THE ROYAL SOCIETY

doi 10.1098/rstb.2001.0964

RNA-binding proteins and circadian rhythms in *Arabidopsis thaliana*

Dorothee Staiger

Institute for Plant Sciences, ETH Zurich, CH-8092 Zurich, Switzerland (dorothee.staiger@ipw.biol.ethz.ch)

An *Arabidopsis* transcript preferentially expressed at the end of the daily light period codes for the RNA-binding protein *At*GRP7. A reverse genetic approach in *Arabidopsis thaliana* has revealed its role in the generation of circadian rhythmicity: *At*GRP7 is part of a negative feedback loop through which it influences the oscillations of its own transcript. Biochemical and genetic experiments indicate a mechanism for this autoregulatory circuit: *Atgrp*7 gene transcription is rhythmically activated by the circadian clock during the day. The *At*GPR7 protein accumulates with a certain delay and represses further accumulation of its transcript, presumably at the post-transcriptional level. In this respect, the *At*GRP7 feedback loop differs from known circadian oscillators in the fruitfly *Drosophila* and mammals based on oscillating clock proteins that repress transcription of their own genes with a 24 h rhythm. It is proposed that the *At*GRP7 feedback loop may act within an output pathway from the *Arabidopsis* clock.

Keywords: *Arabidopsis*; circadian rhythm; RNA binding protein; germin-like protein

1. INTRODUCTION

Physiological processes that are controlled by the circadian clock in *Arabidopsis thaliana* include leaf movement, hypocotyl elongation, stomata movement and photoperiodic flower induction (Engelmann *et al.* 1992; Hicks *et al.* 1996; Somers *et al.* 1998; Dowson-Day & Millar 1999; for a review, see Millar 1999; Somers 1999). Numerous genes have been shown to be rhythmically expressed, including those encoding proteins involved in photosynthetic light absorption and carbon metabolism, nitrate reductase and two catalase genes that are phased opposite during the day (Millar & Kay 1991; Pilgrim *et al.* 1993; Liu *et al.* 1996; Zhong & McClung 1996; for a review, see Staiger & Heintzen 1999). In most cases these transcripts presumably represent hands of the clock rather than regulatory molecules of clock output pathways. The phytochrome B transcript also undergoes circadian oscillations and thus is a target of clock regulation, but additionally serves as a light input receptor to the clock (Kozma Bognar *et al.* 1999).

Recently several molecular components playing a crucial role in the generation of circadian rhythms in *Arabidopsis* have been identified. Among them are two myb-like transcription factors CCA1 and LHY (Schaffer *et al.* 1998; Wang & Tobin 1998), ELF3 (Hicks *et al.* 1996), GIGANTEA (Fowler *et al.* 1999; Park *et al.* 1999), TOC1 (Millar *et al.* 1995; Strayer *et al.* 2000), ZEITLUPE or TOC7 (Millar *et al.* 1995; Somers *et al.* 2000) and FKF1 (Nelson *et al.* 2000). For recent advances in understanding the function of these proteins in the *Arabidopsis* circadian system see McWatters *et al.* (2001).

2. A SEARCH FOR OSCILLATING TRANSCRIPTS

As a way to understand the molecular basis of circadian rhythms, a systematic search for rhythmically expressed genes was undertaken. A potential function of oscillating gene products subsequently can be investigated by manipulating the expression in transgenic plants. Towards this end, a subtractive hybridization of a timed cDNA library with time-of-day-specific probes was performed in the long-day plant *Sinapis alba*, white mustard (Heintzen *et al.* 1994*a*,*b*). This screen identified two transcript groups that undergo circadian oscillations.

3. A CIRCADIANLY REGULATED TRANSCRIPT ENCODING A GERMIN-LIKE CELL WALL PROTEIN

A transcript undergoing steady-state oscillations with peak abundance about 12 h after onset of illumination, roughly antiphase to the *lhc* oscillations, codes for a protein with homology to germin and accordingly was designated *Sa*GLP (*Sinapis alba* germin-like protein) (Heintzen *et al.* 1994*b*). Germin was named for its prevalent expression during germination in wheat (Lane *et al.* 1992). It is almost identical to barley oxalate oxidase and has the same enzymatic activity. Thus, it has been suggested to contribute to cell wall remodelling, by virtue of the enzymatic breakdown of oxalate with concomitant release of hydrogen peroxide (Lane *et al.* 1993). Germin-type proteins that are expressed upon various stress treatments and in response to fungal infection in other monocotyledons have been implicated as part of plant defence mechanisms (Dumas *et al.* 1995; Hurkman & Tanaka 1996).

