similarity to a Thr → Ile change in v-Src versus c-Src at position 338, which corresponds to Thr^{315} in c-Abl. Despite the fact that v-Src and c-Src have almost identical kinase domain sequences (98% identity), v-Src is about 50-fold more resistant than c-Src to kinase inhibition by the Src inhibitor PP1 (*35*).

Although the development of STI-571 resistance presents new therapeutic challenges, the fact that BCR-ABL remains active in STI-571–resistant cells suggests that the chimeric oncoprotein remains a rational drug target. Because several patients examined to date share an identical mutation associated with drug resistance, it may be possible to identify an inhibitor of the mutant *BCR-ABL* allele that would have broad utility. In addition, knowledge of this mutation should permit the development of assays to detect drug-resistant clones before clinical relapse. It should be noted that this study has focused on a small, selected group of patients. Analyses of larger sample sizes are required to determine the true frequency of this mutation among resistant patients.

References and Notes

1. L. Chin *et al.*, *Nature* **400**, 468 (1999).
2. D. W. Felsher, J. M. Bishop, *Mol. Cell* **4**, 199 (1999).
3. C. S. Huettner *et al.*, *Nature Genet.* **24**, 57 (2000).
4. B. J. Druker *et al.*, *N. Engl. J. Med.* **344**, 1038 (2001).
5. B. J. Druker *et al.*, *N. Engl. J. Med.* **344**, 1031 (2001).
6. C. L. Sawyers, *N. Engl. J. Med.* **340**, 1330 (1999).
7. S. Faderl *et al.*, *N. Engl. J. Med.* **341**, 164 (1999).
8. J. B. Konopka *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1810 (1985).
9. G. Q. Daley *et al.*, *Science* **247**, 824 (1990).
10. N. Heisterkamp *et al.*, *Nature* **344**, 251 (1990).
11. B. J. Druker *et al.*, *Nature Med.* **2**, 561 (1996).
12. Peripheral blood and/or bone marrow samples were obtained with appropriate informed consent from CML and Ph-positive ALL patients at the University of California Los Angeles who were enrolled in multicenter clinical trials of STI-571 (sponsored by Novartis Pharmaceuticals, Basel, Switzerland). All patients had >30% blasts in the marrow before treatment. Responding patients had reduction in the percentage of bone marrow blasts to <15% (partial) or <5% (complete), as described (*5*). Progressive disease was defined as an increase in percentage of blasts after an initial response, despite continued STI-571 treatment. Mononuclear cells were isolated by centrifugation through Ficoll-Hypaque, washed twice in phosphate-buffered saline, counted, and used immediately or cryopreserved.
13. S. S. Clark *et al.*, *Science* **235**, 85 (1987).
14. S. S. Clark *et al.*, *Science* **239**, 775 (1988).
15. M. E. Gorre, C. L. Sawyers, unpublished data.
16. J. ten Hoeve *et al.*, *Blood* **84**, 1731 (1994).
17. T. Oda *et al.*, *J. Biol. Chem.* **269**, 22925 (1994).
18. G. L. Nichols *et al.*, *Blood* **84**, 2912 (1994).
19. K. Senechal *et al.*, *J. Biol. Chem.* **271**, 23255 (1996).
20. Cells were lysed in 1% Triton X-100 buffer with protease and phosphatase inhibitors (*36*). Equal amounts of protein, as determined by the BioRad DC protein assay (Bio-Rad, Hercules, CA), were separated by SDS-PAGE, transferred to nitrocellulose, and immunoblotted with phosphotyrosine antibody (4G10, Upstate Biotechnologies, Lake Placid, NY), Abl antibody [pex5, (*36*)], β-actin antibody (Sigma, St. Louis, MO), or Crkl antiserum (Santa Cruz Biotechnology, Santa Cruz, CA). Immunoreactive bands were visualized by ECL (Amersham Pharmacia Biotech, Piscataway, NJ). Several exposures were obtained to ensure linear range of signal intensity. Optimal exposures were quantified by densitometry with ImageQuant software (Molecular Dynamics, Sunnyvale, CA).
21. M. E. Gorre, C. L. Sawyers, data not shown.
22. C. Gambacorti-Passerini *et al.*, *J. Natl. Cancer Inst.* **92**, 1641 (2000).
23. Purified cells were plated at 1×10^6/ml to 10×10^6/ml in RPMI-1640 + 10% human AB serum with varying concentrations of STI-571 for 24 hours. Proteins were extracted and subjected to immunoblot analysis.
