Supporting Text

Supporting Materials and Methods

Materials. The cDNA encoding full-length CPL1 was obtained by RT-PCR as described (1). An EST clone encoding full-length CPL2 (APZL61h09) was obtained from the *Arabidopsis* Biological Resource Center. Bacterial expression plasmids were provided by Y. Matsushita (Tokyo University of Agriculture and Technology) (pGEX-6P-PKA) or purchased from Novagen (pET44a). CaMV35S promoter-GFP expression plasmids pUCGFP-C and pUCNLS-DsRed were provided by I. Hwang (Pohang University, South Korea) (2). *E. coli* BL21 SR cells were prepared by transforming BL21 star (Invitrogen) with a plasmid pRARE (Novagen). The *fry2-1* plant was provided by J.-K. Zhu (University of California, Riverside), and the *cpl2-2* T-DNA insertion mutant (Salk_059753) was obtained from the *Arabidopsis* Biological Resource Center. The sequences of DNA primers used in this study are shown in Table 2.

pENSOTG Plasmid. pENSOTG is a derivative of pENTR2B (Invitrogen, CA). Synthetic $(A_{OCS})_3A_{mas}P_{mas}$ promoter (3), tobacco mosiac virus omega sequence (4), N-terminal-TAP-tag (5), sGFP (6), and nopaline synthese (NOS) terminator sequence (7) were placed between the attL1 and attL2 sites of pENTER2B. Between sGFP and NOS terminator sequence is a multicloning site containing *Sma*I, *Not*I, *Sac*II, and *Sac*I. The sequence of pENSOTG is available from H.K. (koiwa@neo.tamu.edu).

Construction of Recombinant Protein Expression Cassettes. The coding regions of CPL1 and CPL2 cDNAs were amplified with PCR with primer pairs NusCPL1N, NusCPL1C and NusCPL2N, NusCPL2C for CPL1 and CPL2, respectively. The PCR products were digested with *Bgl*II and *Not*I and ligated to pET44a digested with *Bam*HI and *Not*I. Resulting plasmids were termed as pETCPL1 and pETCPL2. GST-CPL1 constructs were prepared by digesting pDESTCPL1 (1) with *Nde*I, filling in the ends with Klenow DNA polymerase, and ligating the blunt-ended fragments to pGEX-6P-PKA digested with *Sma*I (pGST-CPL1N638). pGST-CPL1$_{118-638}$, pGST-CPL1$_{1-419}$, and pGST-CPL1$_{47-419}$ were prepared by digesting pGST-CPL1N638 with *Eco*RI/*Sac*I, *Sty*I/*Sal*I, or *Eco*RI/*Nhe*I, respectively, filling in the ends with DNA polymerase, and ligating the blunt-ended plasmids.

Purification of Recombinant CPL1 and CPL2 Proteins. pET-NusCPL1 and pET-NusCPL2 encoding 6×His-6NusA-CPL fusions were transformed into *E. coli* BL21 SR cells. Recombinant proteins were expressed by inducing bacterial cultures (500 ml) with 1 mM isopropyl β-D-thiogalactoside (IPTG) at 16°C for 12-14 h. His-tagged NusA-CPL fusion proteins were purified from a by Ni^{2+}-NTA agarose (Novagen) chromatography according to the manufacturer's protocol. The 400-mM imidazole eluate fractions were precipitated with 60% saturated $(NH_4)_2SO_4$. The insoluble material was collected by centrifugation, resuspended in 1 ml of storage buffer (50 mM Tris·HCl, pH 7.5/100 mM NaCl/1 mM DTT/10% glycerol/0.01% Triton X-100), and then dialyzed against storage buffer. The dialysate was stored at -80°C. Plasmids expressing GST-CPL fusion proteins were introduced into *E. coli* Top10 cells (Invitrogen). Cultures (500 ml) were induced

with 1 mM IPTG at room temperature for 12-14 h. The recombinant proteins were purified by glutathione-affinity chromatography. The 10 mM glutathione eluate was adjusted to 10% glycerol and stored at -80°C. The concentrations of the full-length GST-CPL1 fusion proteins were gauged by SDS/PAGE analysis of the protein preparations in parallel with various concentrations of a BSA standard. The polypeptides were stained with Coomassie blue dye and the staining intensities of the GST-CPL1 proteins were compared to known amounts of BSA analyzed in parallel.

