

Salomé and McClung, Supplemental Table and Figures

Supplemental Table SI. *Promoter::LUC* fusions used in this study.

Promoter ^a	Starting ^b nt	Ending ^b nt	Reference
<i>LHCBI*1</i>	-376 ^c	-51 ^c	(Millar et al., 1992)
<i>CAT2</i>	-1500	+1	this study
<i>CAT3</i>	-276	-158	(Michael and McClung, 2002)
<i>CCA1</i>	-1070	+1	(Salomé and McClung, 2005)
<i>LHY</i>	-1800	+1	(Salomé and McClung, 2005)
<i>TOC1</i>	-2100	+1	(Salomé and McClung, 2005)
<i>PRR7</i>	-1000	+1	(Salomé and McClung, 2005)

^a *LHCBI*1* and *CAT3* are fused transcriptionally to *LUC* and the constructs do not include 5'UTR sequences. *CAT2*, *CCA1*, *LHY*, *TOC1* and *PRR7* fusions all contain the full 5' UTRs. In the case of *CAT2*, 5' RACE determined that the 5' UTR extended 57 bp upstream of the ATG and therefore overlapped with the sole putative CCA1 binding site. The 5' UTR was therefore included in the promoter fusion. The other 5' UTRs were: *CCA1*: 239 bp; *LHY*: 340 bp; *TOC1*: 398 bp; *PRR7*: 253 bp and, in some cases (*CCA1*, *LHY*, *PRR7*), contained an intron.

^b Relative to the A nucleotide of the ATG translation initiation codon.

^c The *LHCBI*1* promoter fragment is -322 bp to +3 bp relative to the transcription start (Millar et al., 1992), which lies 54 bp upstream of the ATG (Karlin-Neumann et al., 1988).

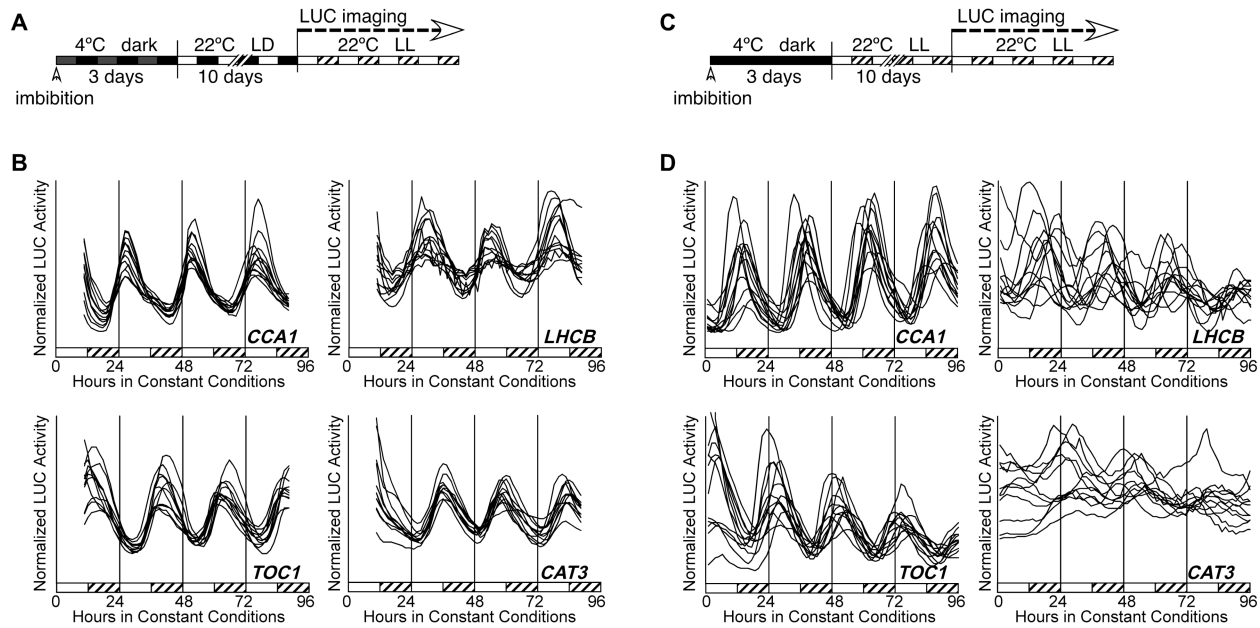


Figure S1. Light-dark cycles are dispensable for rhythmicity but not for synchronization between individuals.

