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Note

Dof transcription factors control the expression of the anaphase promoting complex/cyclosome activator *CCS52A1*

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Abstract Endoreduplication, a modified cell cycle process in which the cellular DNA level increases without subsequent cell division, is a key component of growth and development of plants. CCS52A1, a substrate-specific activator of the anaphase promoting complex/cyclosome (APC/C), is essential for the transition from mitosis to endoreduplication. Here, we show that DNA-binding with one finger (Dof) transcription factors directly regulate the transcription of the *CCS52A1* gene. Dof proteins are plant-specific transcription factors involved in plant growth and development processes, such as phytohormone and light signaling, defense response, and seed germination. We performed yeast one-hybrid screening using an *Arabidopsis* transcription factor library and identified several Dof transcription factors as novel *CCS52A1* promoter-binding proteins. Protoplast transient assay showed that the Dof proteins, such as CDF1, CDF2, CDF3, and COG1, function as transcriptional repressors of *CCS52A1*. All of these Dof transcription factors were localized to the nucleus. Our data imply that CDF1, CDF2, CDF3, and COG1 control plant growth and development through the regulation of *CCS52A1* expression.

Key words: *Arabidopsis*, CCS52A1, cell cycle, Dof transcription factor, endoreduplication.

Plant growth and development depend on the balance of cell proliferation and cell differentiation. Cells proliferate through mitotic cell division in the apical meristem and produce undifferentiated daughter cells, while the cells undergo differentiation during organ formation. The latter process is usually accompanied by entry into a modified cell cycle process, known as endoreduplication or endocycle, in which cellular DNA continues to be synthesized without mitosis; this leads to an increase in the level of DNA ploidy and cell volume in individual cells (Brodsky and Uryvaeva 1977; D'Amato 1964). Down-regulation of mitotic cyclin-dependent kinase (CDK) activity is sufficient for the entry into the endocycle from the mitotic cell cycle (Edgar and Orr-Weaver 2001). A major cause of this downregulation is the degradation of mitotic cyclins, which is mediated by an E3 ubiquitin ligase called anaphase-promoting complex/cyclosome (APC/C) (Heyman and De Veylder 2012; Marrocco et al. 2010). The activity of APC/C is controlled by regulatory proteins, among which activators such as CDH1, Fizzy-related (FZR), and CCS52A promote the onset and progression of the endocycle in human, *Drososphila*, and plants, respectively (Larson-Rabin et al. 2009; Lasorella et al. 2006; Zielke et al. 2008). In *Arabidopsis*, deficiency of *CCS52A1*, one of the two genes for CCS52A, results in a reduced level of DNA ploidy and inhibition of expansion of trichomes and leaf cells, while *CCS52A1* overexpression promotes endoreduplication and increases cell size (Larson-Rabin et al. 2009). These results suggest that *CCS52A1* expression is critical for a transition to the endocycle. Recently, it has been reported that a trihelix transcription factor, GT2-LIKE 1 (GTL1; GT family protein), represses the transcription of *CCS52A1* and stops the progression of the endocycle and ploidy-dependent cell growth in the trichome of *Arabidopsis* (Breuer et al. 2012). However, CCS52A1-mediated control of endoreduplication has not been completely elucidated thus far.

To further reveal molecular mechanisms involved in regulating *CCS52A1* expression and endoreduplication, we screened *Arabidopsis* transcription factors that bind to the *CCS52A1* promoter by using yeast one-hybrid assay. The *CCS52A1* promoter was amplified by polymerase chain reaction (PCR) using the primers 5′-ATG GCG GCC GCT TAT CAT TTG TTT TCT GATT-3′ and 5′-ATG GCG GCC GCT GGT TTT TTT TTT TTG TTGACT-3′ and was cloned into the pINT1-HIS3NB vector (Lopato et al. 2006) to generate a transcriptional

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Table 1. List of transcription factors identified using yeast one-hybrid screening.

