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THEMATIC ARTICLES

Recent Advances in Brassinosteroid Research: From Molecular Mechanisms to Practical Applications

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Brassinosteroids (BRs) are now firmly established as essential regulators of plant growth and development affecting a broad spectrum of processes at the molecular, cellular and physiological levels. The pace of BR research has continued to accelerate since the discovery of BR-insensitive and -deficient mutants in the mid 1990's and progress in understanding mechanistic details of both BR signal transduction and biosynthesis has been particularly dramatic. Studies on the effect of BRs on whole plant physiology, including stress adaptation, continue to build on early experiments in numerous crop species while integrating the advances made in BR molecular genetics in *Arabidopsis*. The ability to manipulate endogenous BR levels in mutant plants affected in BR biosynthetic genes and/or in transgenic plants with altered expression levels of these genes, has allowed testing for causal relationships in BR action that were previously only inferred by application of exogenous BR to wild-type plants. The discovery of specific inhibitors of BR biosynthesis that phenocopy BR-deficient mutants has greatly facilitated these types of studies and has extended their range to species where BR-deficient mutants have yet to be discovered. From a practical perspective, the chemical synthesis of BR analogs

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with high biological activity and increased persistence in the field, suggest that the long-held goal of using BRs as growth regulators in agricultural production still has much potential. In this thematic issue of the *Journal of Plant Growth Regulation*, seven reviews by experts in various aspects of BR research are presented, covering the physiology, molecular biology, biochemistry and practical applications of these unique plant steroids.

The physiology of BRs is reviewed by Jenneth Sasse, focusing primarily on studies conducted since 1999. The identification of endogenous BRs in roots is a significant advance and studies on the organ-specific distribution of transcripts of genes encoding BR-biosynthetic enzymes show good correlation with endogenous BR levels, suggesting a possible molecular mechanism for regulating BR levels in specific tissues. Although the promotion of cell elongation by BRs is well known and continues to be studied in detail, a role for these steroids in cell division, vascular differentiation and seed germination is becoming firmly established. Priti Krishna continues the review of BR physiology by examining the remarkable ability of BRs to confer increased adaptation of plants to various stresses, including high and low temperatures, high salinity, drought and bacterial, fungal and viral pathogens. The probable role of cross-talk between BRs and other plant hormones that modulate stress, such as abscisic acid, ethylene and methyl jasmonate is discussed and proposed molecular mechanisms underlying BR-mediated stress adaptation are presented. Interestingly, BR treatment appears to limit the loss of several components of the translational apparatus during prolonged heat stress and recovery. These effects by BR on translation initiation and elongation factors may present possible avenues of direct interaction between BR signal transduction and the global cellular growth process of protein translation.

Next, Peng Peng and Jianming Li describe the rapidly advancing area of BR signal transduction research. Much of the early work in this field focused primarily on the Brassinosteroid Insensitive 1 (BRI1) leucine-rich repeat receptor kinase, resulting in convincing evidence that BRI1 is an essential component of the BR receptor. In the past two years several novel components of BR signaling have been uncovered including a downstream cytoplasmic kinase that is a negative regulator of BR signal transduction, two related positive regulatory proteins of unknown function that can be nuclear localized in response to BR, and a second leucine-rich repeat receptor kinase that interacts with BRI1 at the cell surface. The emerging picture of BR signal transduction is certainly different from the classical model of animal steroid signaling, but it does share certain similarities to the more recently defined ''non-genomic'' steroid pathways in animals which involve perception of steroids by cell surface receptors. Moreover, it has specific mechanistic similarities to several other non-steroid signaling pathways in *Drosophila* and mammals including those employing receptor tyrosine kinases, as well as the Transforming Growth Factor-β and Wingless/Wnt pathways. In fact, our current knowledge of BR signaling suggests a pathway that has marked similarity to the overall signal transduction logic of several eukaryotic pathways, but with only limited components of the pathway showing sequence homology to their animal counterparts. Thus the very appropriate title of Peng and Li's article, ''Brassinosteroid Signal Transduction: A Mix of Conservation and Novelty.''