CTD Phosphatase Assay. Twenty eight-mer tetraheptad CTD Ser-PO_4 peptides $(YSPTSPS)_4$ and $(YSPTSPS)_4$ were synthesized and purified as described (10). The peptides were dissolved in 10 mM Tris·HCl (pH 7.4), 1 mM EDTA and stored at 4°C. The molar concentrations of the phosphopeptides were initially estimated from the absorbance at 274 nm by using an extinction coefficient of 1.4×10^3 M^{-1} for tyrosine. The Ser-PO_4 content of phospho-CTD peptides was then determined for each peptide, measuring the release of inorganic phosphate after digestion with calf intestinal phosphatase as described (10). CTD phosphatase reaction mixtures (25 μl) containing 50 mM Tris-acetate (pH 5.5), 10 mM $MgCl_2$, CTD phosphopeptide, and CPLs as specified were incubated for 60 min at 37°C. The reactions were quenched by adding 0.5 ml of malachite green reagent. Release of phosphate was determined by measuring A_{620} and interpolating the value to a phosphate standard curve.

Construction of GFP-Fusion Cassettes. The expression cassette encoding GFP-CPL1 and GFP-CPL2 fusion proteins were prepared by inserting the *Sma*I-*Not*I fragment of pETCPL1 and pETCPL2 between *Sma*I-*Not*I sites of pENSOTG. The CPL1 inserts for the constructs encoding GFP:$CPL1_{1-150}$, GFP:$CPL1_{151-639}$, and GFP:$CPL1_{640-967}$ were prepared by PCR using primer pairs CPLG1F, CPLG1R for $CPL1_{1-150}$, CPLG2F, CPLG2R for $CPL1_{151-639}$, and CPLG3F, CPLG3R for $CPL1_{640-967}$, respectively. The PCR products were inserted in pUCGFP-C downstream of the coding region of smGFP (8).

Molecular Analysis of *fry2-1* and *cpl2-2* Mutations. The single point mutation of the *fry2-1* allele at the *CPL1/FRY2* locus in individual plants was determined by PCR amplification of the *CPL1* locus and direct sequencing of the PCR products. Primer pair FRY2F, FRY2R was used for the reaction. The PCR products were isolated after electrophoresis through a 1% agarose gel and then directly sequenced by using the FRY2R primer to distinguish the *fry2-1* point mutation and WT. The genotype of the *CPL2* locus was determined by PCR using primer pairs NusCPL2C, SalkLB1a (9) or CPL2F, NusCPL2C to detect the T-DNA-inserted or WT *CPL2* alleles, respectively. *CPL1/FRY2* and *CPL2* transcripts were detected by RT-PCR using primer pairs FRY2F2, FRY2R2 for *CPL1/FRY2* and CPL2F, NusCPL2C for *CPL2*. *RD29a-LUC* imaging analysis was conducted as described (1).

1. Koiwa, H., Barb, A. W., Xiong, L., Li, F., McCully, M. G., Lee, B.-h., Sokolchik, I., Zhu, J., Gong, Z., Reddy, M., *et al*. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 10893-10898.

2. Jin, J. B., Kim, Y. A., Kim, S. J., Lee, S. H., Kim, D. H., Cheong, G. W. & Hwang, I. (2001) *Plant Cell* **13,** 1511-1526.

3. Ni, M., Cui, D., Einstein, J., Narasimhulu, S., Vergara, C. E. & Gelvin, S. B. (1995) *Plant J.* **7,** 661-676.

4. Gallie, D. R. & Walbot, V. (1992) *Nucleic Acids Res.* **20,** 4631-4638.

5. Rigaut, G., Shevchenko, A., Rutz, B., Wilm, M., Mann, M. & Seraphin, B. (1999) *Nat. Biotechnol.* **17,** 1030-1032.

6. Chiu, W.-L., Niwa, Y., Zeng, W., Hirano, T., Kobayashi, H. & Sheen, J. (1996) *Curr. Biol.* **6,** 325-330.

7. Becker, D. (1990) *Nucleic Acids Res.* **18,** 203.

8. Davis, S. J. & Vierstra, R. D. (1998) *Plant Mol. Biol.* **36,** 521-528.

9. Koiwa, H., Li, F., McCully, M. G., Mendoza, I., Koizumi, N., Manabe, Y., Nakagawa, Y., Zhu, J.-H., Rus, A., Pardo, J. M., Bressan, R. A. & Hasegawa, P. M. (2003) *Plant Cell* **15,** 2273-2284.

10. Hausman, S., Erdjument-Bromage, H. & Shuman, S. (2003) *J. Biol. Chem.* **279,** 10892-10900.