24. E. Weisberg, J. D. Griffin, *Blood* **95**, 3498 (2000).
25. P. le Coutre *et al.*, *Blood* **95**, 1758 (2000).
26. F. X. Mahon *et al.*, *Blood* **96**, 1070 (2000).
27. Interphase and metaphase cells were prepared (*37*) and examined with Locus Specific Identifier *BCR-ABL* dual color translocation probe (Vysis, Downers Grove, IL).
28. gDNA was extracted from purified bone marrow or peripheral blood cells with the QiaAMP Blood Mini Kit (Qiagen, Valencia, CA). We subjected 10 ng of total gDNA to real-time PCR analysis with the iCycler iQ system (Bio-Rad, Hercules, CA). A 361-bp gDNA fragment including ABL exon 3 was amplified with two primers (5′-CAGAGTCAGAATCCTTCAG-3′ and 5′-TTTGTAAAAGGCTGCCCGGC-3′), which are specific for intron sequences 5′ and 3′ of ABL exon 3, respectively. A 472-bp gDNA fragment of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified with two primers (5′-TTCACCACCATGGAGAAGGC-3′ and 5′-CAGGAAATGAGCTTGACAAA-3′), which are specific for sequences in exon 5 and exon 8 of GAPDH, respectively. Fold increase in ABL copy number was determined by calculating the difference between threshhold cycle numbers of ABL and GAPDH for each sample (ΔCt). With control LB3 as reference sample, ΔCt from each sample was subtracted from ΔCt of control to determine Δ(ΔCt). Fold increase was calculated as $2^{-\Delta(\Delta Ct)}$.
29. RNA was extracted from purified peripheral blood or bone marrow cells with Trireagent-LS (Molecular Research Center, Cincinnati, OH). Two milligrams of total RNA was subjected to reverse transcriptase (RT)-PCR with Oligo dT primers. A 1327-bp cDNA fragment was amplified by PCR with a 5′BCR-specific primer (5′-GAAGCTTCTCCCTGGCATCCGT-3′) and a 3′ ABL-specific primer (5′-CCAGGCTCTCGGGTGCAGTCC-3′). In two patients, the BCR-ABL fragment could not be amplified; therefore, a 579-bp fragment was amplified with an alternative 5′ ABL-specific primer (5′-GCGCAACAAGCCCACTGTCTATGG-3′) and the same 3′ ABL primer. PCR products were cloned into the pCR2.1 TA cloning vector (Invitrogen, Carlsbad, CA). Both strands of a 579-bp region were sequenced with the 5′ ABL primer and M13 forward primer or M13 forward and reverse primer set for the 1327-bp and the 579-bp fragments, respectively, on an ABI prism 377 automated DNA sequencer (PE Applied Biosystems, Foster City, CA). Sequence analysis was performed with the ClustalW alignment algorithm (*38*).
30. gDNA was extracted from purified bone marrow or peripheral blood cells with the QiaAMP Blood Mini Kit (Qiagen). A 361-bp DNA fragment was amplified by PCR with two primers (5′-GCAGAGTCAGAATCCTTCAG-3′ and 5′-TTTGTAAAAGGCTGCCCGGC-3′), which are specific for intron sequences 5′ and 3′ of ABL exon 3, respectively. PCR products were cloned and sequenced.
31. T. Schindler *et al.*, *Science* **289**, 1938 (2000).
32. The structure of the kinase domain of Hck in complex with an ATP analog (AMP-PNP) was superimposed onto the model of the Ile315 Abl kinase domain.
33. Full-length p210 BCR-ABL was subcloned into the pSRaMSVtkNeo retrovirus vector (*39*). A fragment containing the C → T mutation at *ABL* nt 944 was made by PCR and swapped with the corresponding sequence in pSRaMSVtkNeo p210 BCR-ABL wild-type to create the pSRaMSVtkNeo p210 BCR-ABL T315I mutant. The resulting construct was confirmed by sequencing.
34. Transient transfection of 293T cells was performed with $CaCl_2$ (*39*). After a 24-hour transfection, cells were incubated with varying concentrations of STI-571 (Novartis) for 2 hours. Proteins were extracted and subjected to immunoblot analysis.
35. Y. Liu *et al.*, *Chem. Biol.* **6**, 671 (1999).
36. A. Goga *et al.*, *Cell* **82**, 981 (1995).
37. E. Abruzzese *et al.*, *Cancer Genet. Cytogenet.* **105**, 164 (1998).
38. J. D. Thompson *et al.*, *Nucleic. Acids Res.* **25**, 4876 (1997).
39. A. J. Muller *et al.*, *Mol. Cell. Biol.* **11**, 1785 (1991).
40. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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Reciprocal Regulation Between *TOC1* and *LHY/CCA1* Within the *Arabidopsis* Circadian Clock

