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PHYSIOLOGY

Bacteria can anticipate the seasons: Photoperiodism in cyanobacteria

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Photoperiodic time measurement is the ability of plants and animals to measure differences in day versus night length (photoperiod) and use that information to anticipate critical seasonal transformations, such as annual temperature cycles. This timekeeping phenomenon triggers adaptive responses in higher organisms, such as gonadal stimulation, flowering, and hibernation. Unexpectedly, we observed this capability in cyanobacteria—unicellular prokaryotes with generation times as short as 5 to 6 hours. Cyanobacteria exposed to short, winter-like days developed enhanced resistance to cold mediated by desaturation of membrane lipids and differential programs of gene transcription, including stress response pathways. As in eukaryotes, this photoperiodic timekeeping required an intact circadian clockwork and developed over multiple cycles of photoperiod. Therefore, photoperiodic timekeeping evolved in much simpler organisms than previously appreciated and enabled genetic responses to stresses that recur seasonally.

Many branches of the eukaryotic tree of life have evolved the ability to not only react to but also to anticipate the changing seasons and proactively adjust their behavior and physiology by measuring seasonally changing photoperiods (PPs) with a timekeeping mechanism that often requires a circadian clock (*1*–*3*). However, prokaryotic organisms with very short generation times have barely been considered to harbor such a prolonged temporal program (*4*, *5*). Cyanobacteria with doubling times as short as 5 to 6 hours have nevertheless become productive model organisms for the study of circadian (daily) rhythms, with a core mechanism comprising only three proteins that can be reconstituted in vitro (KaiABC) (*6*–*9*). Cyanobacteria are found in a wide range of latitudes and, as such, are exposed to pronounced annual changes in light and temperature in their natural environment (fig. S1) (*10*). We therefore tested whether cyanobacteria are capable of timekeeping on a temporal scale that is even longer than daily, namely photoperiodic time measurement (PPTM) in promoting adaptive cold-resistance responses to seasonal changes.

Short days promote cold resistance in cyanobacteria in a clock-dependent manner

Cells of the unicellular cyanobacterium *Synechococcus elongatus* PCC 7942 were grown at 30°C on plates containing solid BG-11 medium and exposed to eight continuous 24-hour cycles of either short days (LD8:16, 8 hours of light followed by 16 hours of darkness), equinox (LD12:12), or long days (LD16:8). After this exposure, the bacteria were resuspended into liquid medium at the middle of their day phase and exposed to ice-cold temperatures for various durations, after which their survival was assessed (Fig. 1A and fig. S2). Prior exposure to short days led to approximately two to three times higher survival of wild-type (WT) cyanobacteria compared with those that were exposed to equinox or long days (Fig. 1B). This differential survival was not observed in arhythmic cells in which the circadian clock genes *kaiA*, *kaiB*, and *kaiC* had been deleted (Δ*kaiABC*; Fig. 1B and fig. S3, A and B) or in mutants that lacked other components of the core clockwork or its output pathways (Δ*kaiA*, Δ*rpaA*, Δ*cikA*, Δ*sasA*; fig. S3, A and C).

To assess whether cold resistance was influenced by the time of day of the assay, we repeated the experiment in the morning, midday, and evening of the LD cycles (Fig. 1C for WT and fig. S4A for Δ*kaiABC*). The survival differential was highest when the assay was performed in the early day, but for all sampled times, WT bacteria survive cold best after exposure to short PPs as compared with long or equinox PPs; arhythmic Δ*kaiABC* cells showed no effect of PP or time of day on survival (Fig. 1C and fig. S4B). We standardized our protocol to midday assays because that daily time is when the cells' circadian phase should be most comparable across different PPs (*11*). In general, PPTM phenomena show differential responses along a continuum of PPs, and we therefore assayed along a gradient of PPs and found that long (LD16:8 and LD14:10) PPs led to poor survival, whereas LD12:12 and shorter PPs progressively led to increased cell survival (Fig. 1D). Survival of the Δ*kaiABC* cells was unaffected by PP. In neither the WT nor the Δ*kaiABC* strain was cell length or volume different when grown in LD8:16 versus LD16:8, indicating that the distributions of cell cycle phase and energy resources were equivalent (fig. S5).