AGI code	Transcription factor family	Description
At5g39660	Dof	CDF2 (CYCLING DOF FACTOR 2)
At1g64620	Dof	AtDof1.8
At3g52440	Dof	AtDof3.5
At5g60850	Dof	AtDof5.4
At5g65590	Dof	AtDof5.7
At3g10000	GT	EDA31 (EMBRYO SAC DEVELOPMENT ARREST 31)
At5g03680	GT	PTL (PETAL LOSS)
At1g76890	GT	GT2
At1g33240	GT	GTL1 (GT2 LIKE 1)
At1g76880	GT	DF1

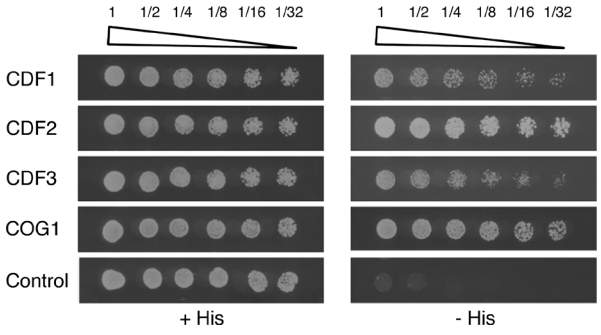

Figure 1. Dof transcription factors bind to the *CCS52A1* promoter. Yeast one-hybrid assay with the *CCS52A1* promoter. Yeast cells harboring *proCCS52A1-HIS3* were transformed with the plasmids carrying *CDF1*, *CDF2*, *CDF3*, and *COG1* fused to the GAL4 activation domain. Yeast cultures were diluted and spotted on media with (His^+) or without (His^-) histidine. The empty vector was used as a control.

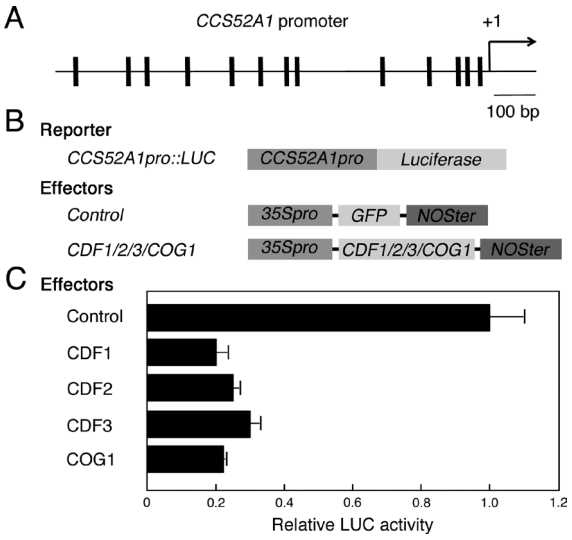

Figure 2. Dof transcription factors repress *CCS52A1* expression. (A) Schematic representation of the *CCS52A1* promoter region. The solid line and the arrow indicate the promoter region and the transcription start site, respectively. Vertical bars represent the sites of consensus binding motif (5′-(A/T)AAAG-3′) for Dof transcription factor in sense and antisense orientations. (B) Schematic diagrams of effector and reporter plasmids; *pro*, promoter; *ter*, terminator; *35S*, CaMV35S; *NOS*, nopaline synthase. (C) Relative luciferase activity in protoplast transient expression assay. The luciferase (LUC) activity was normalized to the *Renilla* luciferase activity exerted from cotransfected normalization construct. Error bars indicate mean±standard deviation (S.D., $n=3$).

fusion with the *HIS3* gene. For the screening, we used a library containing approximately 1,500 *Arabidopsis* transcription factors that are cloned into pDEST_GAD424 to generate a translational fusion with the GAL4 activation domain (Mitsuda et al. 2010). The bait and library plasmids were introduced into the yeast strain AH109 (*MATa, trp1-901, leu2-3, 112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1$_{UAS}$-GAL1$_{TATA}$-HIS3, GAL2$_{UAS}$-GAL2$_{TATA}$-ADE2, URA3::MEL1$_{UAS}$-MEL1$_{TATA}$-lacZ*), and positive colonies were obtained from 1.5×10^5 clones.

