Plant Mol Biol Rep (2010) 28:162–168
DOI 10.1007/s11105-009-0131-2

Interactions between a NAC-Domain Transcription Factor and the Putative Small Protein Encoding *DVL/ROT* Gene Family

Clayton T. Larue · Jiangqi Wen · John C. Walker

Published online: 28 August 2009

Abstract Plant development is a complex process that is regulated by the action of many gene products and small signaling molecules such as plant hormones. Recently, it has become clear that small protein signaling and regulatory molecules are important for many aspects of plant development. The *DVL/ROT* gene family encodes predicted small proteins that have been shown to play a role in *Arabidopsis* development. However, little is known about the genetic pathways in which these family members are active. Here, we show that many transcription factors are responsive to overexpression of a *DVL/ROT* family member. One of these transcription factors was *NAC1*. *NAC1* was shown to genetically interact with a *DVL/ROT* family member, including through an unexpected epigenetic interaction.

Keywords NAC transcription factors · *DVL/ROT* · Plant peptides · Plant development · *Arabidopsis*

Electronic supplementary material The online version of this article (doi:10.1007/s11105-009-0131-2) contains supplementary material, which is available to authorized users.

C. T. Larue · J. Wen · J. C. Walker (✉)
Division of Biological Sciences,
Bond Life Sciences Center and Interdisciplinary Plant Group,
University of Missouri-Columbia,
Columbia, MO 65211, USA
e-mail: walkerj@missouri.edu

C. T. Larue
Department of Plant Biology, University of Illinois,
Urbana, IL 61801, USA

Present Address:
C. T. Larue
Photosynthesis Research Unit, Agriculture Research Service,
United States Department of Agriculture,
Urbana, IL 61801, USA
e-mail: clarue@life.uiuc.edu

Present Address:
J. Wen
Plant Biology Division, The Samuel Roberts Noble Foundation,
Ardmore, OK 73401, USA
e-mail: jwen@noble.org

Introduction

Plant hormones are known to be important in many aspects of plant growth and development. Recent evidence has begun to suggest that a much bigger suite of signaling molecules exist in plants than previously understood (Lindsey et al. 2002). This includes a diverse group of putative small proteins that may function as signaling and regulatory molecules important in various aspects of plant development and responses to external stimuli.

The first well-characterized plant peptide hormone, systemin, was discovered as an 18 amino acid (AA) peptide in wounded tomato leaves responsible for transmitting a systemic signal in response to insect herbivory (Pearce et al. 1991). Systemin is encoded as a single peptide at the C-terminal end of a larger precursor protein (about 200AA; McGurl et al. 1992). Recent work has identified a likely candidate for a systemin receptor in tomato, a receptor with an additional role in brassinolide signaling (Scheer and Ryan 1999, 2002; Montoya et al. 2002). Since the initial characterization of systemin, multiple small proteins that appear to act as hormones have been described with roles in plant development.

For example, in plant development, the *CLAVATA* pathway consists of CLV1, a LRR-RLK (Clark et al.

1997), CLV2, a LRR-containing protein with a transmembrane domain but lacking an intracellular kinase domain (Jeong et al. 1999), and CLV3, a 12-AA-secreted peptide (Fletcher et al. 1999; Ito et al. 2006; Kondo et al. 2006; Sawa et al. 2006). The *CLV* genes appear to act in a self-regulatory loop with the homeobox gene, *WUSCHEL* (*WUS*), to maintain a functional shoot apical meristem (Schoof et al. 2000; Fletcher 2002). The importance of peptides in plant development has become clear as additional peptides and peptide roles have been uncovered. Currently, over 10 families of peptides have been found to play a role in plant development (Farrokhi et al. 2008). However, our understanding of many of these roles is still limited and much additional research is required to determine the functions of these proteins in plant biology.

The *DEVIL* (*DVL*) gene family, encoding a group of putative small proteins in Arabidopsis, has been shown to play a role in plant development. The founding member of this family, *devil1-1 dominant* (*dvl1-1D*), is a dominant, activation-tagged mutant that shows pleiotropic developmental phenotypes (Wen et al. 2004). *dvl1-1D* plants have shorter but wider rosette leaves resulting in a rounded leaf appearance and are shorter in stature compared to WT plants. The flowering inflorescence is more tightly clustered than WT, and the individual flowers have shortened sepals and petals, which results in a protrusion of the pistil prior to flower opening. The flowers are fully fertile, and seed set does not appear to be significantly altered. The valves of the silique show an interesting horn-like protrusion on the distal end of the silique that was the namesake of this mutant.

