Final report: DE-FG02-97ER20264, Genetics of ABA biosynthesis

Project goals

The carotenoid cleavage dioxygenases (CCD) catalyze synthesis of a variety of apo-carotenoid secondary metabolites in plants, animals and bacteria. In plants, the reaction catalyzed by the 11, 12, 9-cis-epoxy carotenoid dioxygenase (NCED) is the first committed and key regulated step in synthesis of the plant hormone, abscisic acid (ABA). ABA is a key regulator of plant stress responses and has critical functions in normal root and seed development. The overall goal of this project was to understand the molecular mechanisms responsible for developmental control of ABA synthesis in plant tissues. Five of the nine CCD genes present in the Arabidopsis genome encode NCED's involved in control of ABA synthesis in the plant. We focused on functional analysis of these five AtNCED genes as a key to understanding developmental regulation of ABA synthesis and dissecting the role of ABA in plant development. For this purpose, we developed a comprehensive set of gene knockouts in the AtNCED genes that facilitate genetic dissection of ABA synthesis. These mutants were used in combination with key molecular tools to address the following specific objectives: 1) the role of ABA synthesis in root development; 2) developmental control of ABA synthesis in seeds; 3) analysis of ATNCED over-expressers, 4) perform preliminary X-ray crystallography of the maize VP14 enyzme.

Analysis of knockouts of the AtNCED gene family in Arabidopsis

A complete set of gene knockouts were identified for the carotenoid dioxygenase gene family in Arabidopsis facilitating genetic dissection of ABA synthesis during development. By screening the UW population and mining the sequence tagged Salk collection complete coverage of all nine genes was achieve with well positioned insertions and multiple alleles in several cases. Subsequent studies focused on the five *AtNCED* genes involved in ABA synthesis. A complete series of *AtNCED* alleles have been isolated in the WS background (Table 1).

Table 1. AtNCED knockout summary (WS alleles)

	Phenotype	**ABA deficient[1]**
AtNCED2-1,-2	none	no
AtNCED3-1	wilty, lateral root growth[2]	yes
AtNCED5-1	small seedling, lateral root growth[2]	no
AtNCED6-1	probable ap3 co-suppression	n.d.
AtNCED9-2	none	n.d.

[1]In stressed and non-stressed seedlings, Jan Zeevaart, unpublished.
[2] Nitrate induced lateral root growth responses (Figure 4).

As expected based on work from the Shinozaki lab, the AtNCED3 has a wilty phenotype and is highly susceptible to water stress. The AtNCED5 knockout is linked to a small seedling phenotype that is discernible in the first two weeks of growth. We are evaluating additional backcross lines and a second AtNCED5 allele to confirm that this phenotype is caused by the AtNCED5 gene.

NCED function in root development

A second key objective was to understand ABA synthesis and functions in roots. Low concentrations of nitrate stimulate lateral root elongation in Arabidopsis, whereas, high levels of nitrate in the media inhibits lateral root initiation and lateral root elongation. We showed that the *AtNCED3* and *AtNCED5*-1 knockouts block separate aspects of nitrate regulated root growth. The *AtNCED3* knockout mutant partially blocks nitrate inhibition of lateral root initiation and

elongation at high nitrate. In contrast, the *AtNCED5* mutant inhibits lateral root elongation at low nitrate. The *AtNCED9* knockout shows a similar trend with a small effect on lateral growth at 1 mM NO_3.

Differential expression of AtNCED genes

In order to quantify expression of *AtNCED* gene expression in dissected plant tissues, we developed and tested a set of sensitive TacMan RT-PCR probes for each of the five *AtNCED* genes. In pair-wise tests, the TacMan probes provided a minimum of 10^4-fold discrimination between all members of the *AtNCED* gene family. Our results confirm evidence that *AtNCED3* is the major stress induced form expressed in leaves, whereas, the *AtNCED2* transcript is stress-induced in young intact seedlings. In contrast, *AtNCED5, AtNCED6* and *AtNCED9* show weak or no induction under water-stress. *AtNCED6* and *AtNCED9* are the predominant genes expressed in developing siliques indicating that these genes are likely to control developmentally regulated synthesis of ABA in developing seeds. To further characterize the developmental regulation of these genes we constructed a complete series of promoter-GUS constructs containing the upstream 1.5 kp of DNA from each AtNCED.

Over-expression of ATNCED2

In order to address the role of ABA in plant development, we over-expressed ATNCED2 in Arabidopsis plants using the 35S promoter. Independent ATNCED2 over-expressing lines (S6, S8, S9) have similar complex developmental phenotypes that vary in severity. The phenotype includes inhibition of lateral root development, delayed flowering, delayed true leaf development in seedlings, partial male sterility and abnormal anthocyanin accumulation. The severity of these phenotypes correlates well with levels of transgene expression but does not correlate with measurable differences in basal or stress-induced ABA content.

We found a striking correspondence between the manifestation of the 35S-ATNCED2 phenotype in developing organs and the localized pattern of AtNCED2 expression in lateral root initials, leaf and flower organ primordial. We have not ruled out the possibility that the over-expresser phenotype is due to co-suppression of endogenous AtNCED2 in these organs. However, two independent AtNCED2 knockout alleles have no discernable phenotype indicating that if co-suppression is the cause, it is not limited to loss of function of the AtNCED2 gene. AtNCED3 is evidently not co-suppressed in leaves because stress-induced ABA synthesis is normal in 35S-AtNCED2 plants.