*Sa*GLP was among the first germin-like proteins identified in dicotyledons (Michalowski & Bohnert 1992; Heintzen *et al.* 1994*b*; Ono *et al.* 1996). Immunogold labelling showed that the protein is located in the cell wall, in accordance with the presence of a signal peptide (Heintzen *et al.* 1994*b*). At present it is not known whether germin-like proteins in dicotyledons also contribute to

remodelling of the cell wall structure during growth, as does germin itself. In particular, no oxalate oxidase activity has yet been demonstrated for the *Arabidopsis* homologue of *Sa*GLP, *At*GER3 (Membre *et al.* 1997, 2000; Staiger *et al.* 1999).

As there is no evidence that *Sa*GLP or *At*GER3 regulate other rhythmic processes, they probably represent a mere output for the clock.

4. CLOCK-CONTROLLED TRANSCRIPTS ENCODING RNA-BINDING PROTEINS

The *Sagrp*1 and *Sagrp*2 cDNAs code for two closely related proteins with an N-terminal 90 amino-acid domain known as the RNA recognition motif of RNA-binding proteins and a C-terminal region enriched in glycines. Their transcript levels show a robust circadian rhythm and reach their maximum 8–12 h after onset of illumination (Heintzen *et al.* 1994*a*). In mustard plants raised under constant illumination, rhythmic temperature shifts can act as alternative *zeitgebers* to light–dark cycles to entrain the *Sagrp* transcript oscillations (Heintzen *et al.* 1994*a*,*b*).

The *Sagrp* transcripts are mainly expressed in meristematic and growing tissue including the shoot apical meristem, procambial strands, the cambial meristem and young leaves. Immunogold labelling demonstrated that the *Sa*GRP proteins are mostly found in the nucleus (Heintzen *et al.* 1994*a*).

That some RNA-binding proteins show circadian rhythmicity is not unexpected, bearing in mind the importance of post-transcriptional processes in shaping the primarily transcriptional clock feedback loops in other organisms (So & Rosbash 1997; Stanewsky *et al.* 1997; Edery 1999). Moreover, rhythmically expressed RNA binding proteins have been implicated in the control of clock output in *Drosophila* and *Gonyaulax* (Mittag *et al.* 1994; Newby & Jackson 1996; McNeil *et al.* 1998). A potential function of the glycine-rich RNA binding proteins in the generation of circadian rhythmicity was therefore tested by manipulating the expression in transgenic plants.

5. THE RNA-BINDING PROTEIN *At*GRP7 AS PART OF A CLOCK-REGULATED NEGATIVE FEEDBACK LOOP

Arabidopsis thaliana was chosen for subsequent experiments, because, in contrast to *Sinapis alba*, it can be transformed with high efficiency. Two homologues of the *Sagrp* transcripts were isolated from an evening-specific *Arabidopsis thaliana* cDNA library (Heintzen *et al.* 1997; D. Staiger, unpublished data). They correspond to *Atgrp*7 (*Arabidopsis thaliana* glycine-rich protein), also termed *ccr2* (cold and circadian regulated), and to *Atgrp*8/*ccr*1, respectively (Van Nocker & Vierstra 1993; Carpenter *et al.* 1994). As observed in mustard, levels of the *Arabidopsis* transcripts oscillate according to a circadian rhythm, peaking in the early evening. The steady-state concentration of the *At*GRP7 protein also undergoes circadian oscillations that are delayed by about 4 h relative to the transcript oscillations: when *At*GRP7 has reached a high level the transcript starts to decline and does not rise again until the protein has reached its minimum (Heintzen *et al.* 1997). This resembles the situation found for the *Drosophila* PER and TIM clock proteins whose rhythms lag behind those of their cognate RNAs by about 6 h. The analogy provoked the speculation that the *At*GRP7 protein similarly may regulate oscillations of its own transcript by negative feedback.