An intriguing aspect of photoperiodic responses in general is that they are often history dependent—i.e., an organism's interpretation of a PP is dependent on the prior exposure conditions (*2*). Consequently, a common characteristic of bona fide PPTM is that multiple 24-hour cycles of inductive PPs are often required to elicit a complete response. This has been termed a "photoperiodic counter" (*12*–*16*). Given their short generation time, we wondered if prokaryotic cyanobacteria could remember and integrate a PP signal that extended over multiple cycles and generations. A single cycle of different PPs did not produce differential survival (Fig. 2A); at least four cycles seemed to be necessary to achieve the full magnitude of the photoperiodic response (Fig. 2B). The development of differential survival among PPs appeared to be mainly caused by a progressive gain of resistance in short days, rather than by a further loss of cold resistance in long days. Therefore, the photoperiodic response required cumulative exposure to multiple cycles and a PP-counting memory. The short-day gain of cold resistance achieved by four PP cycles spanned at least ~3.2 generations for WT cells (Fig. 2C). Again, cells without a functional circadian clock expressed a PP-independent increase in cold resistance as the number of LD cycles increased (fig. S6, A and B). We tested how long the cold resistance lasted after the photoperiodic cues were removed. After our standard pretreatment of eight PP cycles, we transferred the cells to either constant light (LL) or constant darkness (DD) and measured thereafter at 24-hour intervals. Transfer to LL led to a general decrease in survival of the short PP–exposed cells (Fig. 2D and fig. S6F), whereas the opposite was true for release to DD (fig. S6, D and E). In both cases, after 24 hours in constant conditions, short PP–exposed cells tended to survive slightly longer than cells from the other PPs, but these differences were not statistically significant (fig. S6, F and G).

To be certain that our results were dependent on the duration of the PP and not simply a differential exposure to light—which is relevant for a photosynthetic organism—we tested whether changes in light intensity could influence this photoperiodic response. Cells were exposed to fluence rates of 20 μE m^{-2} s^{-1}, 40 μE m^{-2} s^{-1} (our standard light intensity), and 80 μE m^{-2} s^{-1} (Fig. 2E). Recovery of the Δ*kaiABC* cells from cold was a simple function of fluence rate: Higher light intensities led to lower survival than low light intensities (Fig. 2F and fig. S7 for the full survival curves at each light intensity). By contrast, whereas overall survival was also correlated with fluence rate for WT cells, in this case WT cells exposed to short days survived better than those exposed to long days at all intensities (Fig. 2F and fig. S7B),

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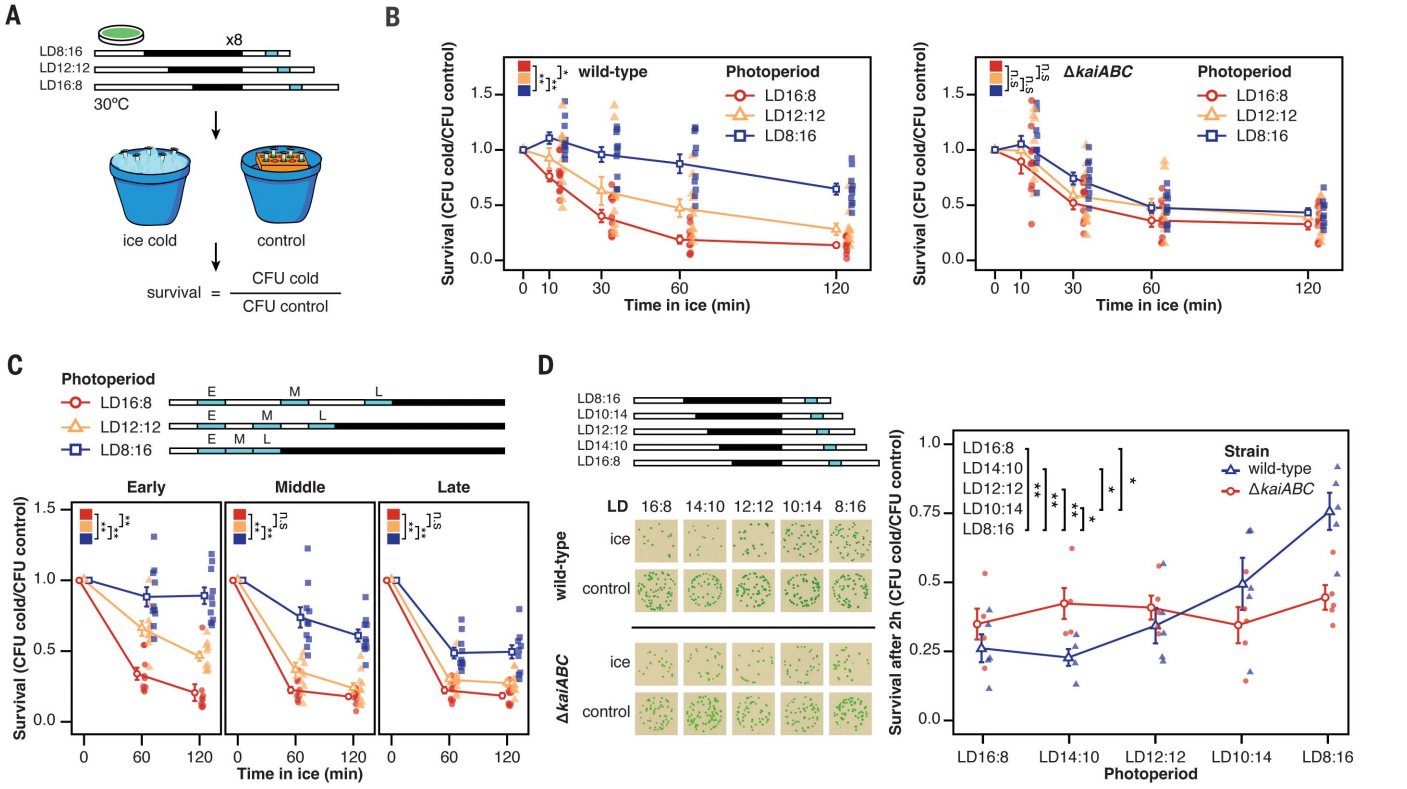