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The interactive regulation between clock genes is central for oscillator function. Here, we show interactions between the *Arabidopsis* clock genes *LATE ELONGATED HYPOCOTYL* (*LHY*), *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*), and *TIMING OF CAB EXPRESSION 1* (*TOC1*). The MYB transcription factors LHY and CCA1 negatively regulate *TOC1* expression. We show that both proteins bind to a region in the *TOC1* promoter that is critical for its clock regulation. Conversely, TOC1 appears to participate in the positive regulation of *LHY* and *CCA1* expression. Our results indicate that these interactions form a loop critical for clock function in *Arabidopsis*.

Circadian clocks represent a widespread endogenous mechanism that allows organisms to time different processes appropriately throughout the day-night cycle. Among the activities controlled by the circadian clock are the regulation of transcription in cyanobacteria, the rhythmic movement of leaves in plants, and more complex activities such as

control of feeding behavior in flies and control of the sleep-wake cycle in humans. The basic molecular mechanisms underlying the generation of circadian rhythms have been significantly deciphered in *Synechococcus elongatus*, *Neurospora crassa*, *Drosophila melanogaster*, and the mouse (*1*). In these four model systems, the oscillator is based on transcriptional-translational negative feedback loops involving a group of clock genes. In *Arabidopsis*, three genes have been suggested as components of the oscillator: *LHY*, *CCA1*, and *TOC1* (*2*). *LHY* and *CCA1* encode highly conserved single-MYB transcription factors which, when expressed at high and constitutive levels, disrupt the normal functioning of the clock (*3, 4*). The third gene, *TOC1*, was initially identified as the *toc1-1* mutant in a screen for plants with altered period length of *cab2::luc* expression (*5*). *toc1-1* is a short-period mutant with altered clock function through the entire life cycle of the plant. Moreover, the effect is independent of light quantity, suggesting a role for TOC1 in the core of the oscillator (*6, 7*). The recent cloning of *TOC1* has allowed us a more detailed characterization of its molecular phenotypes (*7*). Here, we investigate potential interactions between *TOC1* and the MYB genes *LHY* and *CCA1*, because those interactions are likely to shape the *Arabidopsis* oscillator.

We investigated the *TOC1* expression patterns in *lhy* and *CCA1-OX* plants, which constitutively overexpress either *LHY* or *CCA1* from the strong promoter *CaMV 35S* (Fig. 1, A and B). *TOC1* mRNA oscillated with high amplitude in the wild-type parental lines in constant light (LL). The *TOC1* mRNA level was constant in both *lhy* and *CCA1-OX* plants under the same light conditions, as expected for plants in which the oscillator function is mostly disrupted. The transcript level in these mutants was similar to or lower than the trough of wild-type expression. Therefore, high and constant expression levels of either *LHY* or *CCA1* result in low and constant levels of *TOC1* transcript in LL. LHY and CCA1 appear to be negative regulators of *TOC1*. The fact that *TOC1* transcript oscillates 12 hours out of phase with both the *LHY* and *CCA1* transcripts in wild-type plants further supports this idea. The CCA1 protein has been found to cycle with little lag with respect to its transcript (*4*), indicating that maximum CCA1 level coincides with minimum *TOC1* mRNA level, and vice versa.

