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**Luc Genetic Screen Illuminates Stress-Responsive Gene Regulation**

A typical genetic screen for identifying loci associated with plant responses to stress involves searching for mutants that are hypersensitive and/or hyperresistant to a particular stress, on the basis of visual appearance of seedlings compared with the wild-type response. This type of screen has successfully identified numerous genetic loci that are important components of plant responses to biotic and abiotic stresses. However, this approach has limitations. For example, acute stress conditions often are required for a visible phenotype to be manifested, and such conditions may continually fail to generate mutants in important components of stress response pathways. In other cases, visible phenotypes may be completely absent. Because of the complexity of stress response signaling networks, new and innovative types of screens may be necessary to identify all of the components.

**REPORTER GENE SCREENS**

Reporter gene screens typically make use of a reporter gene, such as firefly luciferase (LUC) or β-glucuronidase (GUS), fused to the promoter of a specifically regulated or inducible gene. This type of genetic screen (in use since the late 1980s and early 1990s) represents an important advance in genetic technologies; not only does it create a highly visible phenotypic screen, it also specifically targets genes that encode regulatory and/or signal transduction components that may play key roles in the regulation of plant responses to stress. The Arabidopsis npr1 (nonexpressor of pathogenesis-related genes) mutant provides an example of the importance of this type of screen in plant genetics. To isolate the npr1 mutant, Cao et al. (1994) made use of transgenic Arabidopsis expressing GUS under the control of the Arabidopsis β-1,3-glucanase (BGL2) promoter. BGL2 is a pathogenesis-related gene that is regulated by salicylic acid and is a marker gene for the induction of systemic acquired resistance; thus, plants expressing BGL2-GUS provided a visible phenotype for mutants impaired in the induction of systemic acquired resistance after pathogen attack. Although npr1 alleles also were identified via more conventional genetic screens, the npr1 mutation yielded important information regarding the key role of NPR1 in salicylic acid signaling and the induction of systemic acquired resistance (Cao et al., 1997; Kinkema et al., 2000).

Osmotic stress is one area that has yielded a relative paucity of mutations in traditional genetic screens. Ishitani et al. (1997) described a genetic screen to identify components of osmotic and low temperature plant stress response pathways that makes use of transgenic Arabidopsis expressing the LUC coding sequence under the control of the stress-responsive RD29A promoter. The transformed RD29A-LUC seedlings (referred to as “wild type”) produce strongly bioluminescent leaves under various stress conditions. Seed of these plants were treated with the mutagen ethyl methanesulfonate, and mutants were identified by an alteration in the pattern of LUC expression relative to the wild type under various abiotic stress conditions. A large number of mutants were identified in three major groupings, termed los (low expression of osmotically responsive genes), cos (constitutive expression of osmotically responsive genes), and hos (high expression of osmotically responsive genes; Ishitani et al., 1997).

**los5 IS ABSCISIC ACID DEFICIENT AND ALLELIC TO aba3**

In this issue of *The Plant Cell*, Xiong et al. (pages 2063–2083) characterize two allelic Arabidopsis RD29A-LUC mutants, los5-1 and los5-2, that exhibit greatly reduced RD29A-LUC gene expression in response to dehydration, high salt, or low temperature. Gene expression analysis showed that stress induction of the endogenous RD29A gene and several other known stress response genes (COR15A, KIN1, P5CS, RD22, and COR47) was inhibited completely or partially by the los5 mutation, and importantly, the mutant plants also were found to have reduced tolerance to drought, salt stress, and freezing. The los5 mutants were found to be deficient in abscisic acid (ABA), and subsequently the mutation was shown to be allelic to aba3. The LOS5/ABA3 gene was cloned via map-based cloning and was found to encode an enzyme that functions indirectly in ABA biosynthesis. This enzyme, molybdopterin cofactor sulfurylase, catalyzes the production of sulfurylated molybdopterin cofactor required by aldehyde oxidase (which functions in the last step of ABA biosynthesis). Schwartz et al. (1997) showed previously that the aba3 mutant was blocked at the last step of ABA biosynthesis (conversion of ABA aldehyde to ABA by aldehyde oxidase), and the lesion appeared to be in the sulfurylation of the molybdenum cofactor, because treatment of extracts with Na2S restored ABA aldehyde oxidase activity.

An important feature of the RD29A-LUC screen in this case is that it led to
the identification of hitherto unrecognized characteristics of a previously identified locus. aba3 was identified by Léon-Kloosterziel et al. (1996) and characterized further by Schwartz et al. (1997), but these studies did not examine the possible involvement of this locus in plant stress responses. Llorente et al. (2000) identified another aba3 allele, called fts1, in a screen to identify freezing-sensitive mutants of Arabidopsis, which showed that the aba3 locus has an important function in freezing tolerance in Arabidopsis. However, the nature of the los5 mutation characterized by Xiong et al. facilitated the map-based cloning of the LOS5/ABA3 gene and allowed for further insights into the role of ABA in the regulation of stress-responsive gene expression.

ABA AND STRESS RESPONSE REGULATION

ABA is involved in the regulation of many aspects of plant growth and development, including seed dormancy, embryogenesis, root and shoot development, and stomatal function. ABA also is an important regulatory component of plant responses to abiotic stresses such as drought, salinity, and, possibly, low temperature.