(A) Diagram representing the growth conditions used in **(B)**. Seeds were imbibed at noon on the first day and stratified for three days. At noon on the fourth day, plates were released into LD cycles (lights on at noon; lights off at midnight). Individual seedlings (12 per LUC reporter) were transferred to 96-well plates after 10 days in LD cycle, and moved into constant conditions of continuous light (LL) and constant temperature (22°C) for LUC activity measurement.

(B) Expression of clock (*CCA1*, *LHY* and *TOC1*) and clock-output (*LHCB1*1* and *CAT3*) genes in LL after LD cycles in individual seedlings. To facilitate comparison among genes of different absolute expression levels, LUC activities for each seedling at each time point are expressed relative to the mean LUC activity for that seedling and are presented as “normalized LUC activity.” Open bars represent subjective day and hatched bars represent subjective night.

(C) Diagram representing the growth conditions used in **(D)**. Seeds were imbibed at noon on the first day and stratified for three days. At noon on the fourth day, plates were released into LL. Individual seedlings (12 per LUC reporter) were transferred to 96-well plates after 10 days in constant conditions of continuous light (LL) and constant temperature (22°C) before LUC activity measurement on days 11 through 14.

(D) Expression of clock (*CCA1*, *LHY* and *TOC1*) and clock-output (*LHCB1*1* and *CAT3*) genes in LL in individual seedlings in the absence of entrainment by LD cycles. Open bars represent

subjective day and hatched bars represent subjective night where subjective dawn is defined by release from stratification.

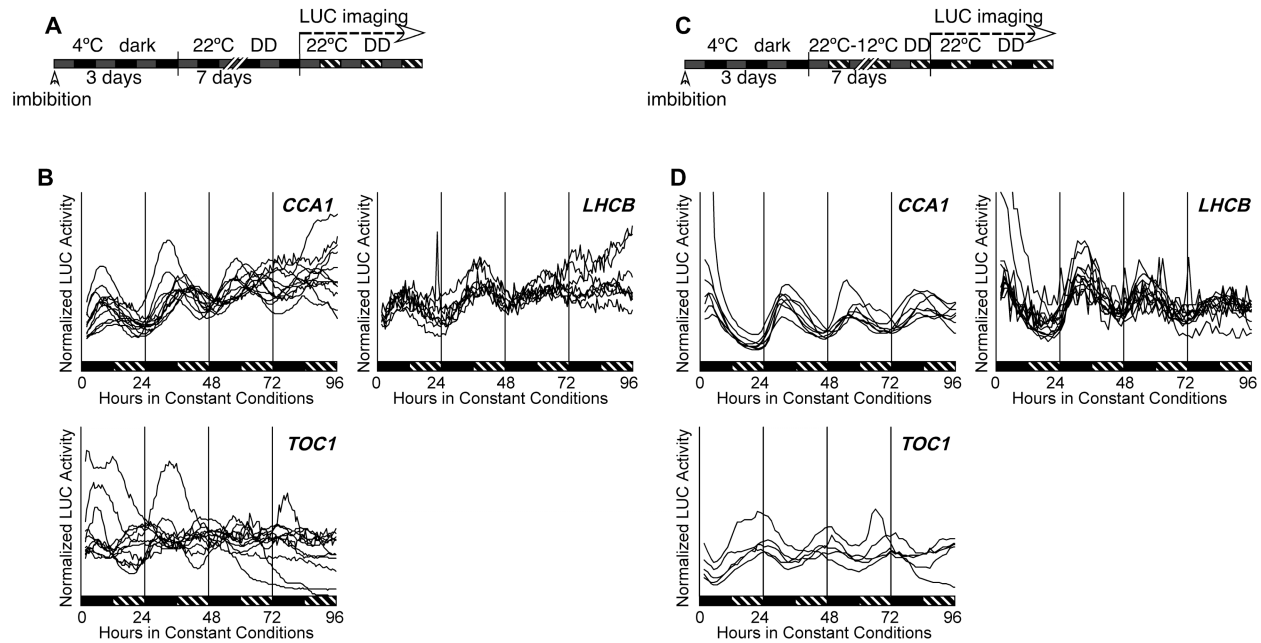


Figure S2. Etiolated seedlings are rhythmic.