elf3-1 plants become arrhythmic due to an arrest of its circadian clock in continuous light (*8, 9*). We investigated the *TOC1* expression profiles in the *elf3-1* mutant under LL conditions (Fig. 1C). Contrary to what we observed for the wild type, the transcript level remained elevated and unchanging in the mutant in free-running conditions. The arrhythmic phenotypes observed in *elf3-1* and in *lhy* and *CCA1-OX* plants in LL are associated with different levels of *TOC1* transcript. Both the *LHY* and *CCA1* mRNA levels were found to be reduced in *elf3-1* plants in LL (*3, 10*), consistent with the elevated *TOC1* transcript levels in this mutant arising from a significant reduction in the amount of the negative regulators LHY and CCA1.

If LHY and CCA1 act directly to negatively regulate *TOC1* expression, they might bind to a functionally important region of the *TOC1* promoter. Accordingly, we sought to identify regulatory regions of the *TOC1* promoter by generating a series of deletions fused to the luciferase gene and analyzing expression of the reporter in *Arabidopsis* plants transformed with these constructs (Fig. 2, A and B). Progressive deletions from −2340 (base pairs from the starting ATG) to −834 had only a slight effect on the mean expression level of the reporter and did not affect its oscillation (Fig. 2A) (*10*). On the other hand, deletion from −834 to −620 abolished rhythmicity. The region from −834 to −620 was sufficient to confer rhythmic expression to the reporter (Fig. 2B). To assess the possibility that LHY and/or CCA1 utilize this region to regulate *TOC1* expression, the ability of these two proteins to bind to the −834 to −620 fragment was tested by performing electrophoretic mobility shift assay (Fig. 2, C and D) (*11*). Addition of extracts from *Escherichia coli* expressing either GST-CCA1 or GST-LHY to a probe corresponding to the −834 to −620 region of the *TOC1* promoter produced DNA species with retarded mobility (*11*); bacterial extracts harboring the empty glutathione *S*-transferase (GST) vector did not produce comparable bands. Competition experiments show that GST-CCA1 and GST-LHY specifically bind to a DNA fragment corresponding to this part of the promoter (Fig. 2, C and D). To delimit further this region, three more deletions were analyzed for the expression of the reporter (Fig. 2B). Removal of the region between −734 and −687 greatly reduced rhythmicity. Therefore, the −734 to −687 region contains *cis* elements important for clock regulation of *TOC1*. Recently, a novel sequence element (evening element or EE) has been identified in the promoter region of 31 cycling genes peaking at the end of the subjective day (*12*). The importance of that element for circadian regulation in vivo was shown for one of those genes, *CCR2*. We found one EE in the −734 to −687 fragment of the *TOC1* promoter. Mutation of the EE in the context of the −834 to −620 fragment caused strong reduction in rhythmicity (Fig. 2B). This indicates that the EE is important for clock regulation of *TOC1*. Given the high similarity between the EE (AAAATATCT) and the motif that CCA1 binds (AAAAATCT) (*13*), we investigated whether the EE was the actual target used by LHY and CCA1 to regulate *TOC1*. Figure 2, C and D, show the specific interaction of both transcription factors with the EE. Taken together, these results strongly suggest that LHY and CCA1 negatively regulate *TOC1* expression by binding to the EE in its promoter region.