Indeed, constitutive overexpression of the protein under control of the strong cauliflower mosaic virus (CaMV) promoter in transgenic *Arabidopsis* plants markedly depressed the oscillations of the endogenous transcript (Heintzen *et al.* 1997). To confirm that the downregulation of the endogenous *Atgrp*7 transcript is due to the enhanced *At*GRP7 protein level, a mutated cDNA was overexpressed: a frameshift was introduced upstream of the RNA recognition motif which leads to six translation termination amino acids after the start so that no functional protein is made. In the transgenic plants a fairly high level of the mutated transcript is expressed but the protein level is not elevated and the oscillations of the endogenous *Atgrp*7 transcript are not influenced (D. Staiger, unpublished data). Taken together, these data reveal that the downregulation of the endogenous transcript depends on an elevated level of the functional RNA-binding protein. Both *Atgrp*7 transcript and *At*GRP7 protein are therefore linked in a negative autoregulatory circuit.

6. THE BIOCHEMICAL MECHANISM OF THE OSCILLATORY *At*GRP7 FEEDBACK LOOP

Negative autoregulatory circuits serving as oscillators in *Drosophila*, *Neurospora* or mammals comprise transcription factors that rhythmically activate clock gene transcription and clock proteins that, after a lag phase, block these activator molecules to shut off transcription of their own genes (Hardin *et al.* 1990; Aronson *et al.* 1994; for a review, see Dunlap 1998, 1999). No precedent has been described for a negative feedback loop centred around an RNA-binding protein. It is therefore of interest to unravel the molecular underpinnings of the *At*GRP7 feedback loop.

*Atgrp*7 oscillations are generated through rhythmic transcriptional activation, as the *Atgrp*7 promoter confers circadian oscillations onto a linked β-glucuronidase reporter gene in transgenic *Arabidopsis* plants with highest levels in the evening (Staiger & Apel 1999).

A deletion analysis uncovered a 264 bp fragment upstream of the *Atgrp*7 transcription start site necessary for high amplitude RNA cycling (Staiger & Apel 1999). Within the −264 bp region, two separate regulatory elements were identified. A minimal clock-responsive element mediating a basal circadian oscillation with peak abundance at the end of the daily light phase is located downstream of position −112. This basal oscillation is augmented by sequences located between positions −178 and −264. Inclusion of the −178/264 bp region enhances the amplitude from about threefold to about 60-fold.

Presumably several transcription factors act in concert *in vivo* to accomplish changes in *Atgrp*7 gene transcription in the course of the day. Two circadianly regulated MYB-like transcription factors, LHY and CCA1, have been implicated in the control of *Atgrp*7 promoter activity

(Schaffer *et al*. 1998; Wang & Tobin 1998). In transgenic *Arabidopsis* plants overexpressing CCA1 the *Atgrp*7 transcript shows irregular fluctuations with a reduced amplitude in continuous light, indicating that *Atgrp*7 is under negative control of CCA1. *Atgrp*7 oscillations are also altered in *cca*1 knockout plants (Green & Tobin 1999). CCA1 has been shown previously to bind to a 36 bp domain of the *lhc*1*1 (*cab*2) promoter sufficient for clock regulation (Carré & Kay 1995; Wang & Tobin 1998). Sequence motifs displaying a 7 out of 8 bp identity to the CCA1 binding site are also present in the *Atgrp*7 promoter TATA proximal region, and thus it will be interesting to know whether CCA1 or related factors would interact efficiently with the element. Recently, the candidate clock protein TOC1 also has been found to influence rhythmic *Atgrp*7 promoter activity (Strayer *et al*. 2000).

As *Atgrp*7 oscillations persist for several days after shifting plants to continuous darkness, the transcript represents a useful tool to probe oscillator function in the dark (Carpenter *et al*. 1994; Strayer *et al*. 2000; D. Staiger, unpublished data). Furthermore, the *Atgrp*7 transcript with its evening peak (similar to *catalase*3), the *Lhc* transcripts preferentially expressed during the early light phase (similar to *catalase*2) and the *Atger*3 transcript peaking in the middle of the night together serve as clock output markers to cover most of the circadian cycle (figure 1).