Rotundifolia4-1D (*rot4-1D*) is another member of the *DVL* family isolated in an activation-tagging screen for modified leaf morphology in *Arabidopsis* (Narita et al. 2004). *rot4-1D* has a similar phenotype as *dvl1-1D* of smaller, rounded leaves. The size of the leaf cells does not appear to be altered. However, the number of cells is reduced, especially in the longitudinal axis of the leaf, resulting in the shorter leaf phenotype. Cloning of the tagged genes uncovered small ORFs encoding predicted small proteins of 51AA (*dvl1-1D*) and 53AA (*rot4-1D*). These genes belong to a larger gene family (in *Arabidopsis*, >20 members) with members found thus far only in the seed plants (Narita et al. 2004; Wen et al. 2004). A domain at the C-terminal end is conserved in members of this gene family, including two highly conserved cysteine residues. In *Arabidopsis*, the *DVL* family members are likely to be partly redundant in function. *dvl1-1D* and *rot4-1D* have similar phenotypes. Ectopic overexpression of other family members also results in similar phenotypes (Wen et al. 2004; Wen and Walker 2006).

Overexpression of *DVL* (*dvlOE*) was shown to result in a downregulation of the MADS-box gene, FUL/AGL8 (Wen et al. 2004). However, our understanding of the genetic interactions in which *DVL* plays a role is still incomplete. To gain a better understanding of these interactions, we developed a large-scale genomic screen using a *dvl* transgene that could be experimentally controlled. In this screen, we uncovered many responses at the mRNA level of genes that play roles in transcription processes. One of these genes, *NAC1*, a NAC-domain transcription factor (Olsen et al. 2005), was further investigated. A loss-of-function (LOF) of *nac1* reverses a *dvl* overexpression phenotype to a WT-like phenotype. Furthermore, this *nac1* LOF results in an epigenetic change that is stable in following generations.

Materials and Methods

To identify genes that are transcriptionally responsive to *DVL* overexpression, microarray analysis to profile different transgenic Arabidopsis lines in a Columbia (Col WT) background was used. These transgenic lines used a *GVG* construct (glucocorticoid inducible) that allows for control of the transgene expression by applications of a steroid hormone, dexamethazone (dex) to the transgenic plants (Aoyama and Chua 1997). A control line was transformed with the *GVG* construct but without a coding sequence inserted in the multiple-cloning site. This line was indistinguishable from Col WT with and without dex treatment or mock treatment. A second line was also transformed with the *GVG* construct containing the *DVL4* coding sequence. Without dex treatment or mock treatment, this line was indistinguishable from Col WT but displayed a typical dvlOE phenotype with dex treatment. The *GVG* construct was generated as described in (Aoyama and Chua 1997) and transformed into Arabidopsis using Agrobacterium-mediated transformation (Clough and Bent 1998).

Plants were grown in artificial greenhouse potting media in standard growth chambers at 22°C and 16-h days. Plants were treated when they reached the six expanded rosette leaf stage (vegetative) with mock treatment or 30-μm dex in water. Three different treatments were completed: Empty vector control with dex treatment; *GVG DVL4* with mock treatment; and *GVG DVL4* with dex treatment. Three biological replicates were collected for each treatment.

At 9–10 h post-treatment, the largest rosette leaf was collected from 20 plants. These leaves were pooled and were considered one biological replicate. A total of three biological replicates (each pool of leaves from 20 plants) were collected for each treatment; thus, three microarray chips were hybridized per treatment. Total RNA was isolated from the leaf samples using TRIzol reagent (Invitrogen) and purified using the RNeasy Midi Kit

(Qiagen) according to manufacturer's instructions. cDNA synthesis and labeling with biotin was completed according to chip manufacturer's instructions (Affymetrix). Hybridization to Affymetrix Arabidopsis ATH1 Genome GeneChips was completed according to chip manufacturer's instructions (Affymetrix) using an Affymetrix GeneChip Hybridization Oven 640 and GeneChip Fluidics Station 450 at the DNA Core, University of Missouri, Columbia. The microarrays were then scanned and data acquired using an Affymetrix GeneChip Scanner 3000 driven by GCOS 1.2 software (Affymetrix) at the DNA core. Computational analysis was completed using dChip software (www.dchip.org). The empty vector control with dex treatment was used to correct for any transcriptional responses due to dex treatment alone. Global analysis of the datasets was completed using MapMan software, version 5.0 (Thimm et al. 2004).

