Preface

Plants need to perceive and accordingly respond to an incredible number of signals from their surrounding environment in order to survive. Aside from the sheer number of them, the nature of these signals can be extremely variable; chemical nutrients in the rhizosphere, day length (or more correctly, night), pathogenic microbes, water shortage, heat, cold, herbivores, gravity, etc. In order to recognize such a varied array of signals, they have evolved a large number of receptors with very different structural characteristics. However, perception of a stimulus is only the first step in a long and complicated process culminating in the production of correct responses that can be as diverse as the original stimuli. Coupling each specific stimulus to the appropriate response is essential and can be the difference between life and death. Aside from specificity, controlling the magnitude of the response is also important and needs to be proportionate to the perceived signal. The hypersensitive response is a very efficient way to combat specific pathogens, but it needs to be restricted so the plant only sacrifices the necessary amount of tissue. Finally, while some responses such as flowering in response to photoperiod can be produced over a relatively wide window of time, in some cases speed is of the essence and stomatas need to be quickly closed in a hot mid-day before dehydration results in irreversible damage to the plant. In order to control the specificity, speed, and magnitude of the response, plants have evolved sophisticated signal transduction mechanisms that in most cases are far from linear events. A single stimulus can trigger one or more receptors, and each activated receptor can initiate several signaling cascades. To complicate it all, signaling to different stimuli can have extensive cross-talk as is the case in the response to hormones.

Even though there have been spectacular advances in our knowledge of signal transduction pathways, we are currently seeing just the tip of what is expected to be a very large iceberg. There are hundreds of receptors in plants and the vast majority of them are still "orphan", i.e., their agonists are not known. In addition, even the same signaling elements can use very different molecular mechanisms in plants and animals as is the case of heterotrimeric G-proteins, a group of vital transducers with totally different associated receptors and activation/deactivation loops in humans and plants [1]. Expanding our knowledge of signal transduction pathways is not only important for basic biology but will ultimately allow precise manipulation of important plant responses to produce improved crops with enhanced resistance to diseases and abiotic stresses, less dependence on chemical fertilizers, and increased yield. This almost untapped crop improvement tool has a huge potential in the development of the new "green" agriculture urgently needed to provide the world with food security while protecting the environment [2].

It is impossible to compile all the methods available for the study of signal transduction pathways; therefore, we have opted for a selection of "classic" as well as newly developed approaches. These approaches expand the fields of molecular biology, biochemistry, physiology, cell biology, genetics, and genomics. Production and analysis of mutants is arguably one of the most useful methods to determine the involvement of specific genes in a signaling pathway. Once a gene and therefore its encoded protein have been identified as part of a signaling pathway, the production and screening of a mutagenized population on the

original mutant background can identify additional members of the signaling pathway. In the first chapter of this volume, Li and Zhang describe how to produce a mutagenized population in *Arabidopsis thaliana* using a chemical mutagen and provide useful insights into the basic principles for the design of suppressor screens. An essential component of the response to a specific stimulus is the induction or repression of a set of genes in the nucleus. It is therefore important to accurately quantify gene expression levels in order to measure disruptions in signaling pathways, and Abdallah and Bauer provide a thorough description of one of the preferred quantification methods, quantitative reverse transcription PCR, in Chapter 2. Transcription factors are essential regulatory genes and final targets of multiple signal transduction pathways. Pose and Levi describe how to determine direct targets of transcription factors using ChIP-seq, a technique that is greatly facilitated by next-generation sequencing (NSG) approaches. In Chapter 4, Amaya and coworkers also use NGS combined with bulk-segregant analysis (BSA) to identify genes determining volatile composition in strawberry. This type of approach (BSA linked to NGS) is very powerful and promises to facilitate the identification of key genes involved in multiple biological processes, either as a consequence of natural variability or generated by induced mutagenesis.