*At*GRP7 may accomplish negative feedback regulation of its own transcript in several ways that are not mutually exclusive. Based on the presence of an RNA recognition motif, an interaction of *At*GRP7 with its own RNA may be inferred. So, following rhythmic transcriptional activation, *At*GRP7 may restrict transcript accumulation by influencing, for example, transcript stability. Alternatively, *At*GRP7 could inhibit transcription of its own gene, either indirectly by interfering with a transcriptional activator—as shown for PER feedback on its transcription (Darlington *et al*. 1998)—or by direct interaction with the promoter, as several RNA-binding proteins are able to bind to DNA and act as transcriptional regulators (Deschamps *et al*. 1992; Michelotti *et al*. 1996).

To determine whether sequence elements mediating *At*GRP7-dependent mRNA cycling would reside within the region conferring rhythmic *Atgrp*7 transcription, the transgenic lines constitutively overexpressing *At*GRP7 under control of the CaMV promoter were crossed with the transgenic plants harbouring the transcriptional *Atgrp*7-β-glucuronidase fusion. In the offspring, an elevated *At*GRP7 protein level does not affect the abundance of the gus mRNA, in contrast to the endogenous *Atgrp*7 transcript. The *Atgrp*7 promoter by itself is therefore not sufficient to mediate the negative feedback, pointing to a post-transcriptional mode of autoregulation (Staiger & Apel 1999).

Negative autoregulation of *Atgrp*7 transcript abundance by *At*GRP7 may occur through a direct interaction, as *At*GRP7 contains a conserved RNA recognition motif. Indeed, bacterially expressed *At*GRP7-glutathione-*S*-transferase fusion protein has been shown to interact specifically with parts of its own transcript *in vitro* (L. Zecca, L. Eckstein and D. Staiger, unpublished data).

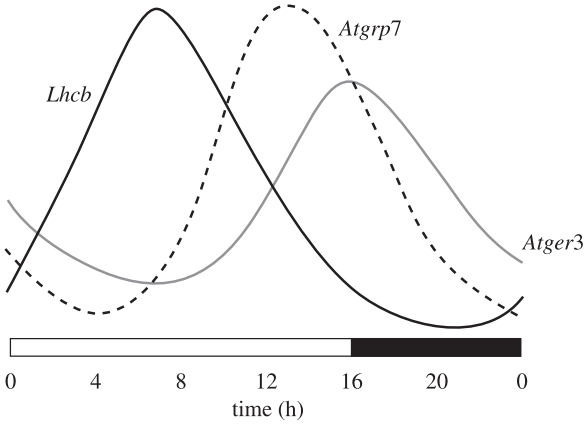

Figure 1. Schematic representation of the phases of *Lhcb*, *Atgrp*7 and *Atger*3 transcript oscillations (based on experimental data shown in Heintzen *et al*. 1997).

7. HOW DOES *At*GRP7 COMPARE WITH OTHER CLOCK-REGULATED RNA-BINDING PROTEINS?

The *Drosophila lark* gene, mutations of which cause adult flies to prematurely emerge from their pupal cases but do not affect locomotor activity rhythms, encodes a putative RNA-binding protein with two conserved RNA recognition motifs but otherwise is not related to *At*GRP7 (Newby & Jackson 1996; McNeil *et al*. 1998). The LARK protein oscillates in abundance, peaking several hours prior to the peak of eclosion, consistent with the assumption that LARK periodically represses eclosion. Steady-state levels of LARK mRNA do not oscillate. Changes in protein therefore may be due to rhythmic translation or altered protein stability. Whether LARK itself is involved in its rhythmic translation and thus would also undergo autoregulation is not known.

Circadian rhythmicity of certain proteins is controlled at the translational level in *Gonyaulax* (Morse *et al*. 1989). An RNA-binding activity specifically interacting with the luciferin binding protein (LBP) 3′ untranslated region has been identified in protein extracts (Mittag *et al*. 1994). Its abundance oscillates with nadir levels at the phase when LBP mRNA is translated, arguing that the RNA-binding activity might act as a translational repressor generating the circadian rhythm in LBP protein concentration and thus may be part of the clock output. Further mechanistic details await purification of the protein and cloning of the cognate gene.