To investigate whether the regulation is reciprocal, we analyzed *LHY* and *CCA1* expression patterns in the *TOC1* alleles *toc1-1* and *toc1-2* (Fig. 3) (*5, 7*). Both the *LHY* and *CCA1* messages cycled with a shorter period length in the two mutants than in wild-type plants, consistent with the short period observed for the expression of

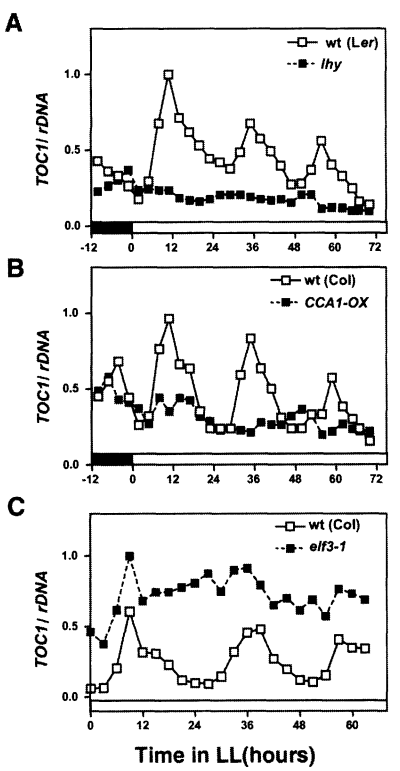

Fig. 1. *TOC1* expression is strongly affected in *lhy*, *CCA1-OX*, and *elf3-1* plants. *lhy* plants and the parental wild type (*Ler*) (**A**), *CCA1-OX* (line o34) and *elf3-1* plants, and the parental wild type (Col-0) [(**B**) and (**C**), respectively] were germinated and entrained to cycles of 12 hours light, 12 hours dark [LD (12, 12)] for 7 days and then released into constant light (LL). *TOC1* RNA levels were analyzed by Northern blot. The dark and white bars at the bottom of each graph represent lights off and on, respectively. Representative data are shown.

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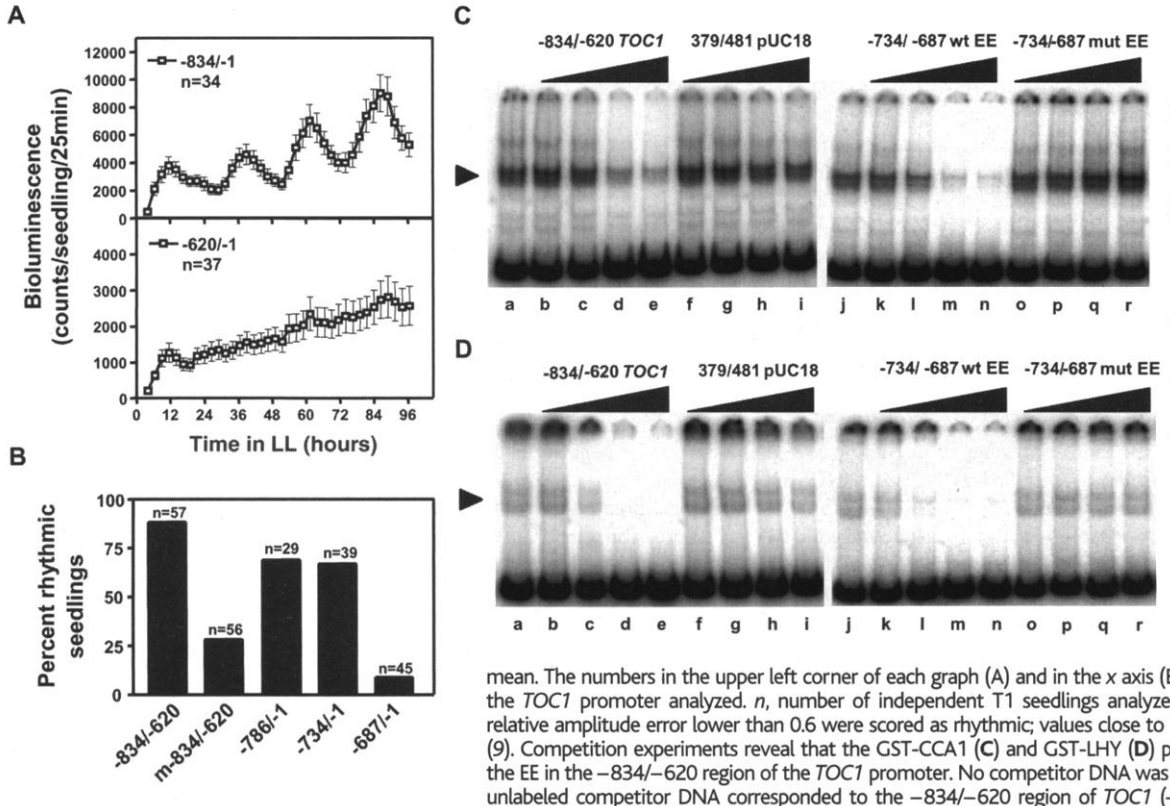

