Molecular Cell, Volume *32*

Supplemental Data

COP1 and ELF3 Control Circadian Function and Photoperiodic Flowering by Regulating GI Stability

Jae-Woong Yu, Vicente Rubio, Na-Yeoun Lee, Sulan Bai, Sun-Young Lee, Sang-Sook Kim, Lijing Liu, Yiyue Zhang, María Luisa Irigoyen, James A. Sullivan, Yu Zhang, Ilha Lee, Qi Xie, Nam-Chon Paek, and Xing Wang Deng

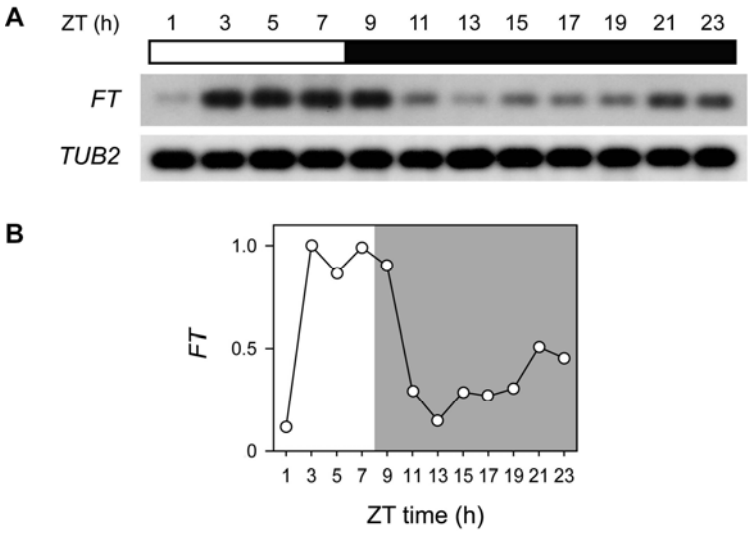

Figure S1. Expression Patterns of *FT* Gene in *elf3-8* Mutant under SD Conditions

(A-B) Total RNA samples were collected every 2 hr from 14-d-old plants entrained in SD (8L/16D). *FT* mRNA abundance was quantified by semiquantitative RT-PCR and expressed relative to the abundance of *TUB2* transcripts. In the line graph (B), grey area behind the traces represents the night period. This experiment was repeated twice with similar results.