An obvious function for a circadianly regulated feedback loop would be to control other rhythmic phenomena. The presence of an RNA recognition motif suggests that *At*GRP7 might interact with downstream transcripts and in this way may confer rhythmicity to them. Therefore selected oscillating transcripts representing different circadian phases were compared in wild-type plants and the *At*GRP7 overexpressing lines. No significant differences in steady-state concentrations were observed for the *lhc* transcripts that peak around noon, for the germin-like protein *Atger*3 that peaks in the late evening, and for *catalase*2 and *catalase*3. However, circadian oscillations of the *Atgrp*8 transcript encoding a related glycine-rich RNA-binding protein that cycles in phase with *Atgrp*7 in wild-type plants were almost fully

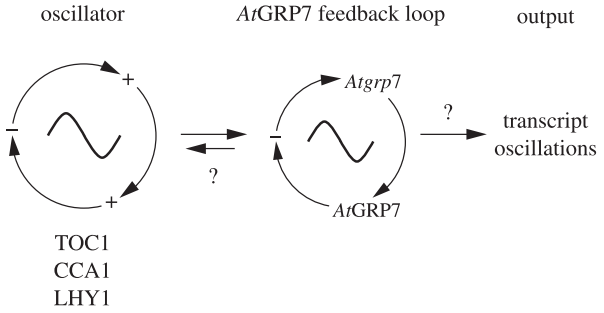

Figure 2. Model of *At*GRP7 negative feedback loop operating downstream of another oscillator in *Arabidopsis* cells. TOC1, CCA1 and LHY have been shown to influence *Atgrp*7 expression in *trans*. For details, see text.

suppressed in the transgenic plants (Heintzen *et al.* 1997). *At*GRP7 therefore is able to regulate transcripts apart from its own. Clearly, most clock-regulated transcripts are not affected and the *At*GRP7 feedback loop presumably is not a main oscillator in *Arabidopsis* cells. Rather, it could operate subordinated to another oscillator (figure 2). It would acquire rhythmicity from this oscillator, conserve it by feedback regulation and transduce it into timed output (Heintzen *et al.* 1997). Such a hierarchical organization of the circadian system comprising 'master' and 'slave' oscillators has indeed been proposed (Pittendrigh 1960, 1981). What might be the significance of a subordinated feedback loop? The output pathways communicating temporal information from the central oscillator into physiological processes may encompass multiple oscillating gene products that in turn regulate the rhythmicity of downstream transcripts. These may have different half-lives, and thus oscillations of downstream transcripts may have a smaller amplitude. Intermediate negative feedback would accomplish timely decay of cycling gene products so that the following signal input from the central oscillator can manifest itself. The suboscillators would not normally generate time information themselves but would counteract a decrease in amplitude and therefore maintain rhythmicity. Dependent on the half-lives of the components they could also direct peak and trough levels to various times of the day so that a single output would suffice to phase rhythms differently.

The identification of further target transcripts of *At*GRP7 regulation may help to define the role this feedback loop plays within the output from the *Arabidopsis* clock.

8. CONCLUSIONS

A systematic search for oscillating transcripts in the long-day plants *Sinapis alba* and *Arabidopsis thaliana* has been performed with the rationale that components contributing to clock function themselves should show circadian regulation (Heintzen *et al.* 1994*a*,*b*). A candidate regulatory RNA-binding protein, *At*GRP7, has been demonstrated subsequently by reverse genetics to be causally involved in rhythm genesis (Heintzen *et al.* 1997).

As characteristic features of proteins involved in rhythm genesis have been defined in other organisms, testing molecules with inferred clock-associated functions in transgenic plants by reverse genetics has yielded valuable insight into the circadian system of *Arabidopsis* (Schaffer *et al.* 1998; Wang & Tobin 1998). This approach will continue to be useful as it will identify proteins with redundant functions in rhythm genesis and sequence information of the entire *Arabidopsis* genome is available.

REFERENCES

Aronson, B. D., Johnson, K. A., Loros, J. J. & Dunlap, J. C. 1994 Negative feedback defining a circadian clock: autoregulation of the clock gene *frequency*. *Science* **263**, 1578–1584.