Fig. 1. Cyanobacteria exposed to short days survive cold treatment better than those exposed to long days. (**A**) Experimental protocol used in the cold survival assay. See fig. S2, A to E, for details. Light blue bars indicate the phase (midday) in which the cold survival assay was performed. CFU, colony-forming units. (**B**) Cold survival curve for WT (n = 10) and Δ*kaiABC* cells (n = 9 for LD16:8; n = 10 for LD12:12 except for 10 min in which n = 9; n = 10 for LD8:16 except for 120 min in which n = 9). The results of statistical comparisons between PPs (determined by pairwise t tests with Bonferroni correction) are expressed as asterisks (*P < 0.05; **P < 0.01), with the comparison indicated with brackets around squares that share a color scheme with the PP data points. n.s., not significant. (**C**) Similar to (B), but showing results for cells tested at early day (2 hours after lights on, n = 10 for LD8:16 and LD12:12, n = 9 for LD16:8), midday [same as (B), n = 10 except for LD16:8 at 60 min, in which n = 9], and late day (2 hours before lights off, n = 10 except for LD8:16 and LD16:8 at 120 min, in which n = 9). E, early day; L, late day; M, midday. (**D**) Original data on the left show CFU for WT (top) and Δ*kaiABC* (bottom) cells exposed to a range of PPs as indicated, tested after eight PP cycles at their respective middays. (Right) Quantification of the survival after 2 hours of exposure to ice (n = 5, 4, 5, 5, and 5 for WT and n = 4, 4, 5, 5, and 5 for Δ*kaiABC*). Significance levels refer to comparisons between WT values (there was no significant difference among Δ*kaiABC* values). For all graphs, error bars show the SEM, and open dots indicate the average.

indicating that WT cells appear to integrate temporal information with light intensity. Taken together, these data indicate that the PP-dependent response to cold exposure develops over multiple LD cycles that span multiple generations of cyanobacteria. Our data also suggest that cells in their natural environment integrate multiple factors (e.g., light intensity) along with PP to program the optimal adaptive response.

Short days lead to lipid membrane desaturation

Prior exposure of cyanobacteria to mild low temperatures increases subsequent survival to colder temperatures and lipid desaturation (which increases membrane fluidity) as an important adaptation to cold (*17*, *18*). In their natural environments, cyanobacteria experience a shortening of the day length in concert with a lowering of temperatures as winter approaches. To confirm that PP and prior temperature exposure both contribute to cold survival, we exposed cells to eight cycles of either short, equinox, or long days at constant 30°C, followed by another cycle of each PP at 20°C, after which the cells were tested for their cold survival (Fig. 3, A and B). Although cells exposed to 20°C increased their survival in comparison with cells that did not receive the 20°C treatment at all PPs, the cells exposed to short days survived better than those exposed to the other PPs (Fig. 3, B and C). In WT cells, the increase in survival caused by short days without any 20°C exposure was comparable to the effect of 20°C exposure to the long-day cells (Fig. 3C). In clockless Δ*kaiABC* cells, 20°C exposure enhanced cold survival, but different PPs did not (fig. S8, A to C).

We tested whether short days in the absence of a temperature change could mimic the molecular adaptive response of cold-induced lipid desaturation. WT and Δ*kaiABC* cells were exposed to the eight cycles of each PP plus 24 hours at 20°C and extracted for lipidomic analyses (Fig. 3D). WT cells exposed to short days had increased the abundance of desaturated lipid species in their cell membranes [especially species in the monogalactosyldiacylglycerol (MGDG) and phosphatidylglycerol (PG) classes; Fig. 3, E and F]. This increase was of the same magnitude as that observed for cells exposed to equinox plus 24 hours at 20°C. In WT cells, exposure to either short-day or 20°C conditions increased abundance of the desaturated species MGDG and PG, whereas short days increased the desaturated digalactosyldiacylglycerol (DGDG) even more than did 20°C exposure (Fig. 3F). In Δ*kaiABC* cells, however, only 20°C exposure led to changes in lipid saturation; different PPs in the absence of temperature changes had no effect in cells without the circadian clock genes (Fig. 3, G and H). Apparently, short days elicit a *kaiABC*-dependent desaturation of membrane lipids in WT cells that could function to anticipate and adapt to seasonally cooling temperatures.