As expected, we identified genes for transcription factors belonging to the GT family, such as *GTL1* (*At1g33240*), *EDA31* (*EMBRYO SAC DEVELOPMENT ARREST 31*; *At3g10000*), *PTL* (*PETAL LOSS*; *At5g03680*), *GT2* (*At1g76890*), and *DF1* (*At1g76880*) (Table 1). In addition, cDNAs encoding several Dof transcription factors *CDF2* (*CYCLING DOF FACTOR2*; *At5g39660*) (Fornara et al. 2009), *AtDof1.8* (*At1g64620*), *AtDof3.5* (*At3g52440*), *AtDof5.4* (*At5g60850*), and *AtDof5.7* (*At5g65590*) were obtained. To check whether other Dof transcription factors can also bind to the *CCS52A1* promoter, we tested the binding capacity of CDF1 (CYCLING DOF FACTOR 1; At5g62430) (Imaizumi et al. 2005), CDF3 (CYCLING DOF FACTOR 3; At3g47500) (Fornara et al. 2009), and COG1 (COGWEEL 1; At1g29160) (Park et al. 2003), the amino acid sequences of which are closely related to those of CDF2 (Yanagisawa 2002). The coding regions of *CDF1*, *CDF2*, *CDF3*, and *COG1* were cloned into the pGADT7 vector (Clontech, Mountain View, CA, USA) to generate a translational fusion with the GAL4 activation domain, and yeast one-hybrid assay was performed as described above. CDF1, CDF3, and COG1 as well as CDF2 were bound to the *CCS52A1* promoter in yeast cells (Figure 1). Most of the Dof proteins consist of an N-terminal conserved DNA-binding domain and a C-terminal domain for transcriptional regulation, in which the DNA-binding domain is highly conserved (Yanagisawa

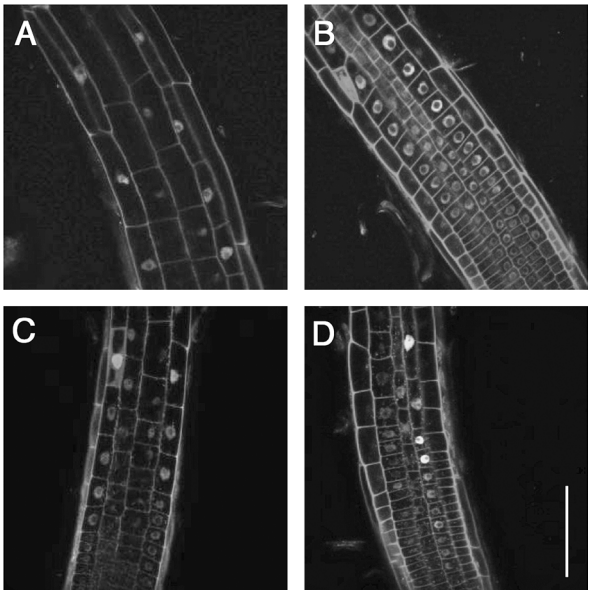

Figure 3. Nuclear localization of the Dof transcription factors. Green fluorescent protein (GFP) was fused in frame to *CDF1*, *CDF2*, *CDF3*, and *COG1* under the CaMV35S promoter and was introduced into *Arabidopsis* wild-type plants. GFP fluorescence was observed in transgenic roots. (A) CDF1-GFP; (B) CDF2-GFP; (C) CDF3-GFP; and (D) COG1-GFP. Scale bar, 100 μm.

2002). Binding-site selection experiments have showed the presence of the (A/T)AAAG sequence motif at the recognition site for Dof proteins (Yanagisawa and Schmidt 1999). We found this consensus sequence at multiple sites in the *CCS52A1* promoter (Figure 2A); this observation supports the above-mentioned finding that Dof proteins bind directly to the *CCS52A1* promoter.