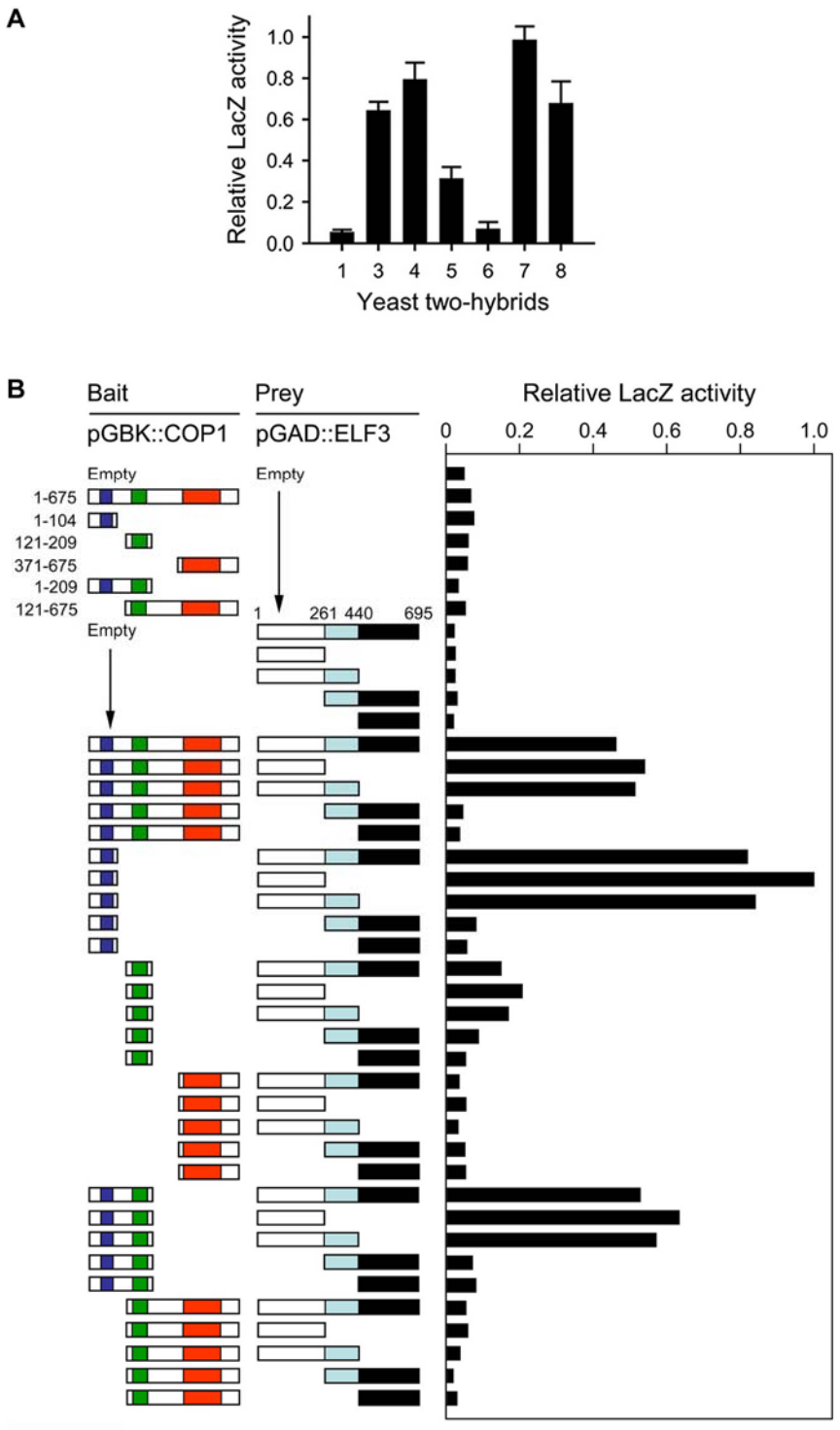

Figure S2. Relative LacZ Activities of Interactions between COP1 and ELF3 Domains in Yeast Two-hybrid Assays

(A) Relative LacZ activities in yeast two-hybrid assays shown in Figure 4B. The numbers (1, 3-8) of the x-axis are identical to those in Figure 4B. Activity values are adjusted relative to the highest value among three replicates of COP1::CCT1 (7). Values are the averages of three colonies and error bars represent standard deviations.

(B) Five regions of COP1 (aa 1-675) were used as baits, according to representative COP1 domains; RING-finger (45-94; blue), coiled-coil (128-209; green), and WD-40 repeats (386-619; red), as previously described (McNellis et al., 1994). ELF3 was divided by five parts, such as N-terminal (N; 1-261; white), middle (M; 261-440; cyan), C-terminal (C; 440-695; black), NM (1-440), and MC (261-695) regions as previously described (Liu et al., 2001).

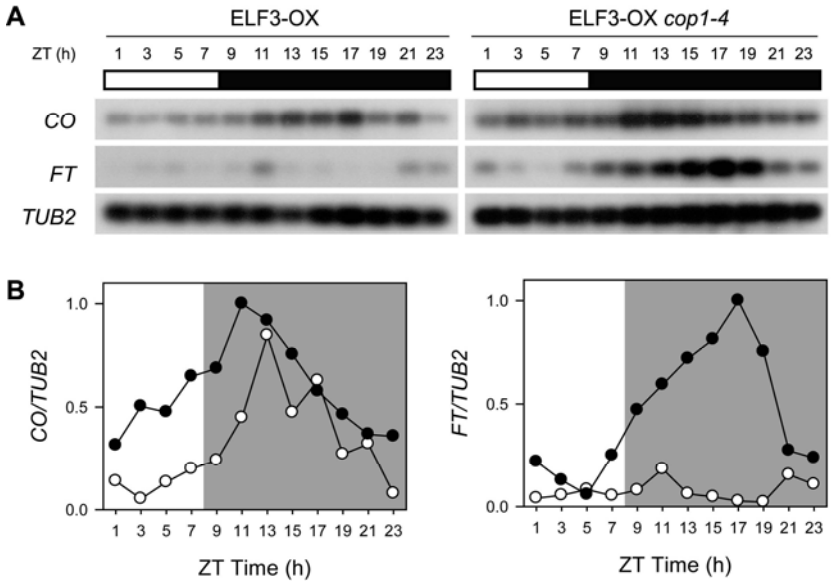

Figure S3. Expression Patterns of *CO* and *FT* Genes in ELF3-OX and ELF-OX *cop1-4* Plants under SD Conditions