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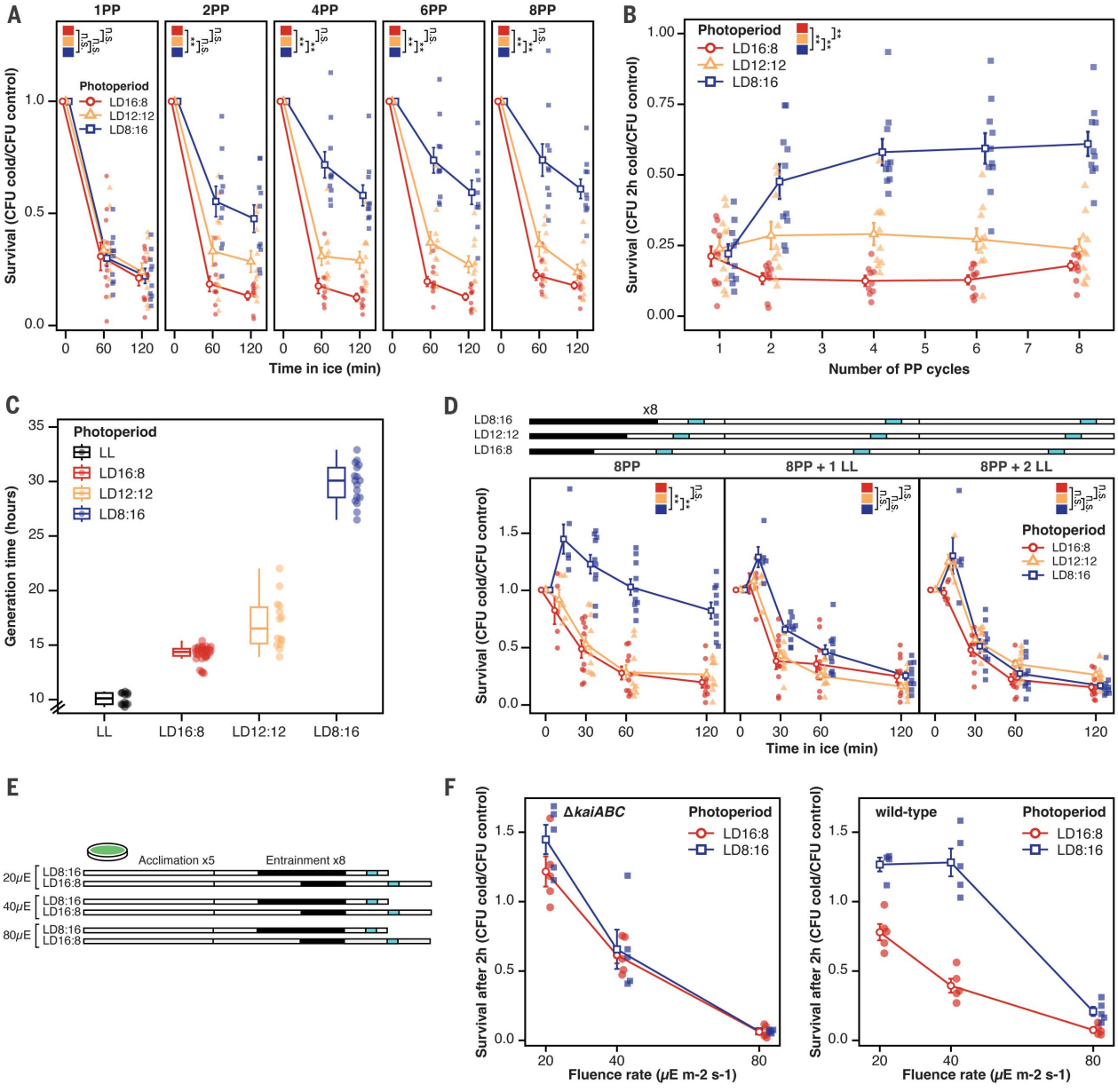

Fig. 2. Cyanobacterial photoperiodic response takes multiple cycles to develop and spans multiple generations. (**A**) Survival curves similar to those in Fig. 1B but for cold survival assays performed after different numbers of PP cycles (n = 10 for all except LD16:8 at two PPs and four PPs, in which n = 9). (**B**) Survival after 2 hours of exposure to cold throughout the different number of PP cycles. (**C**) Generation time of cyanobacterial cells for each of the PPs analyzed, as well as LL (from left to right, n = 12, 32, 14, and 17). (**D**) Diagram and survival curves for the LL assay (n = 10, except for at 10 min, in which n = 5). Blue bars indicate the times at which the survival assay was performed (midday, 24 hours after, and 48 hours after). (**E**) Diagram showing the protocol for the different light intensities and PPs tested. Blue bars indicate the time at which the survival assay was performed. (**F**) Survival after 2 hours of exposure to cold at different fluence rates (n = 5 for all except for WT LD8:16 at 20 μE, for which n = 4). Full survival curves, including other time points, are in fig. S7B. For all survival curves, open symbols denote the average, and error bars denote the SEM. Significance was determined through pairwise t tests with Bonferroni correction.