To examine whether Dof transcription factors control *CCS52A1* expression in plant cells, we performed a protoplast transient expression assay. The reporter construct was created by PCR amplification of the *CCS52A1* promoter by using the primers 5′-ATG CCT GCA GTT ATC ATT TGT TTT CTG ATT-3′ and 5′-CAT GGT CGA CAC ATT GCT TGC TGT AGG ATCTTC-3,′ and was cloned into the 35S-GAL4UAS-LUC vector (Yamaguchi et al. 2010) to generate a transcriptional fusion with the firefly luciferase (*fLUC*) gene (Figure 2B). The effector constructs were created by cloning the coding regions of *CDF1*, *CDF2*, *CDF3*, *COG1*, and *GFP* (as a negative control) into p35SG (Yamaguchi et al. 2010) to generate a transcriptional fusion with the Cauliflower Mosaic Virus 35S (CaMV35S) promoter (Figure 2B). Protoplasts prepared from *Arabidopsis* cell culture were cotransfected with the reporter plasmid, effector plasmids, and the normalization construct carrying the *Renilla* luciferase (*rLUC*) gene under the CaMV35S promoter. Protoplasts were incubated at 22°C for 12 h and then the activities of fLUC and rLUC were measured with the Dual-Luciferase reporter system (Promega, Madison, WI, USA) using a Mithras LB940 microplate reader (Berthold, Bad Wildbad, Germany). Compared to GFP control, CDF1, CDF2, CDF3, and COG1 reduced the Luc activity less than 30% (Figure 2C). This indicates that these Dof proteins repress *CCS52A1* expression in plant cells.

To determine the subcellular localization of CDF1, CDF2, CDF3, and COG1, we generated transgenic *Arabidopsis* plants overexpressing GFP-fused Dof protein under the control of CaMV35S promoter. The transgenic roots showed that the GFP fluorescence for all 4 Dof proteins was detected in the nucleus (Figure 3), which suggested that CDF1, CDF2, CDF3, and COG1 were localized to the nucleus.

Arabidopsis consists of 36 putative genes encoding Dof transcription factors (Lijavetzky et al. 2003), and the expression of some of the genes is regulated in a spatiotemporal manner (Chen et al. 2002; Gualberti et al. 2002; Kang and Singh 2000; Washio 2001). Recent studies have showed diverse functions of these transcription factors in gene expression associated with light signaling (Park et al. 2003), defense response (Chen et al. 1996; Zhang et al. 1995), and seed development and germination (Gualberti et al. 2002). CDF1, CDF2, and CDF3 are involved in the photoperiodic control of flowering (Fornara et al. 2009; Imaizumi et al. 2005), and COG1 regulates phytochrome signaling (Park et al. 2003). Considering that DNA ploidy changes in response to various environmental conditions, it is likely that these Dof proteins link light signaling to the cell cycle control by adjusting the expression level of *CCS52A1*. Further studies using *Arabidopsis* mutants may reveal how Dof transcription factors are involved in controlling endoreduplication in response to internal or external stimuli.

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References

Breuer C, Morohashi K, Kawamura A, Takahashi N, Ishida T, Umeda M, Grotewold E, Sugimoto K (2012) Transcriptional repression of the APC/C activator CCS52A1 promotes active termination of cell growth. *EMBO J* 31: 4488–4501

Brodsky VY, Uryvaeva IV (1977) Cell polyploidy: Its relation to tissue growth and function. *Int Rev Cytol* 50: 275–332

Chen W, Chao G, Singh K (1996) The promoter of an H_2O_2 inducible, *Arabidopsis* glutathione *S*-transferase gene contains closely linked OBF- and OBP1-binding sites. *Plant J* 10: 955–966

Chen W, Provart NJ, Glazebrook J, Katagiri F, Chang HS, Eulgem T, Mauch F, Luan S, Zou G, Whitham SA, et al. (2002) Expression

profile matrix of Arabidopsis transcription factor genes suggests their putative functions in response to environmental stresses. *Plant Cell* 14: 559–574

D'Amato F (1964) Endopolyploidy as a factor in plant tissue development. *Caryologia* 17: 41–52

Edgar BA, Orr-Weaver TL (2001) Endoreplication cell cycles: more for less. *Cell* 105: 297–306