Total RNA samples were collected every 2 hr from 20-d-old plants entrained in SD (8L/16D). Each mRNA abundance was quantified by semiquantitative RT-PCR and expressed relative to the abundance of *TUBULIN2* (*TUB2*) transcripts. Plants were grown at 22^{o}C under cool-white fluorescent light (100 μmole m^{-2} s^{-1}). In the line graphs, grey areas behind the traces represent night periods. Open circle, ELF3-OX; closed circle, ELF3-OX *cop1-4*.

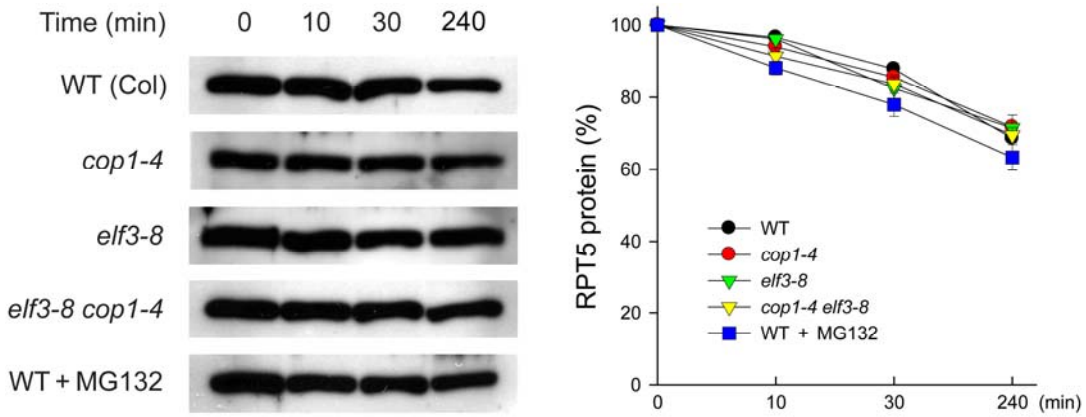

Figure S4. Mutations in COP1 and ELF3 do not affect the degradation rate of proteasome subunit RPT5.

Degradation of endogenous RPT5 in cellular extracts from WT (Col), MG132-treated WT (WT+MG132), *cop1-4*, *elf3-8* or *elf3-8 cop1-4* plants grown under LD and harvested at ZT22, after incubation for the indicated times (min). Mean and standard deviation values of three replicates are shown.

Table S1. Effect of *cop1-4* mutation on flowering time in different mutant backgrounds.