Carpenter, C. D., Kreps, J. A. & Simon, A. E. 1994 Genes encoding glycine-rich *Arabidopsis thaliana* proteins with RNA-binding motifs are influenced by cold treatment and an endogenous circadian rhythm. *Plant Physiol.* **104**, 1015–1025.

Carré, I. A. & Kay, S. A. 1995 Multiple DNA–protein complexes at a circadian-regulated promoter element. *Plant Cell* **7**, 2039–2051.

Darlington, T. K., Wager-Smith, K., Ceriani, M. F., Staknis, D., Gekakis, N., Steeves, T. D. L., Weitz, C. J., Takahashi, J. S. & Kay, S. A. 1998 Closing the circadian loop: CLOCK-induced transcription of its own inhibitors *per* and *tim*. *Science* **280**, 1599–1603.

Deschamps, S., Viel, A., Garrigos, M., Denis, H. & LeMaire, M. 1992 mRNP4, a major mRNA-binding protein for *Xenopus* oocytes is identical to transcription factor FRG Y2. *J. Biol. Chem.* **267**, 13 799–13 802.

Dowson-Day, M. J. & Millar, A. J. 1999 Circadian dysfunction causes aberrant hypocotyl elongation patterns in *Arabidopsis*. *Plant J.* **17**, 63–71.

Dumas, B., Freyssinet, G. & Pallet, K. E. 1995 Tissue-specific expression of germin-like oxalate oxidase during development and fungal infection of barley seedlings. *Plant Physiol.* **107**, 1091–1096.

Dunlap, J. 1998 Circadian rhythms. An end in the beginning. *Science* **280**, 1548–1549.

Dunlap, J. C. 1999 Molecular bases for circadian clocks. *Cell* **96**, 271–290.

Edery, I. 1999 Role of posttranscriptional regulation in circadian clocks: lessons from *Drosophila*. *Chronobiol. Int.* **16**, 377–414.

Engelmann, W., Simon, K. & Phen, C. J. 1992 Leaf movement rhythms in *Arabidopsis thaliana*. *Z. Naturforsch.* **47c**, 925–928.

Fowler, S., Lee, K., Onouchi, H., Samach, A., Richardson, K., Morris, B., Coupland, G. & Putterill, J. 1999 *GIGANTEA*: a circadian clock-controlled gene that regulates photoperiodic flowering in *Arabidopsis* and encodes a protein with several possible membrane-spanning domains. *EMBO J.* **18**, 4679–4688.

Green, R. M. & Tobin. E. 1999 Loss of the circadian clock-associated protein 1 in *Arabidopsis* results in altered clock-regulated gene expression. *Proc. Natl Acad. Sci. USA* **96**, 4176–4179.

Hardin, P. E., Hall, J. C. & Rosbash, M. 1990 Feedback of the *Drosophila period* gene product on circadian cycling of its messenger RNA levels. *Nature* **343**, 536–540.

Heintzen, C., Melzer, S., Fischer, R., Kappeler, S., Apel, K. & Staiger, D. 1994*a* A light- and temperature-entrained circadian clock controls expression of transcripts encoding nuclear proteins with homology to RNA-binding proteins in meristematic tissue. *Plant J.* **5**, 799–813.

Heintzen, C., Fischer, R., Melzer, S., Kappeler, K., Apel, K. & Staiger, D. 1994*b* Circadian oscillations of a transcript encoding a germin-like protein that is associated with cell walls in young leaves of the long-day plant *Sinapis alba* L. *Plant Physiol.* **106**, 905–915.

Heintzen, C., Nater, M., Apel, K. & Staiger, D. 1997 *At*GRP7, a nuclear RNA-binding protein as a component of a circadian-regulated negative feedback loop in *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. USA* **94**, 8515–8520.

Hicks, K. A., Millar, A. J., Carré, I. A., Somers, D. E., Straume, M., Meeks-Wagner, D. R. & Kay, S. A. 1996 Conditional circadian dysfunction of the *Arabidopsis early-flowering3* mutant. *Science* **274**, 790–792.

Hurkman, W. J. & Tanaka, C. K. 1996 Germin gene expression is induced in wheat leaves by powdery mildew infection. *Plant Physiol.* **111**, 735–739.