(A) Diagram representing the growth conditions used in **(B)**. Seeds were imbibed at noon on the first day and sown directly into 96-well plates, then stratified for three days. At noon on the fourth day, plates were released into DD. LUC activity was recorded starting at the beginning of the 11th day.

(B) Expression of clock (*CCA1*, *LHY* and *TOC1*) and clock output (*LHCB1*1*) genes in DD in individual seedlings in the absence of entrainment. Open bars represent subjective day and hatched bars represent subjective night where subjective dawn is defined by release from stratification.

(C) Diagram representing the growth conditions used in **(D)**. Seeds were imbibed at noon on the first day and sowed directly into 96-well plates, then stratified for three days. At noon on the fourth day, plates were released into HC (22°/12°C) cycles in DD. After seven days, plates were released in constant conditions of continuous dark and constant temperature (22°C), and LUC activity was recorded.

(D) Expression of clock (*CCA1*, *LHY* and *TOC1*) and clock output (*LHCB1*1*) genes in individual seedlings in DD after entrainment by HC cycles. Open bars represent subjective day and hatched bars represent subjective night, where subjective dawn is defined by onset of 22°C during entrainment.

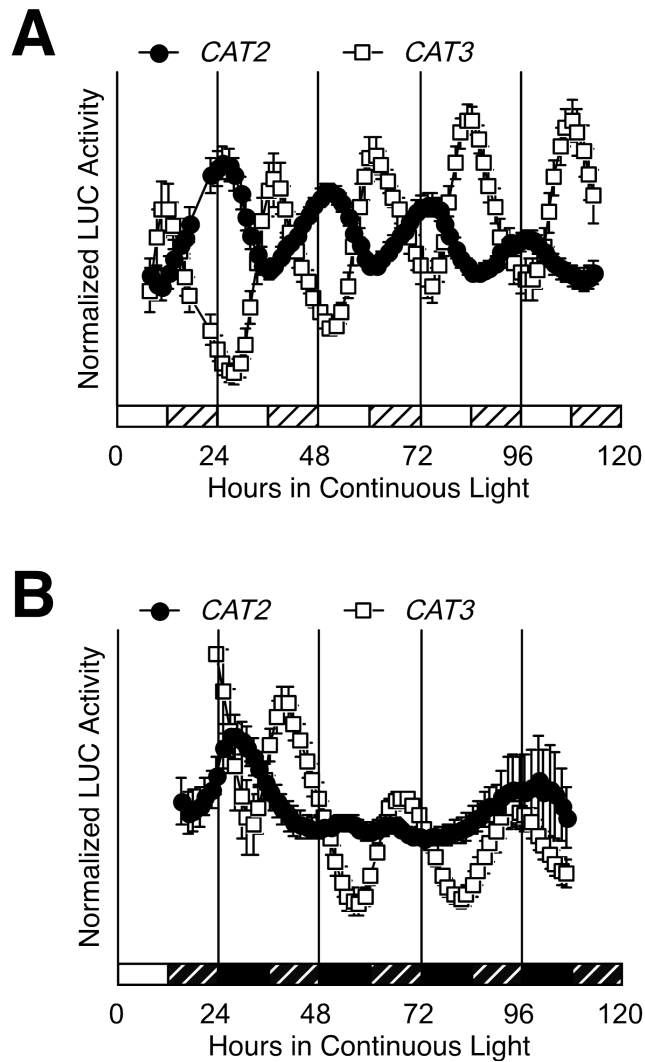


Figure S3. Description of the *CAT2::LUC* reporter.