Fig. 2. Identification of a region in the *TOC1* promoter that is essential for its circadian oscillation and is a target of LHY and CCA1. (**A** and **B**) Analysis of the *TOC1* promoter. The different deleted versions of the promoter were fused to the firefly luciferase+ gene and the resulting constructs were introduced into *Arabidopsis* by *Agrobacterium*-mediated DNA transfer (*21*). Primary transgenic plants were selected under LD (12, 12) cycles for 8 days, transferred to media without selection, and imaged and analyzed as described (*7*). In the mutant promoter [m– 834/–620 in (B)], the last six nucleotides of the EE were changed to CTGCAG. Error bars in (A) represent the standard error of the mean. The numbers in the upper left corner of each graph (A) and in the *x* axis (B) indicate the fragment of the *TOC1* promoter analyzed. *n*, number of independent T1 seedlings analyzed. In (B), seedlings with a relative amplitude error lower than 0.6 were scored as rhythmic; values close to 1.0 indicate weak rhythms (*9*). Competition experiments reveal that the GST-CCA1 (**C**) and GST-LHY (**D**) proteins specifically bind to the EE in the –834/–620 region of the *TOC1* promoter. No competitor DNA was added in lanes a and j. The unlabeled competitor DNA corresponded to the –834/–620 region of *TOC1* (–834/–620 *TOC1*; lanes b through e) or the 379 to 481 region of the pUC18 (379/481 pUC18; lanes f through i). In lanes k through n, the unlabeled competitor DNA corresponded to the –734/–687 region of *TOC1* (–734/–687 wt EE), and the competitor DNA in lanes o through r was the same as in lanes k through n but with the last six nucleotides of the EE changed to CTGCAG (–734/–687 mut EE). Black triangles represent increasing amount of competitor, which was present at 1× (lanes b, f, k, and o), 10× (lanes c, g, l, and p), 100× (lanes d, h, m, and q), and 200× (lanes e, i, n, and r) molar excess of the labeled probe. The arrowhead indicates the protein-DNA complexes.

other genes (*5, 7*). The peak levels of *LHY* and *CCA1* expression were reduced by more than 50% in the *toc1-2* background, and, consequently, the amplitude of oscillation for both transcripts was reduced. The different effects of the *toc1-1* and *toc1-2* mutations on the expression levels of *LHY* and *CCA1* likely reflect the different nature of these alleles. *toc1-1* is semidominant and appears to retain some of the TOC1 function, whereas *toc1-2* is recessive and presumably represents a loss-of-function allele (*5, 7*). The low expression level of both genes might be the cause of the short-period phenotype of *toc1-2*. It could also explain the same phenotype in the *CCA1* loss-of-function allele *cca1-1* (*14*). The semidominant nature of *toc1-1* suggests distinct biochemical abnormalities, such as altered protein-protein interaction, for its defect in period length. The reduction of the transcript peak level for both *LHY* and *CCA1* in *toc1-2* strongly suggests a positive effect of TOC1 on the expression of both genes. Several features of TOC1 protein support this idea: It is localized in nuclear speckles that may represent transcriptional complexes (*7*); it has an acidic-rich region at the COOH-terminal end of the protein, which is commonly found among several