Kozma Bognar, L., Hall, A., Adam, E., Thain, S. C., Nagy, F. & Millar, A. J. 1999 The circadian clock controls the expression pattern of the circadian input photoreceptor, phytochrome B. *Proc. Natl Acad. Sci. USA* **96**, 14 652–14 657.

Lane, B. G., Cuming, A. C., Fregeau, J., Carpita, N. C., Hurkman, W. J., Bernier, F., Dratewka-Kos, E. & Kennedy, T. D. 1992 Germin isoforms are discrete temporal markers of wheat development. *Eur. J. Biochem.* **209**, 961–969.

Lane, B. G., Dunwell, J. M., Ray, J. A., Schmitt, M. R. & Cuming, A. C. 1993 Germin, a protein marker of early plant development, is an oxalate oxidase. *J. Biol. Chem.* **268**, 12 239–12 242.

Liu, Z., Taub, C. C. & McClung, C. R. 1996 Identification of an *Arabidopsis thaliana* ribulose-1,5-bisphosphate carboxylase/oxygenase activase (*RCA*) minimal promoter regulated by light and the circadian clock. *Plant Physiol.* **112**, 43–51.

McNeil, G. P., Zhang, X., Genova, G. & Jackson, F. R. 1998 A molecular rhythm mediating circadian clock output in *Drosophila*. *Neuron* **20**, 297–303.

McWatters, H. G., Roden, L., Staiger, D. 2001 Picking out parallels: plant circadian clocks in context. *Phil. Trans. R. Soc. Lond.* B **356**, 1735–1743. (DOI 10.1098/rstb.2001.0936.)

Membre, N., Berna, A., Neutelings, G., David, A., David, H., Staiger, D., Vasquez, J. S., Raynal, M., Delseney, M. & Bernier, F. 1997 cDNA sequence, genomic organisation and differential expression of three *Arabidopsis* genes for germin/oxalate oxidase-like proteins. *Plant Mol. Biol.* **35**, 459–469.

Membre, N., Bernier, F., Staiger, D. & Berna, A. 2000 *Arabidopsis thaliana* germin-like proteins: common and specific features point to a variety of functions. *Planta* **211**, 354–354.

Michalowski, C. B. & Bohnert, H. J. 1992 Nucleotide sequence of a root-specific transcript encoding a germin-like protein from the halophyte *Mesembryanthmum crystallinum*. *Plant Physiol.* **100**, 537–538.

Michelotti, E. F., Michelotti, G. A., Aronsohn, A. I. & Levens, D. 1996 Heterogeneous nuclear ribonucleoprotein K is a transcription factor. *Mol. Cell. Biol.* **16**, 2350–2360.

Millar, A. J. & Kay, S. A. 1991 Circadian control of *cab* gene transcription and mRNA accumulation in *Arabidopsis*. *Plant Cell* **3**, 541–550.

Millar, A. J. 1999 Biological clocks in *Arabidopsis thaliana*. *New Phytol.* **141**, 175–197.

Millar, A. J., Carré, I. A., Strayer, C. A., Chua, N.-H. & Kay, S. A. 1995 Circadian clock mutants in *Arabidopsis* identified by luciferase imaging. *Science* **267**, 1161–1163.

Mittag, M., Lee, D.-H. & Hastings, J. W. 1994 Circadian expression of the luciferin-binding protein correlates with the binding of a protein to the 3′ untranslated region of its mRNA. *Proc. Natl Acad. Sci. USA* **91**, 5257–5261.

Morse, D., Milos, P. M., Roux, E. & Hastings, J. W. 1989 Circadian regulation of bioluminescence in *Gonyaulax* involves translational control. *Proc. Natl Acad. Sci. USA* **86**, 172–176.

Nelson, D. C., Lasswell, J., Rogg, L. E., Mindy, A., Cohen, M. A. & Bartel, B. 2000 *FKF1*, a clock-controlled gene that regulates the transition to flowering in *Arabidopsis*. *Cell* **101**, 331–340.

Newby, L. M. & Jackson, F. R. 1996 Regulation of a specific circadian clock output pathway by Lark, a putative RNA binding protein with repressor activity. *J. Neurobiol.* **31**, 117–128.