Transgenic lines carrying the *CAT2* and *CAT3::LUC* constructs were grown in 12L:12D photocycles for 10 days before being transferred to 96-well plates. Plates were then released into continuous light (**A**) or continuous darkness (**B**) on the beginning of the 11th day.

(A) Expression of the clock-regulated genes *CAT2* and *CAT3* in LL after entrainment to LD cycles. Mean expression (\pm SE, $n = 12$). Open bars represent subjective day and hatched bars represent subjective night. Open squares, *CAT3::LUC*; filled circles, *CAT2::LUC*.

(B) Expression of the clock-regulated genes *CAT2* and *CAT3* in DD after entrainment to LD cycles. Mean expression (\pm SE, $n = 12$). Filled bars represent subjective day and hatched bars represent subjective night. Open squares, *CAT3::LUC*; filled circles, *CAT2::LUC*.

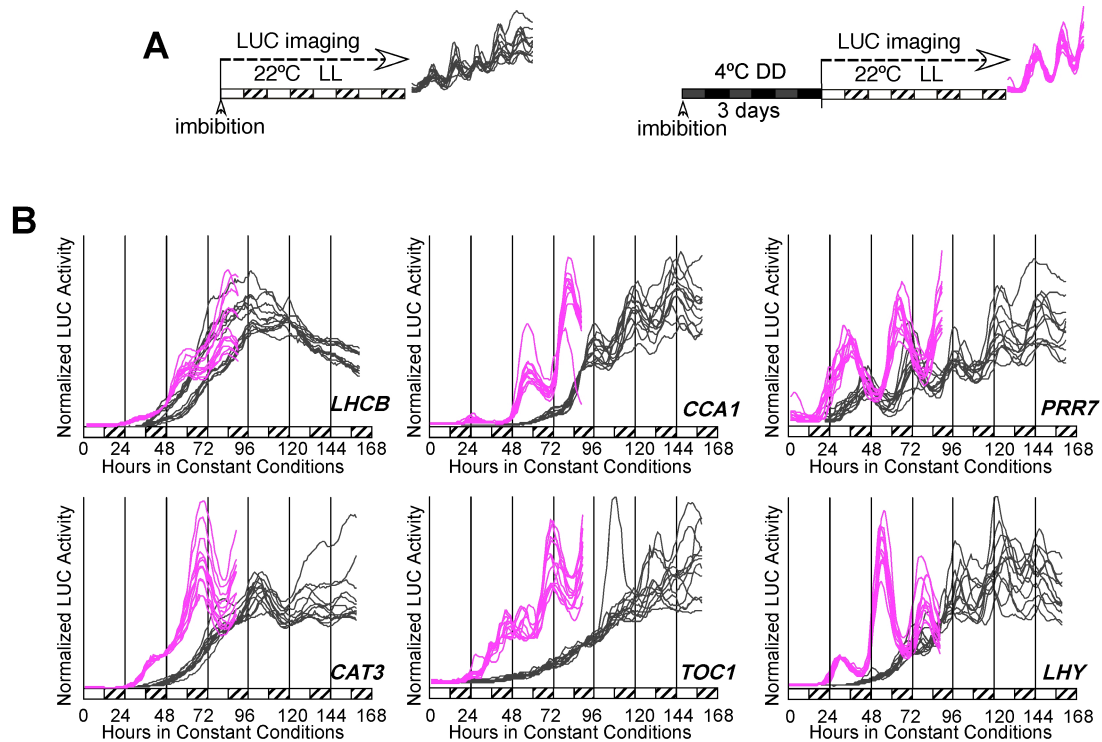


Figure S4. Germinating seeds are rhythmic.

(A) Diagrams representing the growth conditions used in (B). Seeds were imbibed at noon on the first day and sown directly into 96-well plates. One set of plates was then released into LL within four h of imbibition, and LUC activity was recorded. A second set of plates was stratified at 4°C in the dark for 3 days and then released into LL

(C) Expression of clock (*CCA1*, *LHY*, *PRR7*, and *TOC1*) and clock output (*LHCb*, *CAT2*, and *CAT3*) genes in individual germinating seeds. Open bars represent subjective day and hatched bars represent subjective night, where subjective dawn is defined by imbibition. Traces from individuals grown without stratification are shown in dark grey and grown with stratification are shown in magenta.