Quantitative Reverse Transcriptase PCR (QPCR) was completed on total RNA extracts from flower bud tissue. Extracts were made using TRIzol reagent (Invitrogen Life Technologies) per manufacturer's instructions. Contaminating DNA was removed by Turbo DNA-free DNase treatment (Ambion) and cDNA was made using 1 μg of total RNA and ImProm-2 Reverse Transcriptase (Promega) following the manufacturer's instructions. QPCR was completed using an Optican 2 Real-Time PCR machine (MJ Research) and ABsolute QPCR SYBR Green Premix (ABgene) per manufacturer's instructions. All sample c(t) values were log transformed and normalized using *EF1α* control reactions by first calculating the change relative to a control sample and then normalizing with the *EF1α* reactions. Primers are listed in Table S2.

T-DNA insertion LOF *nac1* lines were obtained from the Arabidopsis Biological Resource Center (ABRC; Columbus, OH). Homozygous lines were isolated or confirmed by PCR screening. Primers are listed in Table S2. Direct sequencing identified the insertion sites.

Results and Discussion

Inducible DVL Expression System

To aid in screening for genetic interactions with the *DVL* genes, an inducible system in *Arabidopsis* using the *GVG* vector (Aoyama and Chua 1997) was created. For this inducible system, *DVL4* was cloned and placed downstream of the GVG binding site, allowing for transcriptional activation of *DVL4* with the application of dex to the plant. Multiple homozygous transgenic lines were screened for the absence of any *DVL* phenotype without dex application and a *DVL* overexpression phenotype with the application of dex (Fig. 1). One line with consistent *DVL* overexpression phenotypic responses following dex application was selected for further analysis. Control lines, which only contained an empty vector, were also generated. To confirm that no leaky expression of *DVL4* from the transgene was present before dex application, steady-state *DVL4* transcript levels were tracked using QPCR. Steady-state transcript levels of *DVL4* were identical to WT before the application of dex and strongly induced following foliar dex (30 μM) application (Fig. 2).

Gene Discovery for DVL Interactors

This inducible-*DVL4* system was used in a microarray-based experiment. The inducible-*DVL4* system was used because this should allow the early transcriptional responses to be uncovered without the added complexity of secondary responses that might be associated with the *dvl1-1D* plants. To achieve this, samples were collected shortly after induction of *DVL4* in an attempt to enrich for the rapid changes in gene expression resulting from overexpression of *DVL*.

Control plants (empty vector-transformed plants) and inducible *DVL4* plants were grown in a growth chamber to the six-rosette leaf stage. At this point, all the plants are treated with 30 μM dex or mock treated. Leaf samples were collected at 9–10 h post-treatment. Three replicates of each treatment were prepared, labeled, and hybridized to Affymetrix ATH1 Genome GeneChips. Three different treatments were completed (Table 1). The data were then analyzed using dChip software (www.dchip.org; Li and Wong 2001a, b) that generates a statistical trend for each of the probe sets. Based on the analysis, a common trend was observed in which dex alone induces the expression of many genes. Therefore, an analysis procedure was set up to find all genes that were responsive to dex-induced *DVL4* expression relative to mock treatment and also responded with a significant difference in expression from that of the dex-treated control plants. The full dataset has been deposited at the National Center for Biotechnology Information Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) database under record GSE8975. A summary of the analysis is presented in (Table 2) and a list of the most responsive genes is presented in (Tables S1 and S2).

In order to gain a clearer picture of the responses found in the dex-treated inducible *DVL4* plants, the microarray datasets were further analyzed using MapMan software (Thimm et al. 2004). MapMan software graphically organizes the datasets based on functional classifications. In this analysis, it was found that genes involved in transcription regulation were overrepresented in these datasets. Indeed, transcription factors made up one of the largest subgroups of the genes involved in regulatory processes that were responsive to *DVL4* overexpression (Fig. S3).