ABA biosynthesis in plants branches off of the xanthophyll cycle. One of the key steps is cleavage of 9-cis-neoxanthin to yield xanthoxin, which is catalyzed in maize by the VP14 gene product. VP14 homologs in various species are induced by drought and other abiotic stresses, and this gene is thought to represent a key regulatory step in ABA biosynthesis (Taylor et al., 2000). Xiong et al. found that the LOS5/ABA3 gene was expressed at relatively low levels in all plant parts examined and, interestingly, was induced in response to dehydration, ABA treatment, and osmotic stress. The LOS5/ABA3 promoter region was found to contain putative ABA response elements (ABRE) and dehydration response elements (DRE). It also has been shown that ABA1 (Marin et al., 1996) and AAO3 (Seo et al., 2000), which encode enzymes that catalyze two other steps in ABA biosynthesis immediately prior to the LOS5/ABA3 reaction, are induced in response to drought stress. These observations support the conclusion that ABA biosynthesis is controlled at multiple steps in response to various stresses.

ABA is thought to have a significant role in plant responses to drought and osmotic stress. In addition to its direct effect on stomatal closure, ABA also has a role in the regulation of expression of drought and desiccation tolerance genes in seed and vegetative tissues. ABA-deficient and ABA-insensitive mutants also are marked by a characteristic dehydration-sensitive “wilty” phenotype. ABA also may play a role in cold acclimation and the development of freezing tolerance, although the importance of this role is somewhat controversial. Thomashow (1999) stated that the available evidence suggested a minor role for ABA in cold acclimation and discussed the possibility that plants deficient in ABA are unable to cold acclimate simply because they are unhealthy.

Several genes that are induced by drought, salinity, or cold temperature are not induced in response to exogenous ABA treatment, suggesting that there are ABA-independent as well as ABA-dependent stress response pathways. In addition, some genes that are ABA responsive, such as RD29A, KIN1, and COR47, also are induced by certain stress conditions in ABA-deficient or ABA-insensitive mutants. Yamaguchi-Shinozaki and Shinozaki (1994) showed that the RD29A promoter contains both ABRE and DRE motifs, and the DRE, which is not ABA responsive, was found to be essential for RD29A induction under drought, salt, and low temperature conditions. The presence of functional ABRE and DRE elements in numerous stress-responsive genes suggests that both ABA-dependent and ABA-independent pathways are important in regulating the stress responses of these genes.

DOES ABA INFLUENCE ABA-INDEPENDENT PATHWAYS?

There is growing evidence that the ABA-dependent and ABA-independent pathways are interconnected in a complex and subtle manner. Ishitani et al. (1997) presented evidence for such interconnection when they identified los and hos mutants that showed reduced or enhanced RD29A-LUC expression, respectively, in response to ABA treatment and osmotic stress as well as low temperature stress conditions. The results of Xiong et al. provide further evidence for the idea that there is communication between ABA-dependent and ABA-independent stress-responsive signal transduction pathways. For example, it is perhaps surprising that a mutation in the ABA biosynthetic pathway would completely inhibit the osmotic stress induction of RD/COR/KIN genes, because previous research has shown that osmotic stress induction of these genes is controlled by both ABA-dependent and ABA-independent pathways. In an ABA-deficient mutant such as los5/aba3, we might expect to see some degree of osmotic stress induction of these genes because the ABA-independent pathway should not be affected. This is the case for other ABA-deficient and ABA-insensitive mutants, such as various aba1 and abi1 alleles that have been examined.

Xiong et al. (1999a) showed that salt and ABA have a synergistic effect on wild-type RD29A-LUC expression in a manner that is temperature dependent. Bostock and Quatrano (1992) found that salt and ABA interacted synergistically to regulate the expression of the Em gene in rice. They hypothesized
CONVERGENCE OF OSMOTIC STRESS AND LOW TEMPERATURE SIGNALING AT THE FIERY LOCUS

One of the RD29A-LUC mutants isolated in the screen devised by Ishitani et al. (1997) showed superinduction of RD29A-LUC expression and was named fiery1 (fry1) because of the high level of LUC activity induced in response to cold stress, low temperature, osmotic stress, exogenous ABA, and NaCl (Xiong et al., 2001b). In contrast, the hos1 and hos2 mutations affected only the cold stress response (Ishitani et al., 1998; Lee et al., 1999, 2001), and the hos5 mutation affected only the osmotic stress response (Xiong et al., 1999b). Interestingly, although the fry1 mutants showed superinduction of ABA- and stress-inducible gene expression, they were compromised in their tolerance to freezing, drought, and salt stress. FRY1 was found to encode an inositol polyphosphate 1-phosphatase, suggesting a role for phosphoinositols in ABA and stress signaling (Xiong et al., 2001b). Because the fry1 mutation affects ABA, osmotic stress, and cold stress-responsive signaling, it raises the interesting possibility that phosphoinositol signaling provides the link between ABA-dependent and ABA-independent pathways.

Plant stress responses involve a remarkable array of signal transduction pathways that must be integrated successfully to allow for the normal growth and development of an organism that cannot move to escape predators or adverse environmental conditions. Interactive genetic screens, like the RD29A-LUC reporter screen, are helping to unravel the complex interactions that make up plant stress signaling networks.

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