Fornara F, Panigrahi KC, Gissot L, Sauerbrunn N, Rühl M, Jarillo JA, Coupland G (2009) *Arabidopsis* DOF transcription factors act redundantly to reduce *CONSTANS* expression and are essential for a photoperiodic flowering response. *Dev Cell* 17: 75–86

Gualberti G, Papi M, Bellucci L, Ricci I, Bouchez D, Camilleri C, Costantino P, Vittorioso P (2002) Mutation in the Dof zinc finger genes *DAG2* and *DAG1* influence with opposite effects the germination of Arabidopsis seeds. *Plant Cell* 14: 1253–1263

Heyman J, De Veylder L (2012) The anaphase-promoting complex/cyclosome in control of plant development. *Mol Plant* 5: 1182–1194

Imaizumi T, Schultz TF, Harmon FG, Ho LA, Kay SA (2005) FKF1 F-box protein mediates cyclic degradation of a repressor of *CONSTANS* in *Arabidopsis*. *Science* 309: 293–297

Kang HG, Singh K (2000) Characterization of salicylic acid-responsive, Arabidopsis Dof domain proteins: overexpression of OBP3 leads to growth defects. *Plant J* 21: 329–339

Larson-Rabin Z, Li Z, Masson PH, Day CD (2009) *FZR2/CCS52A1* expression is a determinant of endoreduplication and cell expansion in Arabidopsis. *Plant Physiol* 149: 874–884

Lasorella A, Stegmüller J, Guardavaccaro D, Liu G, Carro MS, Rothschild G, de la Torre-Ubieta L, Pagano M, Bonni A, Iavarone A (2006) Degradation of Id2 by the anaphase-promoting complex couples cell cycle exit and axonal growth. *Nature* 442: 471–474

Lijavetzky D, Carbonero P, Vicente-Carbajosa J (2003) Genome-wide comparative phylogenetic analysis of the rice and Arabidopsis Dof gene families. *BMC Evol Biol* 3: 17

Lopato S, Bazanova N, Morran S, Milligan AS, Shirley N, Langridge P (2006) Isolation of plant transcription factors using a modified yeast one-hybrid system. *Plant Methods* 2: 3

Marrocco K, Bergdoll M, Achard P, Criqui MC, Genschik P (2010) Selective proteolysis sets the tempo of the cell cycle. *Curr Opin Plant Biol* 13: 631–639

Mitsuda N, Ikeda M, Takada S, Takiguchi Y, Kondou Y, Yoshizumi T, Fujita M, Shinozaki K, Matsui M, Ohme-Takagi M (2010) Efficient yeast one-/two-hybrid screening using a library composed only of transcription factors in *Arabidopsis thaliana*. *Plant Cell Physiol* 51: 2145–2151

Park DH, Lim PO, Kim JS, Cho DS, Hong SH, Nam HG (2003) The *Arabidopsis COG1* gene encodes a Dof domain transcription factor and negatively regulates phytochrome signaling. *Plant J* 34: 161–171

Washio K (2001) Identification of Dof proteins with implication in the gibberellin-regulated expression of a peptidase gene following the germination of rice grains. *Biochim Biophys Acta* 1520: 54–62

Yamaguchi M, Ohtani M, Mitsuda N, Kubo M, Ohme-Takagi M, Fukuda H, Demura T (2010) VND-INTERACTING2, a NAC domain transcription factor, negatively regulates xylem vessel formation in *Arabidopsis*. *Plant Cell* 22: 1249–1263

Yanagisawa S (2002) The Dof family of plant transcription factors. *Trends Plant Sci* 7: 555–560

Yanagisawa S, Schmidt RJ (1999) Diversity and similarity among recognition sequences of Dof transcription factors. *Plant J* 17: 209–214

Zhang B, Chen W, Foley RC, Büttner M, Singh KB (1995) Interactions between distinct types of DNA binding proteins enhance binding to *ocs* element promoter sequences. *Plant Cell* 7: 2241–2252

Zielke N, Querings S, Rottig C, Lehner C, Sprenger F (2008) The anaphase-promoting complex/cyclosome (APC/C) is required for rereplication control in endoreplication cycles. *Genes Dev* 22: 1690–1703