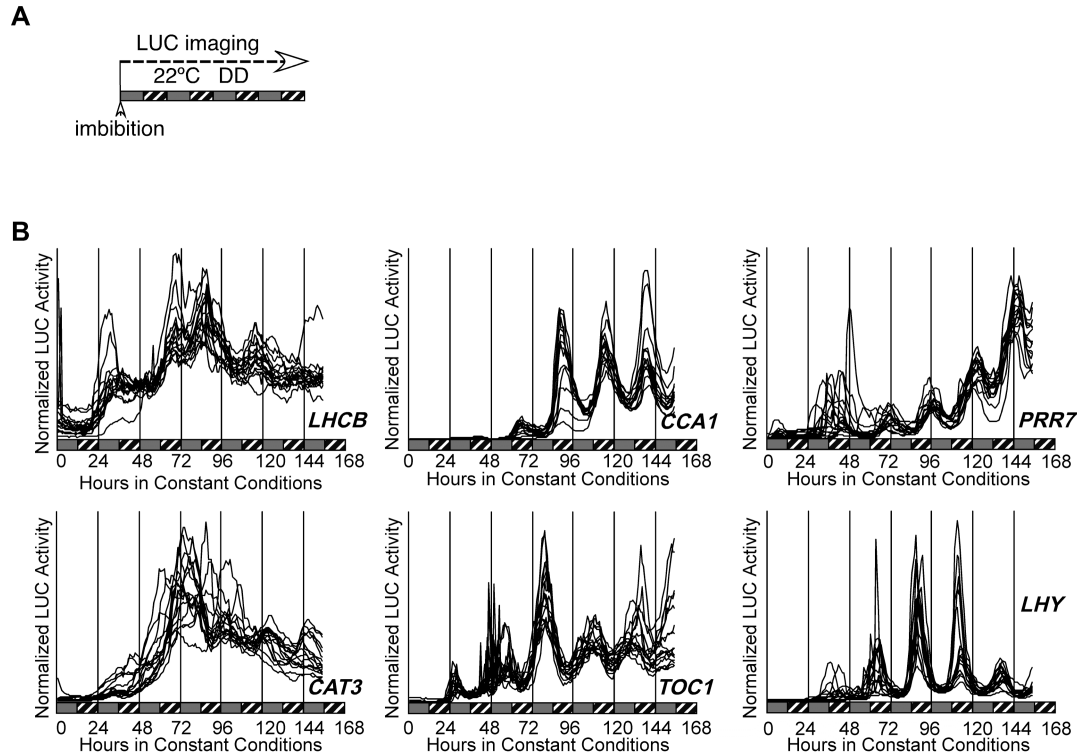


Figure S5. Stratification is dispensable for rhythmicity of etiolated seedlings.

(A) Diagram representing the growth conditions used in (B). Seeds were imbibed at noon on the first day and sown directly into 96-well plates, then released into DD. LUC activity was recorded immediately for 7 days.

(B) Expression of clock (*CCA1*, *LHY*, *PRR7*, and *TOC1*) and clock output (*LHCBI*1* and *CAT3*) genes in individual seedlings in DD in the absence of entrainment and stratification. Hatched bars represent subjective night when noon is taken as ZT0.