PP determines global programs of gene expression

Do PPs differentially affect only lipid desaturation, or are other responses involved? To test whether distinctive gene expression patterns underlie photoperiodic adaptation, we performed RNA sequencing (RNA-seq) on WT and Δ*kaiABC* cells that had been exposed to either one PP cycle [sufficient to synchronize circadian oscillations (*19*) but not to evoke the photoperiodic response; Fig. 2, A and B], four PP cycles (long enough for photoperiodic responses to develop; Fig. 2, A and B), or eight PP cycles (our usual testing condition) (Fig. 4A). Global programs of gene expression were differential in cells exposed to short versus long days (708 genes were differentially expressed in WT at 8PP, which constitutes ~25.5% of the genome), and more than half of the differentially expressed genes achieved that status by four PPs (Venn diagrams in Fig. 4B, volcano plots in Fig. 4C, and principal components analysis and distance matrix in fig. S13). In a comparison between the expression of WT short day–exposed cells and that of WT long day–exposed cells in each of the PP cyclic conditions, there was no obvious correlation between the $\log_2$(fold change)

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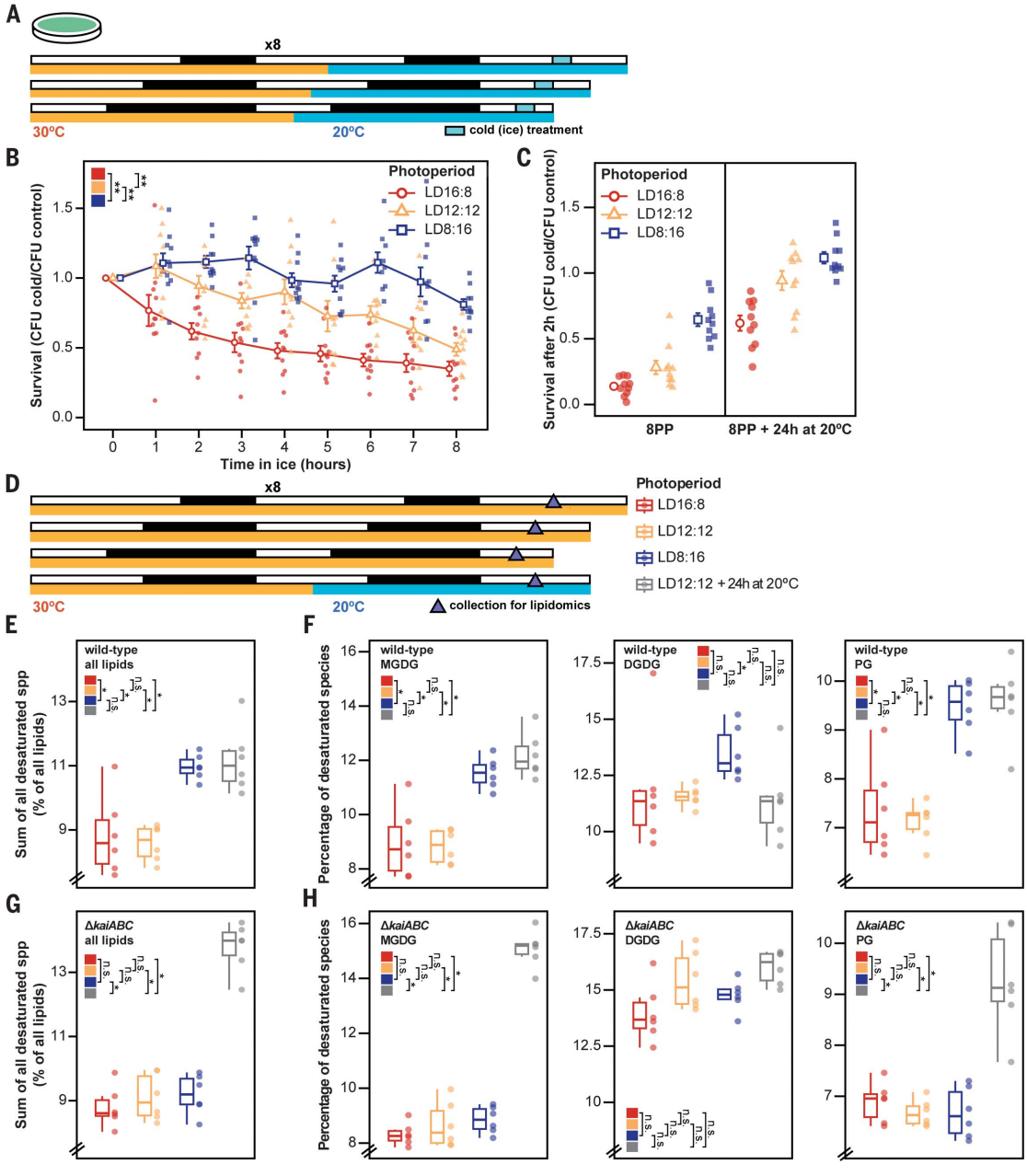