Chapters 5–7 introduce tomato as an alternative model system to Arabidopsis. Even though the advantages of Arabidopsis as a model system are widely acknowledged, even Arabidopsis has limits. Tomato has emerged in recent times as a useful model system for the study of a number of developmental processes and the established model to study fleshy fruit development. Tomato is an important commercial crop, and some varieties such as Micro-Tom are becoming very popular in laboratories around the world. In Chapter 5, Shikata and Ezura describe a quick and easy transformation method for Micro-Tom as well as the use of a valuable public resource: the "TOMATOMA" mutant database. In Chapter 6, Rothan et al. reveal the "secrets" of how to grow healthy populations in the glasshouse as well as important information in order to correctly perform reproducible fruit developmental studies. Fruit development is also the focus of Chapter 7 where Fantini and Giuliano describe how to use Virus-Induced Gene Silencing as a high-throughput reverse genetic tool to the study of gene function in tomato fruits. Production and analysis of tomato fruit volatiles is described later in the volume by Kamiyoshihara et al.

Cell biology methods such as the isolation and transfection of protoplast provide a fast and convenient platform for the study of gene function and can be especially useful for the study of signaling events. A method for the production and transfection of Arabidopsis leaf mesophyll protoplasts is described in Chapter 8 by Schapiro and Lois. This method has been especially designed to streamline the process and avoid most of the time-consuming steps present in other protoplast and transfection protocols. In Chapter 9, Zhu and colleagues describe very specialized protocols to produce and perform electrophysiological studies on guard cell protoplasts. Even more specialized is the method described by Wan et al. in Chapter 10 where they describe the use of variable angle total internal reflection fluorescence microscopy to study molecular events in the vicinity of the plasma membrane.

The first step in signal transduction is often carried out by membrane-associated receptors. These proteins are especially difficult to isolate, a necessary pre-requisite for their subsequent study and characterization. Kadota et al. provide an efficient method for the isolation of membrane proteins by immunoprecipitation in Chapter 11. Signaling cascades are mostly dependent on the sequential interaction of a number of proteins, and a number of methods exist to study such interactions. While most methods focus on interactions

between two proteins, in Chapter 12 Maruta et al. describe a method to study interactions involving three proteins. Microscopy methods are also becoming very popular to study protein interactions as they can provide information about not only the strength but also the cellular location in which the interaction takes place. Nevertheless, the rapid adoption of such methods by numerous laboratories lacking the necessary expertise has resulted in the publication of "less than rigorous" results that are not only embarrassing for the authors but can also mislead the rest of the scientific community by providing the wrong conclusions. Tunc-Ozdemir et al. provide a very useful set of guidance rules for nonspecialists who wish to use two of the most common microscopy-based protein interaction techniques: Bimolecular Fluorescence Complementation (BiFC) and Förster Resonance Energy Transfer (FRET) in Chapter 13.

Proteins not only interact with other proteins during signal transduction, and many signaling peptides change their affinities for different lipids in response to diverse stimuli. In Chapter 14, Perez-Sancho et al. describe how to purify functional C2 domains (common in eukaryotic proteins targeted to cell membranes) and assay them for lipid binding partners. Phosphorylation is arguably the most important as well as the most common method to transmit cellular signals, and protein kinase cascades control the response to numerous stimuli in plants. In Chapter 15, Wang and Zhu describe an in-gel protein kinase assay to detect protein phosphorylation activity of specific protein kinases. Another important posttranscriptional modification is glycosylation that can change the physical nature of a protein from soluble to volatile. Kamiyoshihara et al. provide a protocol to identify uridine diphosphate-dependent glycosyltransferases targeting volatile compounds in Chapter 16.

Understanding of the defence mechanisms used by plants against a myriad of diverse pathogens not only is important for the study of signal transduction mechanisms but can also have important practical applications in the development of crops naturally resistant to diseases. Some of the receptors involved in pathogen recognition have been characterized, but many of the steps leading to deployment of the defence response are still unknown. To determine the possible involvement of a specific gene/protein in the defence signaling, it is essential to determine disease progression in a quantitative and reproducible way, and Chapters 17 and 18 by Macho et al. and Trusov et al. describe simple but reliable methods for three very different pathogens: bacterial, fungal, and viral.

Last but not least, plant responses to many different environmental stimuli are mediated and coordinated by hormones. Biotic and abiotic stresses, developmental processes (such as fruit ripening), and cellular processes such as elongation or division are controlled by the simultaneous action of one or more hormones. It is therefore important to determine endogenous hormonal levels to ascertain whether phenotypic alterations are due to either differences in sensitivity to specific hormones or altered hormonal levels. In Chapter 19, Vallarino and Osorio provide a sensitive, reliable, and inexpensive method to quantify the endogenous levels of several phytohormones.

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