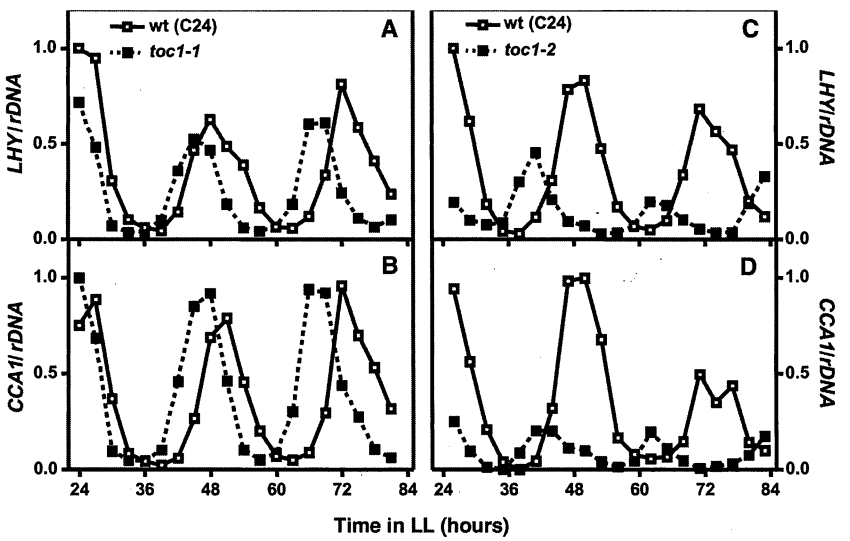

Fig. 3. *LHY* and *CCA1* expression is reduced in *toc1-2* but not in *toc1-1*. *toc1-1* (**A** and **B**), *toc1-2* (**C** and **D**), and wild-type plants (C24) were grown in LD (12, 12) cycles for 7 days and then released into LL. *LHY* and *CCA1* RNA levels were analyzed by Northern blot. Representative data are shown.

types of eukaryotic transcriptional activators (*7, 15*); and it bears homology to the CONSTANS protein of *Arabidopsis*, which has been implicated in transcriptional regulation (*16*). The ability of two *Arabidopsis* response regulators to activate transcription has been described (*17*). The fact that both *LHY* and *CCA1* transcripts continue to oscillate in *toc1-2* indicates that TOC1 is not the only factor controlling expression of both genes. It has also been suggested that the basic helix-loop-helix transcription fac-

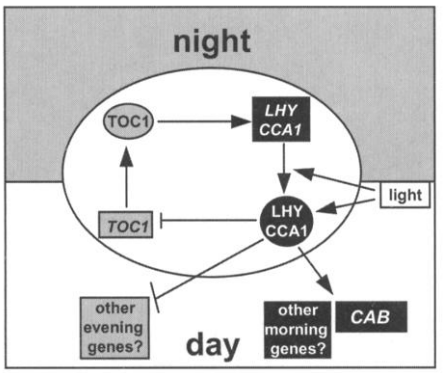

Fig. 4. Model showing the proposed interactions between *TOC1* and the MYB genes *LHY* and *CCA1* within the *Arabidopsis* circadian clock. Light activates *LHY* and *CCA1* expression at dawn (*10*). Posttranslational regulation by light has been suggested for CCA1 (*4*). Hence, light reinforces the function of LHY and CCA1 at dawn. CCA1, and probably LHY, activate *CAB* expression (*4, 13, 14*); they may activate other genes that cycle with a phase similar to *CAB*. LHY and CCA1 simultaneously repress *TOC1* and potentially other evening genes. Progressive reduction of *LHY* and *CCA1* expression levels during the day (*3, 4*) allows *TOC1* transcript levels to rise and reach maximum levels toward the end of the day, when *LHY* and *CCA1* expression levels are lowest (*3, 4, 7*). TOC1, either directly or indirectly, appears to augment the expression of *LHY* and *CCA1*, which reach maximum levels at dawn, starting the cycle again.

tor PIF3 acts as a positive element in *LHY* and *CCA1* expression, at least in etiolated seedlings in response to light pulses (*18*). The possibility remains that TOC1 function is, to some degree, redundant with some of the *TOC1*-like genes found in *Arabidopsis*, one of which oscillates with a phase very similar to *TOC1* (*19*). The low level of TOC1 function expected in *CCA1-OX* could explain, in part, the low level of endogenous *CCA1* and *LHY* messages found in these plants (*4*). However, the intermediate levels of endogenous *LHY* transcript found in the *lhy* allele lhy^{TN104} (*3*) suggest that positive elements other than TOC1 may still be active; an alternative, but not mutually exclusive explanation would be that the effects of *lhy* and lhy^{TN104} on *TOC1* expression are different.