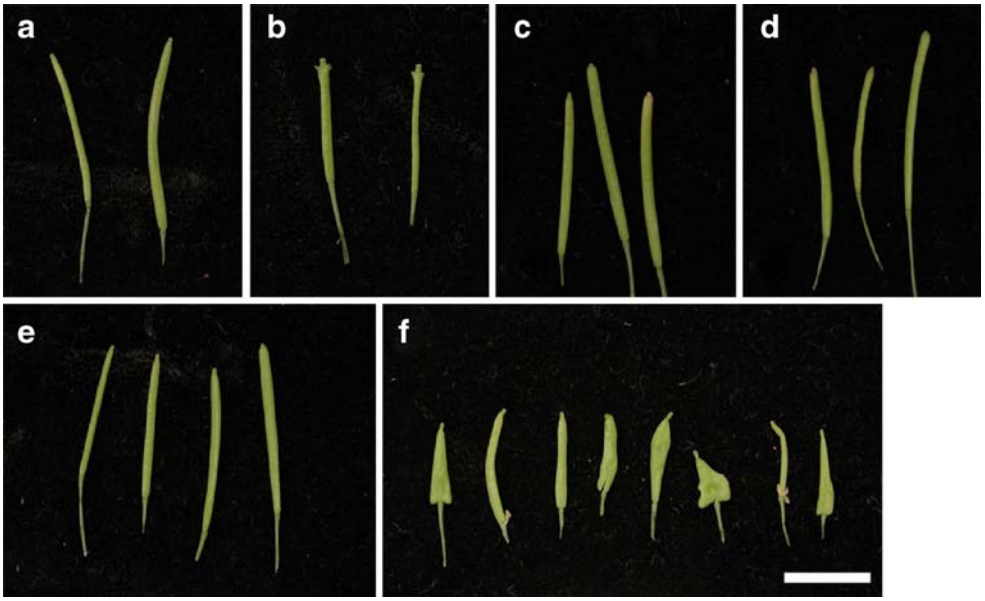

Fig. 1 Inducible *GVG DVL4* transgenic plants. Silique phenotypic responses in inducible *GVG DVL4* and control plants after dex treatment. Columbia WT (**a**); *dvl1-1D* (**b**); control (empty vector) plants, no dex treatment (**c**); control plants, dex treatment (**d**); inducible *GVG DVL4* plants, no dex treatment (**e**); inducible *DVL4* plants, dex treatment (**f**). This range of silique shapes in (**f**) is similar to that seen in CaMV 35S *DVL* transgenic plants (Wen and Walker 2006). *Scale bar*: 1 cm

QPCR was used to confirm the responses seen in the microarray samples for *NAC1* (discussed below) and At2g41070, a basic region/leucine zipper motif transcription factor (Jakoby et al. 2002). At2g41070 mRNA was found to overaccumulate approximately 30-fold in the dex-induced *DVL4* line relative to the dex-treated control line, confirming the trend from the microarray data. Therefore, we decided to focus in greater detail on this subgroup.

NAC1 Loss-of-Function Mutants Disrupt the dvlOE Phenotype

One of the genes in the transcription factor subgroup was selected for further study: *NAC1* (At1g56010). *NAC1* is a NAC-domain transcription factor that has been shown to play a role in auxin-mediated lateral root development along with its targeting microRNA, *miRNA164* (Xie et al. 2000; Guo et al. 2005). QPCR confirmed the microarray data showing *NAC1* expression is increased in dex-induced *DVL4* plants. Available T-DNA insertion mutagenesis pools were searched, and three putative loss-of-function *nac1* lines were found. Inserts were confirmed in the *NAC1*-coding sequence for two of these lines (salk_098992 and salk_135734) by PCR, and the insertion sites were determined by sequencing (Fig. S2).

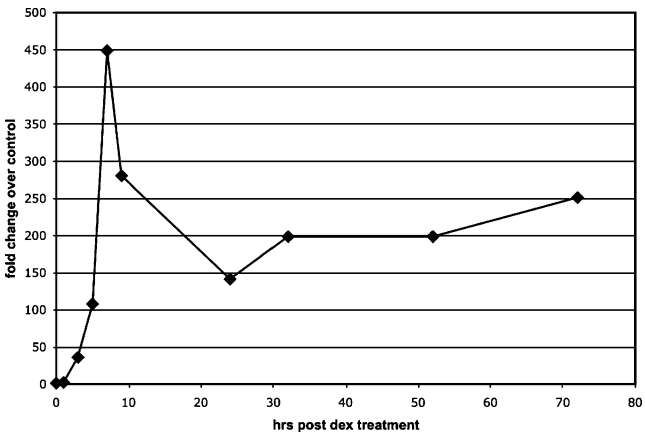

Fig. 2 *DVL4* transcript accumulation in dex-treated inducible *GVG DVL4* plants. QPCR was used to quantify the amount of *DVL4* transcript present, relative to WT Columbia. A biological replicate showed a similar trend and is presented in Fig. S1