Fig. 3. Exposure to short days leads to an increase in membrane desaturation akin to that resulting from exposure to low temperatures. (**A**) Diagram of the experiment testing the interaction of PP and intermediate cold temperatures on survival. (**B**) Survival curve for WT cells treated as in (A) (n = 10 except for LD8:16 at 6 hours, in which n = 9). (**C**) Comparison between the survival of WT cells after 2 hours of exposure to cold, with and without prior treatment for 24 hours at 20°C (n = 10). (**D**) Diagram of the lipidomics collection and prior treatment. (**E**) Sum of all desaturated lipid species for WT [n = 6, data in fig. S9, panels B (DGDG 30:2, 32:2, and 34:2), C (MGDG 32:2 and 34:2), and E (PG 32:2 and 34:2)]. (**F**) Percentage of desaturated species within each lipid class in WT [n = 6, data in fig. S10, panels A (DGDG 30:2, 32:2, and 34:2), B (MGDG 32:2 and 34:2), and C (PG 32:2 and 34:2)]. (**G**) Sum of all desaturated lipid species for Δ*kaiABC* [n = 6, data in fig. S11, panels B (DGDG 30:2, 32:2, and 34:2), C (MGDG 32:2 and 34:2), and E (PG 32:2 and 34:2)]. (**H**) Percentage of desaturated species within each lipid class in Δ*kaiABC* [n = 6, data contained in fig. S12, panels A (DGDG 30:2, 32:2, and 34:2), B (MGDG 32:2 and 34:2), and C (PG, 32:2 and 34:2)]. For (B), open symbols denote the mean and error bars denote the SEM. Significance was determined through pairwise t tests with Bonferroni correction.

at one versus eight PP cycles [adjusted coefficient of determination (R^2_{adj}) ≤ 0.01; Fig. 4B, left], but the differences in expression at four PPs correlated well with those at eight PPs (R^2_{adj} = 0.48, P < 0.001; Fig. 4B, right). Some of the changes in gene expression at one PP may reflect immediate differences in entrainment, whereas four to eight PP cycles are necessary to fully develop the PP-specific genetic programs. Gene expression of Δ*kaiABC* cells in short versus long days was significantly different, but the number of differentially affected genes was almost two times greater in WT cells (708 in WT, 384 in Δ*kaiABC* at eight PPs, and the overlap between these groups is only 283 genes; fig. S14). Therefore, many more genes are differentially expressed between short days and long days in WT than in Δ*kaiABC* cells.

Some genes of particular interest are highlighted in Fig. 4D, especially genes related to transcription, fatty acid metabolism, cell cycle, and stress responses. In prokaryotes, sigma factors regulate many genes synergistically to respond to environmental conditions (*20*), and we observed that the sigma factors *rpoD2* and *sigC* were differentially expressed; long days stimulated *rpoD2* expression, whereas short days promoted expression of *sigC* (Fig. 4D). Both *rpoD2* and *sigC* are direct targets of the circadian transcriptional factor *rpaA* (*21*) and could relay circadian and photoperiodic information to the rest of the genome. We tested whether sigma factors were directly related to the cold-sensitivity response by performing the photoperiodic assay in knockout cells of the sigma factors, *rpoD2*, *rpoD3*, *rpoD4*, and *sigC* (fig. S3D). Although there were no significant differences in the cold-sensitivity response of Δ*sigC*, Δ*rpoD3*, or Δ*rpoD4*, all cells of the Δ*rpoD2* strain regardless of photoperiodic treatment became cold sensitive, suggesting that *rpoD2* might have a role in PP output such that deleting *rpoD2* severs the photoperiodic response. Many of the genes of interest from our RNA-seq analyses have been