X-ray crystallography and structure of VP14

Because the carotenoid cleavage dioxygenases comprise a new family of enzymes, knowing the structure of these proteins would be extremely valuable in understanding their functions. We finally achieved this goal in 2010 through a long and ultimately fruitful collaboration with Simon Messing, a graduate student in Mario Amstel's lab at Johns Hopkin. The effort was initiated with support from this DOE project and continued with support from NSF. The resulting structure provides a basis for functional analyses of plant carotenoid cleavage dioxygenases.

Publications:

1: Messing SA, Gabelli SB, Echeverria I, Vogel JT, Guan JC, Tan BC, Klee HJ,
McCarty DR, Amzel LM. Structural insights into maize viviparous14, a key enzyme in the biosynthesis of the phytohormone abscisic acid. Plant Cell. 2010 22:2970-80.

2: Vogel JT, Tan BC, McCarty DR, Klee HJ. The carotenoid cleavage dioxygenase 1 enzyme has broad substrate specificity, cleaving multiple carotenoids at two different bond positions. J Biol Chem. 2008 283:11364-73.

3: Suzuki M, Latshaw S, Sato Y, Settles AM, Koch KE, Hannah LC, Kojima M, Sakakibara H, McCarty DR. The Maize Viviparous8 locus, encoding a putative ALTERED MERISTEM PROGRAM1-like peptidase, regulates abscisic acid accumulation and coordinates embryo and endosperm development. Plant Physiol. 2008 146:1193-206.

4: Cao X, Costa LM, Biderre-Petit C, Kbhaya B, Dey N, Perez P, McCarty DR, Gutierrez-Marcos JF, Becraft PW. Abscisic acid and stress signals induce Viviparous1 expression in seed and vegetative tissues of maize. Plant Physiol. 2007 143:720-31.

5: Auldridge ME, McCarty DR, Klee HJ. Plant carotenoid cleavage oxygenases and their apocarotenoid products. Curr Opin Plant Biol. 2006 9:315-21.

6: Auldridge ME, Block A, Vogel JT, Dabney-Smith C, Mila I, Bouzayen M, Magallanes-Lundback M, DellaPenna D, McCarty DR, Klee HJ. Characterization of three members of the Arabidopsis carotenoid cleavage dioxygenase family demonstrates the divergent roles of this multifunctional enzyme family. Plant J. 2006 45:982-93.

7: Suzuki M, Settles AM, Tseung CW, Li QB, Latshaw S, Wu S, Porch TG, Schmelz EA, James MG, McCarty DR. The maize viviparous15 locus encodes the molybdopterin synthase small subunit. Plant J. 2006 45:264-74.

8: Suzuki M, Ketterling MG, McCarty DR. Quantitative statistical analysis of cis-regulatory sequences in ABA/VP1- and CBF/DREB1-regulated genes of Arabidopsis. Plant Physiol. 2005 139:437-47.

9: Suzuki M, Ketterling MG, Li QB, McCarty DR. Viviparous1 alters global gene expression patterns through regulation of abscisic acid signaling. Plant Physiol. 2003 132:1664-77.

10: Tan BC, Joseph LM, Deng WT, Liu L, Li QB, Cline K, McCarty DR. Molecular characterization of the Arabidopsis 9-cis epoxycarotenoid dioxygenase gene family. Plant J. 2003 35:44-56.

11: Schwartz SH, Tan BC, McCarty DR, Welch W, Zeevaart JA. Substrate specificity and kinetics for VP14, a carotenoid cleavage dioxygenase in the ABA biosynthetic pathway. Biochim Biophys Acta. 2003 1619:9-14.

12: Nambara E, Suzuki M, Abrams S, McCarty DR, Kamiya Y, McCourt P. A screen for genes that function in abscisic acid signaling in Arabidopsis thaliana. Genetics. 2002 161:1247-55.

13: Suzuki M, Kao CY, Cocciolone S, McCarty DR. Maize VP1 complements Arabidopsis abi3 and confers a novel ABA/auxin interaction in roots. Plant J. 2001 28:409-18.

14: Tan BC, Cline K, McCarty DR. Localization and targeting of the VP14 epoxy-carotenoid dioxygenase to chloroplast membranes. Plant J. 2001 27:373-82.

15: Burbidge A, Grieve TM, Jackson A, Thompson A, McCarty DR, Taylor IB. Characterization of the ABA-deficient tomato mutant notabilis and its relationship with maize Vp14. Plant J. 1999 17:427-31.

16: Tan BC, Schwartz SH, Zeevaart JA, McCarty DR. Genetic control of abscisic acid biosynthesis in maize. Proc Natl Acad Sci U S A. 1997 94:12235-40.

17: Schwartz SH, Tan BC, Gage DA, Zeevaart JA, McCarty DR. Specific oxidative cleavage of carotenoids by VP14 of maize. Science. 1997 276:1872-4.