Homozygous lines have been isolated for both alleles. No obvious developmental phenotypes were observed for either allele. To determine if *NAC1* plays a role in the dvlOE phenotype, both of these alleles were crossed to the *DVL1* overexpressing line, *dvl1-1D* (Fig. 3). This line was used due to its stable phenotype through multiple generations. F1 plants from these crosses do not show any dvlOE phenotypes, which is unexpected since *dvl1-1D* is a dominant gene (Fig. 4). In a F1 generation, the heterozygous *dvl1-1D* normally results in a typical dvlOE phenotype. In the segregating F2 populations of the cross with salk_098992 and *dvl1-1D*, there is a significant disruption in expected segregation ratios (chi-square <0.005) with no $nac1^{-/-}/dvl1\text{-}1D^{+/+}$ plants being recovered (Fig. 3). However, in the F3 lines, a $nac1^{+/-}/dvl1\text{-}1D^{+/+}$ parent does yield $nac1^{-/-}/dvl1\text{-}1D^{+/+}$ plants at a slightly lower, but not significantly reduced ratio. These $nac1^{-/-}/dvl1\text{-}1D^{+/+}$ plants appear indistinguishable from Col WT plants.

Table 1 Summary of treatments

Sample	Plant genotype	Treatment
Vector control	Empty *GVG* vector transgenic	30 μM Dex
Negative control	*GVG dvl4* transgenic	Mock
Experimental	*GVG dvl4* transgenic	30 μM Dex

Table 2 Summary of microarray data

Genes changed by dex-induced *dvl4* with dex-responsive genes removed	
2× up	233
2× down	181
Total	414
Genes changed by dex-induced *dvl4* without dex-responsive genes removed	
2× up	447
2× down	359
Total	806
Genes changed by dex alone	
2× up	187
2× down	92
Total	279

The number of genes recovered from three sets of analysis that show a two-fold or greater change are noted in the column on the right

Surprisingly, all the plants in both the F2 and F3 generations show a Col WT-like phenotype, including the $NAC1^{+/+}/dvl1\text{-}1D^{+/+}$ plants, which would be expected to show a dvlOE phenotype (Figs. 3 and 4). To check for silencing of *dvl1-1D*, transcript levels were examined. Unexpectedly, real time analysis of the F3 $NAC1^{+/+}/dvl1\text{-}1D^{+/+}$ flower buds showed an overaccumulation of *DVL1* transcript, similar as that found in the original *dvl1-1D* line (>100,000-fold overaccumulation) although the dvlOE phenotype was not present. This suggests that *NAC1* likely has an epigenetic effect on *dvlOE*; however, this effect does not seem to function by repression of *DVL* transcription, at least on a tissue scale. This epigenetic effect was not found in F1 plants of *dvl1-1D* crossed with homozygous