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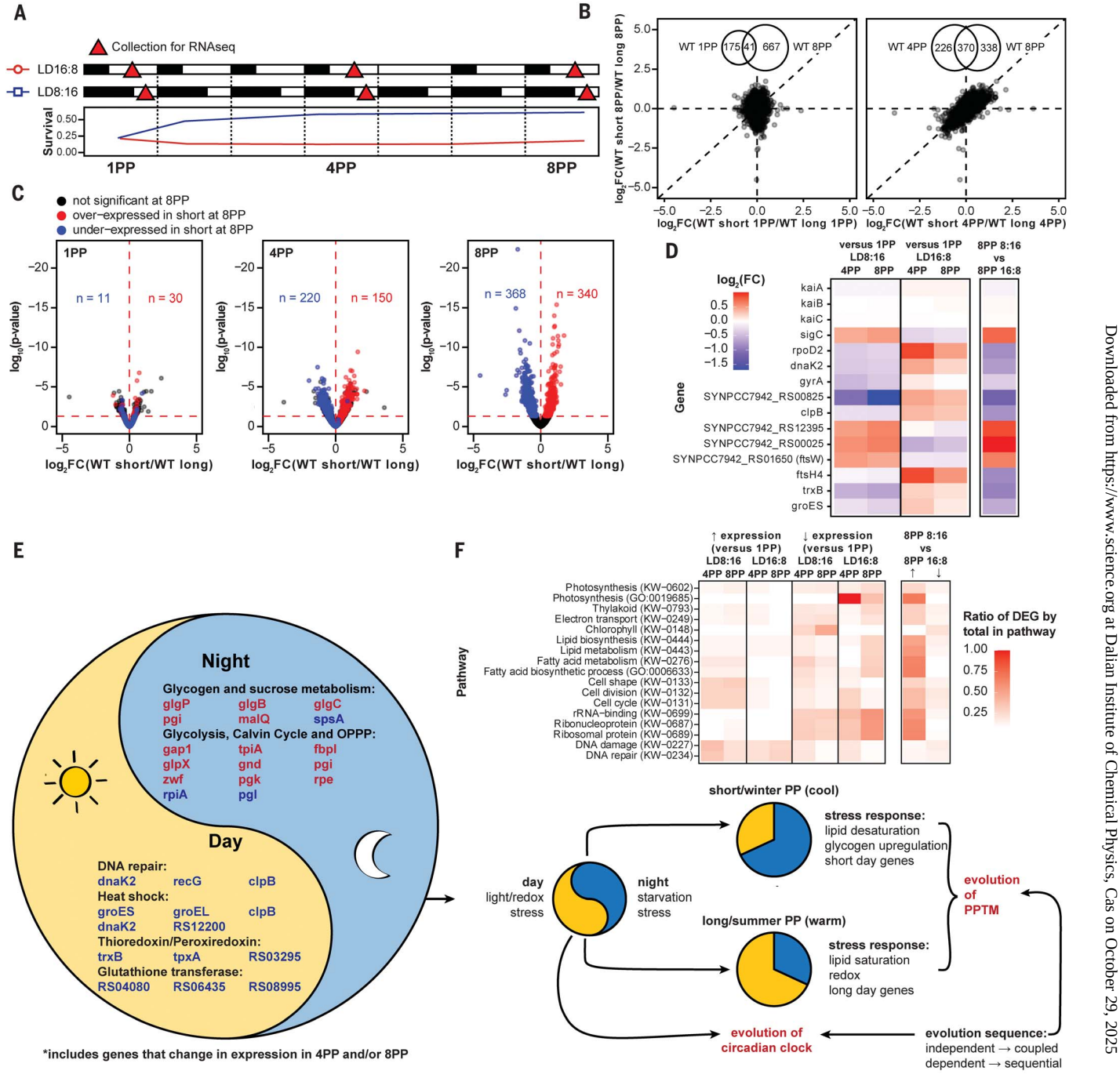

Fig. 4. Exposure to short and long days leads to global alterations in gene expression that are evident after multiple PP cycles. (**A**) Diagram showing time points collected for RNA-seq (n = 3). (**B**) Correlation plot showing the $\log_2$(FC) in expression of short-day cells divided by long-day cells, comparing either eight PPs with one PP (left) or eight PPs with four PPs (right). The Venn diagrams show the number of differentially expressed genes shared between each condition (not including genes differentially expressed in opposite ways). Vertical and horizontal dashed lines indicate the y and x zero intercepts, and the diagonal line shows $y = x$. FC, fold change. (**C**) Volcano plots showing the differentially expressed genes for WT at one PP, four PPs, and eight PPs, colored by the difference in expression at eight PPs. The numbers in blue on the bottom plot refer to the total number of genes underexpressed in short days for WT after eight PPs, whereas those in red show the genes overexpressed. On the middle and top plots, the numbers refer to genes under- or overexpressed in WT after either four PPs or one PP that are also under- or overexpressed in WT after eight PPs. (**D**) Subset of genes of interest (fig. S15), showing the circadian clock genes *kaiA*, *kaiB*, and *kaiC* (which did not show significant changes in expression) as well as genes related to DNA metabolism, fatty acid metabolism, and stress. The first four columns show the $\log_2$(FC) in expression in WT cells within each PP of either four or eight PPs against one PP. The remaining column on the right shows the resulting differential expression between WT cells exposed to eight PPs of short days versus eight PPs of long days. (**E**) Model of the evolution of adaptive PPTM in relation to the circadian clock. Stresses that occur primarily in the day versus the night over the daily cycle are expanded and/or contracted as the daily PP changes. This leads to differential stress response activation over the annual cycle, from which a PPTM mechanism could evolve. (**F**) Similarly to (D), this panel shows a selection of the pathways of interest identified by gene ontology analysis within our dataset of genes of

