

METHODS IN MOLECULAR BIOLOGY™

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Plant Transcription Factors

Methods and Protocols

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Preface

Transcription factors (TFs) are regulatory proteins that generally function by modulating local protein concentrations of limiting factors at the target promoters. TFs operate alone or in complexes with other molecules to activate or repress the recruitment of the basal transcriptional machinery to specific genes, thereby controlling transcription of DNA to mRNA. Close to 10% of higher plant genes encode TFs. The large numbers of TF genes in plant genomes are the result of higher rate of expansion in specific TF superfamilies compared to those in other biological kingdoms. TFs are pivotal in the regulation of plant development, reproduction, intercellular signaling, response to environment, cell cycle, and metabolism. Despite the importance of TFs and the rapid expansion of TF genes discovered through genomics and bioinformatics, only a few TFs have been subjected to extensive functional characterization. A monumental challenge facing plant molecular biologists is the mechanistic understanding of TF functions. This task is particularly challenging considering that TFs may bind to different *cis* elements throughout the genome with varying affinities and often function in a combinatorial fashion with other regulatory factors. Nevertheless, in recent years we have seen a significant advancement in the development of enabling technologies that facilitate the study of TFs.

The purpose of *Plant Transcription Factors* is to introduce the basic concepts and the detailed protocols of a series of commonly used tools for investigating plant TFs. In editing this volume, we envision that the readership will include seasoned plant molecular biologists as well as scientists new to the field of TFs. The chapters are contributed by many leading scientists in the respective areas. Sufficient experimental details are provided to minimize the need to consult additional references.

The organization of this volume first provides an initial discussion of select TF families in plants, with a focus on plant development. The chapters in Section II focus on approaches to identify a TF, either based on homology to TFs/TF families of interest, in association with a particular *cis* element, or via a phenotype (developmental, response to environment, metabolic consequence) of interest. Once identified, it is important to verify the function of the TF in control of a particular process or the ability to impact on gene expression (topics in Section III). Because TFs are most often members of large families, loss-of-function analysis can be hindered by redundancy. [Chapter 5](#) introduces one approach that can address this redundancy.

TFs act in combinatorial fashion and particular complexes can have different consequences for gene expression. Therefore, it is important to identify protein interactions in which TFs are involved and how the interaction is mediated (Section IV, [Chapters 8, 9, 10, and 11](#)). Other post-transcriptional or post-translational control mechanisms of TFs occur and an example of this is covered in [Chapter 12](#). Increasing examples of TFs that function non-cell-autonomously are being discovered and methods to assess intercellular trafficking are addressed in the chapter by Ahmad et al. ([Chapter 13](#)).

Section V examines TF interaction with DNA. Many TFs associate with DNA in a sequence-specific manner and [Chapters 14 and 15](#) present approaches to characterize these DNA *cis* motifs. Finally a key to understanding how TFs actually impact on a process, be it

developmental or response to a stimulus, involves determining what genes are directly controlled by the TF or TF complex of interest. Evaluation of the transcriptome in response to TF accumulation is one piece of this puzzle ([Chapter 7](#)), but assessment of direct interaction with regulatory regions of genes is also important and approaches to ascertain this are presented in [Chapters 16, 17, and 18](#).

Finally, how can we use or even improve TFs to meet the current challenges we face in agriculture? Using TFs to modify specific metabolic pathways in order to improve crop quality is increasingly viewed as a powerful approach in plant biotechnology. However, because TFs commonly regulate more than one gene, to avoid undesirable (unintended) consequences it is critical that a TF is functionally characterized prior to use for the manipulation of plant pathways. Once understood, it is even possible to improve TFs and the final section of this volume discusses directed evolution to generate TFs that can more efficiently control desired processes.

These are exciting times in which to be investigating functions and roles of TFs. With the ever-increasing numbers of organisms with available genome sequence information and the technologies that come with this information, it is becoming possible to obtain a global or near-global view of TF function at a specific developmental stage or in response to a particular stimulus. A challenge in the future will be integrating all of this information to identify regulatory networks and mechanisms underpinning particular processes. We hope *Plant Transcription Factors* provides access of many necessary methods to all scientists who are interested in exploring the functions of TFs. We would like to thank Kathy Shen for her expert assistance in editing this volume. We also would like to thank all the authors who so enthusiastically contributed to this volume and acknowledge their patience and responsiveness to our requests for clarification.

Ling Yuan
Sharyn E. Perry

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