses to environmental challenges. She has contributed to generate genomic resources in diverse woody plant species, including their small non-coding RNA transcriptomes, and her research has been published in over 50 papers in internationally recognized journals.

Prof. Tamas Dalmay is Head of School of Biological Sciences at the University of East Anglia, Norwich. His research group has been working on small non-coding RNAs since 2002 and published about 120 papers on the topic. He worked on small RNAs of many different species and contributed to the UEA workbench, a freely available bioinformatics platform to analyse next-generation sequencing data of small RNAs.

Dr. Inês Chaves is Senior Researcher at iBET and ITQB NOVA, Oeiras, Portugal. Her work is dedicated to the study of development and stress response of woody plants. Her approach is mainly biochemical, but since 2006 she has focused her attention on the role of microRNAs as well as other non-coding small RNAs during these processes. She is also involved in the development of bioinformatic tools to be used in model and non-model species. She has over 20 international publications.

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Part I
Studying microRNAs in Plants

Chapter 1
Regulation of Plant microRNA Biogenesis

Aleksandra Grabowska, Susheel Sagar Bhat, Aleksandra Smoczynska, Dawid Bielewicz, Artur Jarmolowski and Zofia Szweykowska Kulinska

Abstract miRNAs play important regulatory roles in various plant metabolic pathways. Similar to any key regulator, miRNA genes are under a variety of transcriptional and post-transcriptional controls. Another layer of this regulation is provided by the regulation of Microprocessor protein components as well as their interactions with other regulatory proteins. When in cytoplasm, miRNA mode of action and turnover is also influenced by various protein partners that either bind directly to the miRNA or interact with them indirectly. We put together currently available data to provide a comprehensive overview regarding miRNA biogenesis, mode of action, and turnover in plants.

Keywords microRNA · Biogenesis regulation · Microprocessor · Posttranscriptional *MIR* gene regulation · microRNA action and turnover · Plants

1.1 Introduction

microRNAs (miRNAs) are small (21–24 nucleotides) regulatory RNAs that are products of RNA Polymerase II transcription. miRNAs are transcribed as long precursors called primary-miRNAs (pri-miRNAs). pri-miRNAs contain a double-stranded hairpin loop region and are processed by miRNA biogenesis machinery to release miRNA/miRNA* duplexes (Xie 2005; Kim et al. 2011). One strand of the miRNA/miRNA* duplex is then incorporated into Argonaute 1 (AGO1) protein which becomes a part of RNA-induced silencing complex (RISC) that executes the post-transcriptional regulation of messenger RNAs (mRNAs) (Vaucheret et al. 2004; Baumberger and Baulcombe 2005; review: Vaucheret 2008). The regulation can be achieved in two different ways: cleavage of mRNAs or translational inhibition (Llave et al. 2002; Palatnik et al. 2007; Brodersen et al. 2008; Eamens et al. 2012).

Aleksandra Grabowska and Susheel Sagar Bhat contributed equally to this work.

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miRNA biogenesis in plants starts from the transcription of *MIR* genes by RNA Pol II (Lee et al. 2004; Xie 2005). *MIR* genes in plants are mostly located in intergenic regions and are independent transcriptional units (Reinhart et al. 2002). Similar to other RNA Pol II transcripts pri-miRNAs also carry a 5′ 7 methylguanosine cap and 3′ polyadenylated tail (Jones-Rhoades and Bartel 2004; Xie 2005).

The long pri-miRNA transcripts are known to generally fold over to form dsRNA structures containing imperfect hairpin loops that house miRNA/miRNA* duplex. Generally, one pri-miRNA gives rise to one mature miRNA species but 'polycistronic' pri-miRNAs (one pri-miRNA gives rise to more than one miRNA) have also been reported (Talmor-Neiman et al. 2006; Merchan et al. 2009; Zhang et al. 2009; Baldrich et al. 2016). miRNA biogenesis is also affected by general transcriptional regulating factors like the Mediator complex (Kim et al. 2011). Absence of Mediator complex leads to lower levels of primary and mature miRNAs owing to lower RNA Pol II occupancy on *MIR* genes. In addition, *MIR* gene promoters have motifs that allow for binding of various transcription factors, which in turn allow for the regulation of miRNA biogenesis according to various developmental and/or environmental cues (Megraw et al. 2006; Yamasaki et al. 2009; Yant et al. 2010; Rogers and Chen 2013; Wang and Perry 2013; Barciszewska-Pacak et al. 2015a; Stepien et al. 2017). Certain transcriptional factors are noteworthy in this regulation and are discussed further.