	Rosette leaves		Days to flowering	
Genotype	LD	SD	LD	SD
Wild type (Col)	11.7 ± 0.8	46.9 ± 3.6	22.3 ± 0.9	52.7 ± 2.3
cop1-4	12.5 ± 0.7	12.3 ± 0.9	24.8 ± 1.1	36.6 ± 1.0
cop1-6	11.2 ± 0.8	11.5 ± 0.9	23.3 ± 0.9	35.7 ± 1.2
DN-COP1	12.3 ± 0.9	13.6 ± 1.0	23.6 ± 0.5	34.2 ± 1.3
phyB-9	9.2 ± 0.5	38.1 ± 2.9	20.0 ± 0.4	48.3 ± 2.1
phyB-9 cop1-4	10.5 ± 0.7	9.8 ± 1.0	21.4 ± 0.8	31.7 ± 1.1
cry2-1	27.4 ± 1.3	48.3 ± 3.9	31.3 ± 1.5	53.7 ± 2.5
cry2-1 cop1-4	11.5 ± 0.9	12.6 ± 0.8	24.0 ± 1.3	36.7 ± 2.7
elf3-8	7.4 ± 0.5	8.1 ± 0.9	17.4 ± 0.5	30.1 ± 1.2
elf3-8 cop1-4	7.9 ± 0.7	8.0 ± 0.9	18.1 ± 0.6	29.7 ± 1.0
gi-1	38.4 ± 2.3	52.1 ± 2.7	34.7 ± 0.9	55.2 ± 2.9
gi-1 cop1-4	37.1 ± 2.0	51.2 ± 3.3	38.2 ± 1.7	57.3 ± 2.9
*co-1 COP1_* F_{3-1}	35.2 ± 1.7	44.9 ± 2.5	34.9 ± 1.3	53.6 ± 2.5
co-1 cop1-4 F_{3-1}	26.0 ± 1.7	35.7 ± 2.4	33.8 ± 1.6	45.2 ± 4.3
*co-1 COP1_* F_{3-2}	30.6 ± 1.8	39.2 ± 2.4	32.4 ± 1.7	49.7 ± 2.1
co-1 cop1-4 F_{3-2}	21.6 ± 1.9	29.1 ± 3.5	30.8 ± 1.9	41.1 ± 3.7
ft-1	43.6 ± 2.6	69.4 ± 4.0	40.0 ± 1.4	59.1 ± 4.2
ft-1 cop1-4	29.4 ± 1.8	36.3 ± 2.8	33.6 ± 0.7	48.2 ± 2.4
fca-9	63.8 ± 4.3	>120	51.8 ± 3.9	ND*
fca-9 cop1-4	28.9 ± 2.7	26.0 ± 2.6	35.0 ± 1.5	45.2 ± 2.2
ld-1	57.6 ± 3.2	>120	45.2 ± 3.2	ND
ld-1 cop1-4	39.1 ± 2.3	38.5 ± 3.5	41.5 ± 2.5	51.3 ± 3.7
*FRI-Sf2 COP1_* F_3	68.7 ± 3.8	>120	56.8 ± 3.4	ND
FRI-Sf2 cop1-4 F_3	36.4 ± 2.2	34.6 ± 2.0	39.4 ± 2.2	49.8 ± 2.7
soc1-1	24.7 ± 1.2	60.7 ± 4.3	26.7 ± 0.9	57.8 ± 4.0
soc1-1 cop1-4	20.0 ± 1.2	19.5 ± 1.5	27.4 ± 0.7	37.3 ± 1.3
ft-1 soc1-1	49.7 ± 3.8	81.8 ± 6.2	41.5 ± 1.1	69.1 ± 4.3
ft-1 soc1-1 cop1-4	47.4 ± 2.6	84.9 ± 5.6	43.8 ± 2.1	72.8 ± 5.1
spy-3	8.7 ± 0.5	34.9 ± 2.5	18.8 ± 0.6	42.7 ± 1.9
spy-3 cop1-4	7.3 ± 0.8	7.6 ± 0.7	20.1 ± 0.9	30.0 ± 1.1

Wild type (Col)	13.2 ± 0.3	48.5 ± 3.4	ND	ND
phyA-KO	13.4 ± 0.5	52.1 ± 4.3	ND	ND
cop1-4	13.1 ± 0.5	13.6 ± 0.7	ND	ND
phyA-KO cop1-4	14.0 ± 1.3	20.3 ± 2.1	ND	ND

In each genotype, at least 25 plants were analyzed. Due to different ecotypes of *co-1* (Lansberg) and *FRI-Sf* from *cop1-4* (Columbia), segregating F_3 plants were measured. $F_{3\text{-}1}$ and $F_{3\text{-}2}$ indicate F_3 progenies produced from two homozygous *co-1* F_2 plants by a cross of *cop1-4* and *co-1* plants, and F_3 indicate those from a homozygous *FRI-Sf* F_2 plant by a cross of *cop1-4* and *FRI-Sf* plants. Flowering time was measured as the number of rosette leaves at bolting. Data represent mean ± standard deviation.

* ND, not determined.

Supplemental References

Liu, X.L., Covington, M.F., Fankhauser, C., Chory, J., and Wagner, D.R. (2001). *ELF3* encodes a circadian clock-regulated nuclear protein that functions in an *Arabidopsis PHYB* signal transduction pathway. Plant Cell *13*, 1293-1304.

McNellis, T.W., von Arnim, A.G., Araki, T., Komeda, Y., Misera, S., and Deng, X.W. (1994). Genetic and molecular analysis of an allelic series of cop1 mutants suggests functional roles for the multiple protein domains. Plant Cell 6, 487–500.