As mentioned above, high levels of *TOC1* expression correlate with low levels of *LHY* and *CCA1* transcripts in the *elf3-1* mutant in LL (Fig. 1C) (*3, 10*). However, the fact that the level of both transcripts remains low suggests that TOC1 fails to regulate positively their expression in the absence of the ELF3 function. Otherwise, the steady-state levels of *TOC1*, *LHY*, and *CCA1* transcripts would reach intermediate values due to the simultaneous action of positive (TOC1) and negative (LHY and CCA1) factors.

The results presented here show interactions among genes involved in the *Arabidopsis* oscillator. The data reveal different roles for LHY and CCA1 versus TOC1. The MYB factors act as negative elements that repress *TOC1* expression, and, conversely, TOC1 appears to be a positive element for *LHY* and *CCA1* expression (Fig. 4). LHY and CCA1 appear to have a dual role in the *Arabidopsis* clock. It has been suggested that CCA1 acts as a positive regulator of *CAB* gene expression (*4, 13, 14*). Given the overall similarity between *CCA1* and *LHY*, it is likely that the LHY protein is also a positive regulator of *CAB* genes. Furthermore, both of these proteins also act as negative regulators of one evening-phased gene, *TOC1*. We predict that LHY, CCA1, or both may also negatively regulate other genes having EE in their promoter regions, which include, for example, *GIGANTEA* and *CCR2* (*12*). Similar to a mechanism proposed for *Drosophila* (*20*), we suggest a model in which these MYB factors simultaneously regulate genes that are phased to different times of the day. The analysis of TOC1 biochemical properties and the identification of TOC1-interacting proteins will help us to close the loop. Our results suggest that the interactive regulation between these genes define the basic framework for the clock mechanism in *Arabidopsis*.

References and Notes

1. J. C. Dunlap, *Cell* **96**, 271 (1999).
2. D. E. Somers, *Plant Physiol.* **121**, 9 (1999).
3. R. Schaffer *et al.*, *Cell* **93**, 1219 (1998).
4. Z. Y. Wang, E. M. Tobin, *Cell* **93**, 1207 (1998).
5. A. J. Millar, I. A. Carré, C. A. Strayer, N.-H. Chua, S. A. Kay, *Science* **267**, 1161 (1995).
6. D. E. Somers, A. A. R. Webb, M. Pearson, S. Kay, *Development* **125**, 485 (1998).
7. C. A. Strayer *et al.*, *Science* **289**, 768 (2000).
8. K. A. Hicks *et al.*, *Science* **274**, 790 (1996).
9. H. G. McWatters, R. M. Bastow, A. Hall, A. J. Millar, *Nature* **408**, 716 (2000).
10. D. Alabadí, M. J. Yanovsky, P. Más, S. A. Kay, unpublished data.
11. Supplementary material is available at *Science* Online at www.sciencemag.org/cgi/content/full/293/5531/880/DC1.
12. S. L. Harmer *et al.*, *Science* **290**, 2110 (2000).
13. Z. Y. Wang *et al.*, *Plant Cell* **9**, 491 (1997).
14. R. M. Green, E. M. Tobin, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 4176 (1999).
15. K. B. Singh, *Plant Physiol.* **118**, 1111 (1998).
16. A. Samach *et al.*, *Science* **288**, 1613 (2000).
17. H. Sakai, T. Aoyama, A. Oka, *Plant J.* **24**, 703 (2000).
18. J. F. Martínez-García, E. Huq, P. H. Quail, *Science* **288**, 859 (2000).
19. A. Matsushika, S. Makino, M. Kojima, T. Mizuno, *Plant Cell Physiol.* **41**, 1002 (2000).
20. N. R. Glossop, L. C. Lyons, P. E. Hardin, *Science* **286**, 766 (1999).
21. S. J. Clough, A. F. Bent, *Plant J.* **16**, 735 (1998).
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