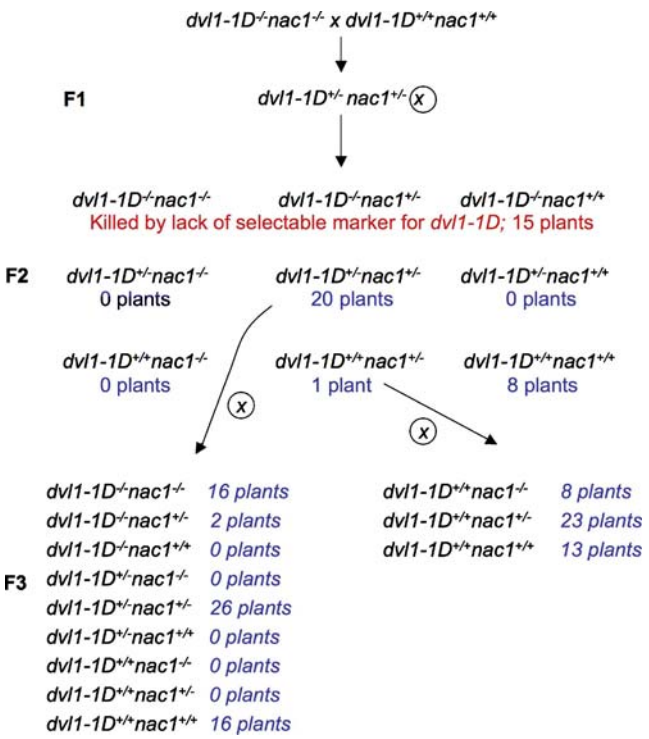

Fig. 3 Segregation analysis of *dvl1-1D* and *nac1* crosses. A crossing diagram and genotypic analysis of the progeny of the *dvl1-1D x nac1* (Salk 098992) cross shows unexpected segregation ratios in both the *F2* and *F3* generations. In the *F2* generation, those plants which did not carry at least one copy of the *dvl1-1D* allele were killed by herbicide selection. The remaining progeny were genotyped by PCR. In the *F3* generation from selected *F2* parents, no herbicide selection was used. An *encircled X* indicates self pollination. All plants in the *F1*, *F2*, and *F3* generations showed only a Col WT-like phenotype

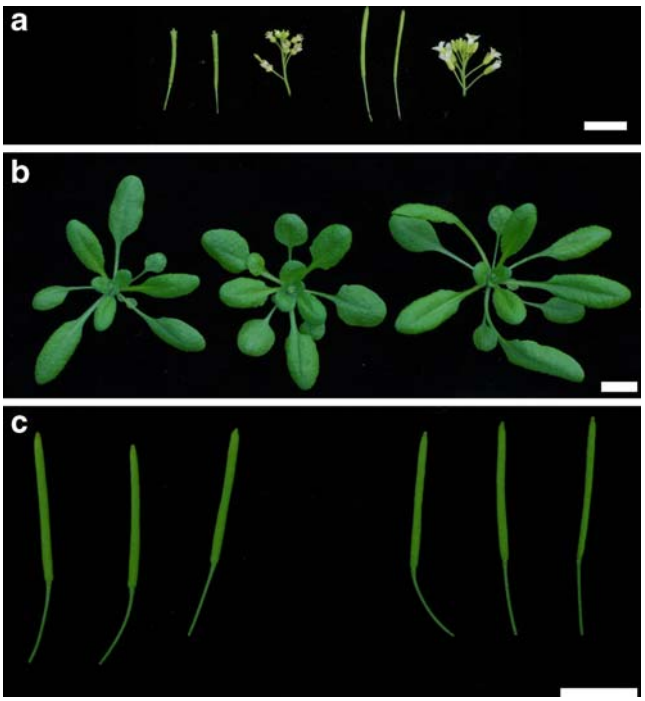

Fig. 4 *nac1* LOF suppresses the *dvl1-1D* phenotypes. *dvl1-1D* x *nac1* F1 plants do not show any dvlOE phenotypes including the protruding pistils and silique horns (**A**), *dvl1-1D left*, *dvl1-1D x nac1* (Salk 098992 shown) F1 *right*. In addition, the rosette phenotype of smaller, rounder leaves is also suppressed in this cross (**B**), Col WT *left*, *dvl1-1D center*, *dvl1-1D x nac1* (Salk 135734 shown) F1 *right*. The LOF of *nac1* results in an epigenetic effect in which the *dvl1-1D* phenotype is suppressed in a F3 plant from the *dvl1-1D x nac1* (Salk 098992) cross with the $NAC1^{+/+}$ $dvl1\text{-}1D^{+/+}$ genotype in both the F2 and F3 generations (**C**) Col WT *left*, $NAC1^{+/+}$ $dvl1\text{-}1D^{+/+}$ *right*. *Scale bar* 1 cm

salk_107861, a line predicted to contain an insert in another NAC transcription factor, *NAC46* (At3g04060). This suggests some specificity in this epigenetic response, occurring only in both alleles of *nac1*.

