Methods in Molecular Biology

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Plant Circadian Networks

Methods and Protocols

Edited by

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Preface

As many organisms, plants evolved an endogenous timekeeper, the biological or "circadian" clock (from Latin *circa diem*, about a day), to synchronize their life with environmental cycles of light and dark and associated temperature cycles. The circadian clock generates an internal time structure, causing major processes in the cell to occur at regular 24-h intervals. This rhythmic component of physiological, biochemical, and molecular processes in the plant has long been mostly neglected when conceiving experiments or analyzing data. The volume "Plant Circadian Networks" provides a collection of protocols that describe how to monitor circadian rhythms at the molecular, biochemical, and physiological level, how to evaluate the data, and how to integrate the data to obtain an overarching picture of circadian networks in the cell.

Chronobiologists, those scientists who occupy their time with studying biological timing (from Greek χρόνος, time or the God of time), have long sought to uncover the molecular underpinnings of endogenous rhythms. More recently, the question why such an endogenous timekeeper may be of benefit for an organism has been addressed.

Plant chronobiology entered the molecular era when the first circadian transcript pattern was described by Klaus Kloppstech about three decades ago. Circadian gene expression experiments require a large number of data points, in other words RNA preparation, around the clock for several cycles of subjective day and night. Thus, they inherently were more laborious than an on/off situation that is measured in experiments looking at the impact of an external stimulus and required a higher precision because subtle differences in expression levels had to be disclosed. A major advance for the field was the development of the luciferase reporter as a noninvasive marker by Andrew Millar, Steve Kay, and coworkers, opening a way to automatization and large genetic screens, and thus leading to the identification of the first clock mutant in *Arabidopsis thaliana*.

Later on, the use of microarrays and next-generation sequencing greatly advanced the field, moving from the analysis of a handful of rhythmic genes to the entire circadian transcriptome. The tight interconnection between endogenous timing and hormone signaling, responses to abiotic stress, and pathogen threat add another level of complexity.

Moving from the model plant *Arabidopsis thaliana* to other systems allowed for identifying common design principles and peculiarities of the clock in different species that may relate to the particular requirements, e.g., seasonal control in trees.

This volume provides a collection of protocols, both standard techniques and the most recent technical developments, to investigate clock-controlled parameters including transcript and small RNA levels, promoter activity using luciferase reporters, protein levels and posttranslational modification, protein–protein interaction, in vivo DNA–protein interaction and RNA–protein interaction, cellular redox state, Ca^{2+} levels, and innate immune responses. Other topics are seasonal processes like flowering time control. Particular emphasis is on the circadian system in the model plant *Arabidopsis thaliana*. In addition, techniques applied in trees, moss, and algae are covered.

Several chapters deal with computational biology. Tools to identify transcription factor binding sites, or small RNA binding sites, and to visualize alternative splicing patterns in RNA-Seq data are covered. The use of BioDare (Biological Data repository) for data storage, data sharing, and processing as well as identification of rhythmic patterns in large data sets is described. Furthermore, it is illustrated how mathematical models can help to understand the design principles of the circadian oscillator and allow to make experimentally testable predictions, ultimately leading to refined oscillator models.

The book is designed for the plant chronobiology community dealing with circadian biology. As the clock has a pervasive effect on all aspects of plant physiology, I hope that the protocols will be of general use to plant biologists.

Bielefeld, Germany ***Dorothee Staiger***

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