The terminal end of BR signal transduction consists of the specific genes that are regulated by interaction with nuclear localized BR signaling components. Carsten Müssig and Thomas Altmann take on the task of surveying the numerous candidates for BR-regulated genes that have been identified both by classical methods of studying differential gene expression such as subtractive hybridization, and more recently by global analysis of gene expression using DNA microarrays. Some of these genes are specifically regulated by BRs, whereas others show regulation by additional hormones such as auxin, gibberellins, ethylene and jasmonic acid; and by environmental stimuli including light, temperature and mechanical perturbation. Several genes are consistently reported as BR-regulated while some are specific to particular growth conditions, plant genotypes and BR application methods. Müssig and Altmann raise a cautionary note about how one defines a gene as truly BR-regulated. Application of exogenous BR to different tissues is quite common but may not reflect endogenous regulation because of problems with uptake and distribution of the applied compound. Moreover, wild-type plants may already have optimal levels of endogenous BRs and might not respond to exogenous treatment. One can use BR-deficient mutants as the treated plants, but the severe phenotype of these plants might also yield secondary effects that are not directly related to BR regulation.

The availability of multiple signal transduction and biosynthetic mutants in *Arabidopsis* has been the foundation of much recent BR research. However, many of the corresponding mutants have also been identified in several crop plants. Gerard Bishop discusses the conserved and unique features of BR-insensitive and -deficient mutants in pea, tomato and rice. For example, the BR-insensitive tomato mutant *curl3* is strikingly similar to *Arabidopsis bri1* in appearance and root response to various hormones. Surprisingly, tomato BRI1 is identical to SR160, which has been shown by biochemical methods to bind the tomato peptide hormone systemin, involved in defense responses, suggesting that tomato BRI1 is a bifunctional receptor able to perceive both peptide and steroid ligands. The availability of several rice mutants defective in BR signaling or biosynthesis now allows the phenotypic comparison of monocot and dicot BR mutants. Moreover, the functional significance of specific BRI1 domains can also be assessed by comparing sequence conservation in BRI1 proteins from four different species. The kinase domain shows the highest sequence conservation followed by the second set of paired cysteines and the membrane spanning domain. The island domain and the juxtamembrane region both show similar levels of identity between *Arabidopsis* and tomato (75%) and between *Arabidopsis* and rice (46%). Interestingly, the LRRs flanking the island domain are more highly conserved than those closer to the N-terminus.

Next, Tadao Asami and colleagues describe the chemistry and biological applications of BR biosynthesis inhibitors. Brassinazole, a potent inhibitor of BR biosynthesis that binds to the DWARF4 steroid hydroxylase, has been extremely useful in phenocopying a BR-deficient mutant phenotype in wild-type plants. This allows induction of BR deficiency at various stages of development, a scenario not achievable with true BR-deficient mutants. Such inhibitors also allow creation of pseudo-BR-deficient mutants in species where genetic mutants have not been identified. Asami and others describe several applications of this chemical genetic approach, including studies on photomorphogenesis, vascular differentiation and disease resistance. Molecular genetic applications in *Arabidopsis*, including screens for brassinazole resistance that have revealed novel signaling components, and global gene expression analyses in plants treated with or without the inhibitor, are also discussed.

Finally, Thomas Back and Richard Pharis describe the considerable progress that has been achieved in the structure-activity analysis of BRs using chemically modified BR analogs coupled with the rice leaf lamina inclination bioassay. Brassinolide is the most active naturally occurring BR, but modification of C-24 in the side chain with cyclopropyl or cyclobutyl groups results in ''superbrassinolide'' analogs that are more active than brassinolide in the rice lamina bioassay. One problem in the practical application of BRs to crops in the field is the metabolism of BRs to less active compounds. Back and Pharis discuss a variety of BR analogs in which ring and side chain hydroxyl groups have been derivatized, that should have greater persistence in the field. A second problem in agricultural application of BRs is the expense of their chemical synthesis. Non-steroidal mimetics of brassinolide that are more efficient to synthesize but which retain BR activity when co-applied with naphthaleneacetic acid have now been developed and these compounds have shown promise in actual field trials. Besides their obvious practical implications, the availability of a wide range of BR analogs with varying biological activity should be useful in understanding which functional groups are essential in the binding of brassinolide to its receptor.

I would like to thank all of the authors for their efforts in thoroughly reviewing the seven topics covered in this special thematic issue. Although a number of BR reviews have appeared recently, we hope the scope and depth of coverage in these seven articles will provide a useful resource for plant hormone biologists and graduate students in plant physiology and biochemistry.