Since *NAC1* was found to be overexpressed following dex-induced *DVL4* expression, we hypothesized that a LOF allele of *NAC1* would likely change the dvlOE phenotype to a more WT-like phenotype. Indeed, this is what was observed. Crosses with *NAC1* LOF alleles resulted in a WT-like phenotype. However, these unusual segregation ratios, epigenetic effects and lack of observed dvlOE phenotypes, even when the plants had segregated to a *NAC1* WT genotype, is an interesting and unexpected discovery. Further insights into the mechanism underlying this epigenetic effect may provide a better understanding of the role of *DVL* and *NAC1* in plant development. Particularly intriguing is the fact that this effect does not appear to be functioning through a repression of transcription since *DVL1* was still found to be overexpressed, although the dvlOE phenotype was absent. Since a repression of transcription of *DVL1* does not seem to be responsible for this observed epigenetic effect, further studies could investigate other aspects. Perhaps translation of the *DVL* mRNA is perturbed resulting in little production of DVL protein despite high mRNA copy number. Additionally, there could be post-translational effects in which DVL protein could be generated but improperly processed and thus not active or not present at the proper location. Indirect effects through epigenetic changes on additional unknown players that may be downstream in the response to *dvlOE* could also result in the observed phenotype. Thus, several possible mechanisms through which this epigenetic effect may function can be envisioned.

Conclusions

Since the *DVL* gene family is well conserved across many seed plants (Narita et al. 2004; Wen et al. 2004), it is likely that this family plays a significant developmental role in general for plants. Therefore, a better understanding of this gene family will not only yield results applicable to the small proteins in plants but also will likely give us greater insights into plant development in general. Global expression analysis revealed that many transcription factors show perturbed expression levels in response to *DVL4* overexpression. One of these transcription factors, *NAC1*, was investigated in further detail. We hypothesized that a LOF of *nac1* would revert a dvlOE phenotype to a more WT-like phenotype. Indeed, this was shown using LOF *nac1* lines. However, unusual epigenetic effects were observed suggesting that the *dvl* and *NAC1* interaction is more complex than expected. Further investigation into this effect may yield insights into developmental and epigenetic processes during plant development.

Acknowledgments We would like to acknowledge Huachun Wang and members of the Walker lab for useful comments and suggestions. Shuqun Zhang provided assistance in setting up the GVG expression system. We would also like to acknowledge the help of the University of Missouri DNA Core Facility, including Ming-Yi Zhou who helped with the microarray experiments. We thank the Salk Institute Genomic Analysis Laboratory for providing the sequence-indexed Arabidopsis T-DNA insertion mutants and the ABRC for distributing these lines. This research was supported by a Department of Energy, Energy Biosciences grant, DE-FG02-05ER15652, and the University of Missouri Food for the 21st Century Program to JCW. CTL was supported by a graduate research fellowship from the Monsanto Corp.

References

Aoyama T, Chua N (1997) A glucocorticoid-mediated transcriptional induction system in transgenic plants. Plant J 11:605–612. doi:10.1046/j.1365-313X.1997.11030605.x

Clark S, Williams R, Meyerowitz E (1997) The *CLAVATA1* gene encodes a putative receptor kinase that controls shoot and floral meristem size in Arabidopsis. Cell 89:575–585. doi:10.1016/S0092-8674(00)80239-1

Clough S, Bent A (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J 16:735–743. doi:10.1046/j.1365-313x.1998.00343.x

Farrokhi N, Whitelegge JP, Brusslan JA (2008) Plant peptides and peptidomics. Plant Biotechnol J 6:105–134. doi:10.1111/j.1467-7652.2007.00315.x

Fletcher JC (2002) Shoot and floral meristem maintenance in Arabidopsis. Annu Rev Plant Biol. 53:45–66. doi:10.1146/annurev.arplant.53.092701.143332

Fletcher JC, Brand U, Running MP, Simon R, Meyerowitz EM (1999) Signaling of cell fate decisions by *CLAVATA3* in Arabidopsis shoot meristems. Science 283:1911–4. doi:10.1126/science.283.5409.1911

Guo HS, Xie Q, Fei JF, Chua NH (2005) MicroRNA directs mRNA cleavage of the transcription factor *NAC1* to downregulate auxin signals for arabidopsis lateral root development. Plant Cell 17:1376–1386. doi:10.1105/tpc.105.030841

Ito Y, Nakanomyo I, Motose H, Iwamoto K, Sawa S, Dohmae N, Fukuda H (2006) Dodeca-CLE peptides as suppressors of plant stem cell differentiation. Science 313:842–845. doi:10.1126/science.1128436

Jakoby M, Weisshaar B, Droge-Laser W, Vicente-Carbajosa J, Tiedemann J, Kroj T, Parcy F (2002) bZIP transcription factors in Arabidopsis. Trends Plant Sci 7:106–111. doi:10.1016/S1360-1385(01)02223-3