Ono, M., Sage-One, K., Inoue, M., Kamada, H. & Harada, H. 1996 Transient increase in the level of mRNA for a germin-like protein in leaves of the short-day plant *Pharbitis nil* during the photoperiodic induction of flowering. *Plant Cell Physiol.* **37**, 855–861.

Park, D. H., Somers, D. E., Kim, Y. S., Choy, Y. H., Lim, H. K., Soh, M. S., Kim, H. J., Kay, S. A. & Nam, H. G. 1999 Control of circadian rhythms and photoperiodic flowering by the *Arabidopsis GIGANTEA* gene. *Science* **285**, 1579–1582.

Pilgrim, M. L., Caspar, T., Quail, P. H. & McClung, C. R. 1993 Circadian and light-regulated expression of nitrate reductase in *Arabidopsis*. *Plant Mol. Biol.* **23**, 349–364,

Pittendrigh, C. S. 1960 Circadian rhythms and the circadian organization of living systems. *Cold Spring Harb. Symp. Quant. Biol.* **25**, 159–184.

Pittendrigh, C. S. 1981 Circadian rhythms: general perspective. In *Handbook of behavioral neurobiology: biological rhythms* (ed. J. Aschoff), pp. 57–80. New York: Plenum Press.

Schaffer, R., Ramsay, N., Samach, A., Corden, S., Putterill, J., Carré, I. A. & Coupland, G. 1998 The *late elongated hypocotyl* mutation of *Arabidopsis* disrupts circadian rhythms and the photoperiodic control of flowering. *Cell* **93**, 1219–1229.

So, W. V. & Rosbash, M. 1997 Post-transcriptional regulation contributes to *Drosophila* clock gene mRNA cycling. *EMBO J.* **16**, 7146–7155.

Somers, D. E., Webb, A. A. R., Pearson, M. & Kay, S. A. 1998 The short-period mutant, *toc*1–1, alters circadian clock regulation of multiple outputs throughout development in *Arabidopsis thaliana*. *Development* **125**, 485–494.

Somers, D. E. 1999 The physiology and molecular bases of the plant circadian clock. *Plant Physiol.* **121**, 9–19.

Somers, D. E., Schultz, T. F., Milnamow, M. & Kay, S. A. 2000 ZEITLUPE encodes a novel clock-associated PAS protein from *Arabidopsis*. *Cell* **101,** 319–329.

Staiger, D. & Apel, K. 1999 Circadian clock-regulated expression of an RNA-binding protein in *Arabidopsis*: characterisation of a minimal promoter element. *Mol. Gen. Genet.* **261**, 811–819.

Staiger, D. & Heintzen, C. 1999 The circadian system of *Arabidopsis thaliana*—forward and reverse genetic approaches. *Chronobiol. Int.* **16**, 1–16.

Staiger, D., Apel, K. & Trepp, G. 1999 The *Atger*3 promoter confers circadian clock-regulated transcription with peak expression at the beginning of the night. *Plant Mol. Biol.* **40**, 873–882.

Stanewsky, R., Jamison, C. F., Plautz, J. D., Kay, S. A. & Hall, J. C. 1997 Multiple circadian-regulated elements contribute to cycling *period* gene expression in *Drosophila*. *EMBO J.* **16**, 5006–5018.

Strayer, C., Oyama, T., Schultz, T. F., Raman, R., Somers, D. E., Mas, P., Panda, S., Kreps, J. A. & Kay, S. A. 2000 Cloning of the *Arabidopsis* clock gene *TOC1*, an autoregulatory response regulator homolog. *Science* **289**, 768–771.

Van Nocker, S. & Vierstra, R. 1993 Two cDNAs from *Arabidopsis thaliana* encode putative RNA binding proteins containing glycine-rich domains. *Plant Mol. Biol.* **21**, 695–699.

Wang, Z.-Y. & Tobin, E. M. 1998 Constitutive expression of the *CIRCADIAN CLOCK ASSOCIATED* 1 (*CCA*1) gene disrupts circadian rhythms and suppresses its own expression. *Cell* **93**, 1207–1217.

Zhong, H. H. & McClung, C. R. 1996 The circadian clock gates expression of two *Arabidopsis* catalase genes to distinct and opposite circadian phases. *Mol. Gen. Genet.* **251**, 196–203.