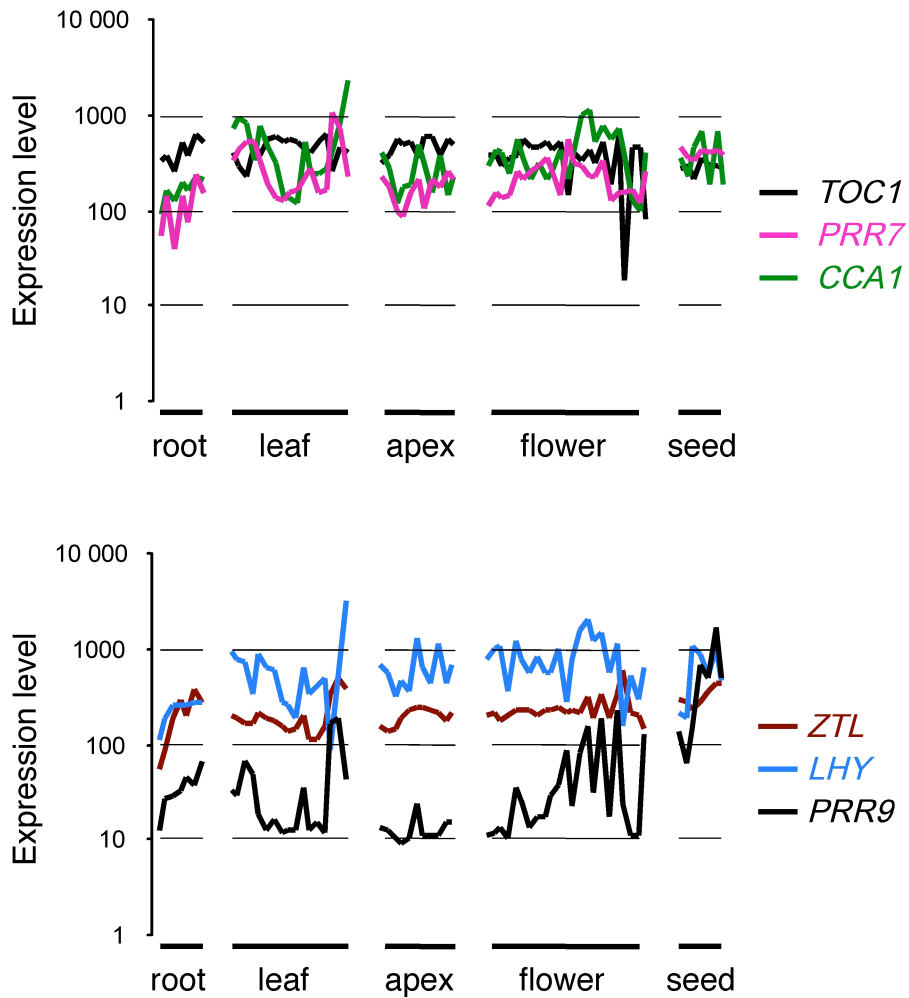


Figure S6. Expression of the clock genes *CCA1*, *LHY*, *TOC1*, *ZTL*, *PRR7* and *PRR9* in the developmental series of the AtGenExpress tool.

Expression values from microarray experiments sampling expression from various tissues and developmental stages (root, leaf, apex, flower and see) were obtained from the AtGenExpress tool (<http://jsp.weigelworld.org/expviz/expviz.jsp>). All genes are well expressed in seeds, with expression values above 100, and averaging 400.

Supplemental Video. *LHY::LUC* is expressed in the cotyledons of etiolated seedlings.

Seeds bearing the *LHY::LUC* transgene were stratified on plates for 3 days in darkness and released into continuous dark at 22°C. Luciferase activity was then recorded in constant darkness at 22°C by a Hamamatsu ORCA II ER CCD camera. See Figure 4. QuickTime Player (<http://quicktime.free-download4u.net/?tid=qp1>) is necessary to view this video.

REFERENCES

- Karlin-Neumann GA, Sun L, Tobin EM** (1988) Expression of light-harvesting chlorophyll *a/b*-protein genes is phytochrome-regulated in etiolated *Arabidopsis thaliana* seedlings. *Plant Physiol.* **88**: 1323-1331
- Michael TP, McClung CR** (2002) Phase-specific circadian clock regulatory elements in *Arabidopsis thaliana*. *Plant Physiol.* **130**: 627-638
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- Salomé PA, McClung CR** (2005) *PRR7* and *PRR9* are partially redundant genes essential for the temperature responsiveness of the *Arabidopsis* circadian clock. *Plant Cell* **17**: 791-803