Negative on TATA less 2 (NOT2a) is a core member of the Carbon Catabolite Repression 4 (CCR4)-NOT complex. NOT2a and NOT2b (previously known as Vire2 interacting protein2) are homologous proteins that act as general transcription factors and associate with RNA Pol II to regulate RNA Pol II based transcription. NOT2a and NOT2b can form homo or heterodimers and interact with Dicer Like 1 (DCL1, discussed later), RNA Pol II and other miRNA biogenesis factors like Serrate (SE) and Cap Binding protein 80/20. Absence or lower levels of NOT2 proteins leads to decreased accumulation of both primary and mature miRNAs (Wang et al. 2013). Cell Division Cycle 5 (CDC5) is another transcription factor that associates with *MIR* gene promoters and positively regulates *MIR* gene transcription (Zhang et al. 2013). Other transcription factors that provide conditional regulation to *MIR* gene expression include, but are not limited to, auxin response factors (ARFs; provide auxin sensitivity) (Megraw et al. 2006), Squamosa Promoter binding protein Like 7 (SPL7; copper sensitivity) (Yamasaki et al. 2009), MYB2 transcriptional factor (phosphate sensitivity) (Baek et al. 2013), Apetala 2 (AP2) (Yant et al. 2010) and Fusca 3 (Wang and Perry 2013) for organ-specific miRNA expression.

miRNA biogenesis is also affected by processes like splicing and alternative polyadenylation (Bielewicz et al. 2013; Schwab et al. 2013; Knop et al. 2017). It has been shown that presence of introns stimulates miRNA biogenesis when miRNA is present in the exonic region, an effect that is enhanced by the presence of active 5′ splice site (5′ ss) rather than splicing itself. In contrast, biogenesis of selected intron-derived miRNAs is enhanced when 5′ ss is inactive or splicing is inhibited. These effects have been annotated to the association between U1 small nuclear ribonucleoprotein (U1 snRNP, component of the spliceosome complex) and Microprocessor components (SE) and Cap Binding Complex (CBC).

pri-miRNAs are processed by the RNase III endonuclease type enzyme DCL1 (Reinhart et al. 2002; Park et al. 2005). DCL1 cleavage is dependent on structural features of pri-miRNAs, especially the stem loop (Song et al. 2010; Werner et al. 2010). The imperfect pairing of the stem loop below the miRNA/miRNA* duplex plays an important role in the DCL1 cleavage (Bologna et al. 2009). pri-miRNAs are processed by DCL1 in a two-step process. In the first step, DCL1 cleaves pri-miRNAs and a precursor-miRNA (pre-miRNA) containing the stem loop (carrying miRNA/miRNA*) with 2nt 3′ overhang and 5′ phosphate group is released (Kurihara and Watanabe 2004). The second cleavage step then releases the miRNA/miRNA* duplex. The precision of this cleavage is also dependent on whether the processing happened from base to loop or loop to base. This bi-directional activity is also attributed to the heterogeneity of pri-miRNA structures (Bologna et al. 2009, 2013; Song et al. 2010; Werner et al. 2010). DCL1 activity also requires several other proteins for proper functioning. Hyponastic Leaves 1/Double stranded RNA Binding protein 1 (HYL1/DRB1) is a double-stranded RNA binding protein that is thought to probably bind the miRNA/miRNA* double-stranded region and guide proper pri-miRNA cleavage (Kurihara, 2005). Similarly, zinc finger protein SE, binds single-stranded RNA regions of pri-miRNAs and helps in proper positioning of pri-miRNAs at the catalytic site of DCL1 (Lobbes et al. 2006; Laubinger et al. 2008). Another protein Tough (TGH) binds single-stranded RNA and is shown to promote DCL1 activity (Ren et al. 2012b). All these proteins, DCL1, HYL1, SE, and TGH are known to interact physically. The plant Microprocessor is largely considered to be formed by DCL1, HYL1, and SE; while the inclusion of TGH in the Microprocessor complex is not yet definitive.

Recently, Chromatin Remodeling Factor (CHR2) has also been shown to be involved in miRNA biogenesis (Wang et al. 2018b). CHR2 is a member of the SWI/SNF chromatin remodeling complex and has ATPase activity. Wang and colleagues show that CHR2 positively affects *MIR* gene transcription and hence leads to higher levels of pri-miRNAs, but when it associates with SE it remodels pri-miRNAs in a way that they are no more suitable substrates for DCL1 mediated cleavage. This remodeling of pri-miRNAs is a result of non-canonical RNA helicase activity of CHR2. The processing of pri-miRNAs thus can also be affected by any modifications that can result in altered structures of pri-miRNAs or can be identified by some specific proteins. Methylation of adenosine at N6 position (m^6A) is one such modification that has been shown to positively affect miRNA production in animals (Alarcón et al. 2015). A recent *Arabidopsis* based study showed that mRNA adenosine methylase (MTA), catalytic component of m^6A methyltransferase complex, binds to and methylates pri-miRNAs and affects their processing. MTA also interacts with RNA Pol II and TGH and thus the possibility that it may also affect *MIR* gene transcription cannot be ruled out (Bhat et al. 2019). Evidence of another regulatory step came in the form of retrograde signaling from chloroplast (Fang et al. 2019). Fang and colleagues showed that nuclear Exoribonuclease 2 (XRN2) degrades pri-miRNAs and it is inhibited by 3′-phosphoadenosine 5′-phosphate (PAP). The levels of PAP are further influenced by tocopherols produced in chloroplast. Another example of retrograde signaling that regulates miRNA biogenesis came from a study done on