Jeong S, Trotochaud AE, Clark SE (1999) The Arabidopsis *CLAVATA2* gene encodes a receptor-like protein required for the stability of the *CLAVATA1* receptor-like kinase. Plant Cell 11:1925–1934

Kondo T, Sawa S, Kinoshita A, Mizuno S, Kakimoto T, Fukuda H, Sakagami Y (2006) A plant peptide encoded by *CLV3* identified by in situ MALDI-TOF MS analysis. Science 313:845–848. doi:10.1126/science.1128439

Li C, Wong WH (2001a) Model-based analysis of oligonucleotide arrays: Expression index computation and outlier detection. Proc Natl Acad Sci USA 98:31–36. doi:10.1073/pnas.011404098

Li C, Wong WH (2001b) Model-based analysis of oligonucleotide arrays: model validation, design issues and standard error application. Genome Biol 2(8):Research0032.1–Research0032.11

Lindsey K, Casson S, Chilley P (2002) Peptides: new signaling molecules in plants. Trends Plant Sci 7:78–83. doi:10.1016/S1360-1385(01)02194-X

McGurl B, Pearce G, Orozco-Cardenas M, Ryan CA (1992) Structure, expression, and antisense inhibition of the systemin precursor gene. Science 255:1570–1573. doi:10.1126/science.1549783

Montoya T, Nomura T, Farrar K, Kaneta T, Yokota T, Bishop GJ (2002) Cloning the tomato *curl3* gene highlights the putative dual role of the leucine-rich repeat receptor kinase *tBRI1/SR160* in plant steroid hormone and peptide hormone signaling. Plant Cell 14:3163–3176. doi:10.1105/tpc.006379

Narita N, Moore S, Horiguchi G, Kubo M, Demura T, Fukuda H, Goodrich J, Tsukaya H (2004) Overexpression of a novel small peptide *ROTUNDIFOLIA4* decreases cell proliferation and alters leaf shape in Arabidopsis thaliana. Plant J 38:699–713. doi:10.1111/j.1365-313X.2004.02078.x

Olsen A, Ernst H, Leggio L, Skriver K (2005) NAC transcription factors: structurally distinct, functionally diverse. Trends Plant Sci 10:79–87. doi:10.1016/j.tplants.2004.12.010

Pearce G, Strydom D, Johnson S, Ryan CA (1991) A polypeptide from tomato leaves induces wound-inducible proteinase inhibitor proteins. Science 253:895–898. doi:10.1126/science.253.5022.895

Sawa S, Kinoshita A, Nakanomyo I, Fukuda H (2006) CLV3/ESR-related (CLE) peptides as intercellular signaling molecules in plants. Chem Rec. 6:303–310. doi:10.1002/tcr.20091

Scheer JM, Ryan CA (1999) A 160-kD systemin receptor on the surface of lycopersicon peruvianum suspension-cultured cells. Plant Cell 11:1525–1536

Scheer JM, Ryan CA (2002) The systemin receptor *SR160* from Lycopersicon peruvianum is a member of the LRR receptor kinase family. Proc Natl Acad Sci USA 99:9585–9590. doi:10.1073/pnas.132266499

Schoof H, Lenhard M, Haecker A, Mayer K, Jurgens G, Laux T (2000) The stem cell population of Arabidopsis shoot meristems in maintained by a regulatory loop between the *CLAVATA* and *WUSCHEL* genes. Cell 100:635–644. doi:10.1016/S0092-8674(00)80700-X

Thimm O, Blasing O, Gibon Y, Nagel A, Meyer S, Kruger P, Selbig J, Muller LA, Rhee SY, Stitt M (2004) MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. Plant J 37:914–939. doi:10.1111/j.1365-313X.2004.02016.x

Wen J, Walker JC (2006) DVL peptides are involved in plant development. In: Kastin AJ (ed) Handbook of biologically active peptides. Academic, Boston, pp 17–22

Wen J, Lease K, Walker J (2004) DVL, a novel class of small polypeptides: overexpression alters Arabidopsis development. Plant J 37:668–677. doi:10.1111/j.1365-313X.2003.01994.x

Xie Q, Frugis G, Colgan D, Chua NH (2000) Arabidopsis *NAC1* transduces auxin signal downstream of *TIR1* to promote lateral root development. Genes Dev 14:3024–3036. doi:10.1101/gad.852200