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interest. Instead of $\log_2$(FC), we show in this panel the ratio of differentially expressed genes (DEGs) by the total amount of genes that were annotated as part of that pathway. The first four columns show the ratio of overexpressed genes within each pathway and are akin to the first four columns in panel (D). The four columns afterward show the ratio of underexpressed genes. The remaining two columns on the left show the resulting ratio of over- and underexpressed genes. GO, gene ontology annotation; KW, UniProt keywords.

implicated in stresses that occur in the day (bright light, UV, redox stress, hotter temperatures) versus night (metabolic stress) over the daily cycle (Fig. 4E).

Gene ontology analysis through DAVID (*22*, *23*) identified differentially controlled pathways of interest, including the key processes of lipid and fatty acid metabolism, photosynthesis, cell cycle, DNA recombination and repair, and ribosomal activity (Fig. 4F). Overall, the RNA-seq results showed that exposure to short versus long days led to distinctive programs of gene expression, caused partially by entrainment itself (changes common to cells exposed to one and eight PPs) and partially by direct responses to short days and long nights (changes common to WT and Δ*kaiABC* cells). However, there were at least 696 genes regulated by the *kaiABC*-dependent PP timekeeping mechanism (i.e., those that occurred only in WT cells exposed to either four or eight PP cycles; tables S1 and S2 and fig. S15). This group of PPTM-regulated genes includes those related to (i) DNA metabolism (*dnaK*, *gyrA*, *clpB*, the sigma factors *sigC* and *rpoD2*, and the class I S-adenosyl methionine (SAM)–dependent methyltransferase SYNPCC7942_RS00825), (ii) temperature [the chaperone *clpB* is a heat shock protein related to thermotolerance in cyanobacteria (*24*), but it is also strongly induced by cold (*25*)], (iii) photosynthesis [thylakoid, electron transport, chlorophyll, photosynthesis (*26*)], and (iv) stress responses. In the stress response category, stimulation by long days increased expression of genes in multiple pathways associated with light or heat stress that accompanies longer exposure to light, namely *ftsH4* [high light (*27*)], *trxB* [oxidative stress (*28*)], *groES* [heat shock (*29*)], and the stringent response of cyanobacteria [redox stress mediated through changes in (p)ppGpp (*30*, *31*)] (fig. S16). Survival in darkness is associated with glycogen in cyanobacteria (*32*), and the gene encoding the rate-limiting step for glycogen synthesis (*glgC*, encoding glucose-1-phosphate adenylyltransferase) showed increased expression in short days (fig. S17). Similarly, multiple gene-related pathways associated with glycogen metabolism (glycolysis, sucrose metabolism, the Calvin cycle, and the oxidative pentose phosphate pathways) were differentially expressed between short and long days (fig. S17), indicating that exposure to different PPs regulated genes encoding crucial metabolic enzymes. The clock genes *kaiA*, *kaiB*, *kaiC*, and *rpaA* were not differentially expressed between short and long days at any of the PP cycles measured, which implies that stability of the core circadian clockwork is conserved while PP changes seasonally. Furthermore, expression levels prominently changed for genes related to lipid and fatty acid metabolism and membrane transport, such as the fatty acid desaturase SYNPCC7942_RS12395, the permease SYNPCC7942_RS00025, and the flippase *ftsW* (SYNPCC7942_RS01650) (*33*, *34*). These genetic changes are consistent with our observation of molecular increases in the desaturation of membrane lipids, particularly in MGDG and PG levels (Fig. 3, E to H, and figs. S9 to S12).

Discussion

These results show that cyanobacteria exposed to winter-like PPs survive low temperatures two to three times better than those exposed to summerlike PPs and that this response requires a functional circadian clock. Short versus long days promote distinctive transcriptional programs, and cells exposed to short days experience an adaptive change in the saturation of membrane lipids akin to that seen in cells exposed to cool temperatures. The amplitude of the cold photoperiodic survival can be modulated by several environmental factors—namely, the daily phase of testing, light intensity, and previous exposure to cooler temperatures. In nature, as winter approaches, organisms face the shortening of day length [and lower light intensity (*35*)] that precedes a gradual lowering of average temperature by ~1 to ~2 months (*36*, *37*). In their natural environment, cyanobacteria likely integrate multiple environmental factors to establish the timing and magnitude of their photoperiodic response. At first, it seems unexpected that an organism with such a short generation time has evolved a mechanism to keep track of seasons and adaptively respond preemptively, but when selection is considered to be acting upon the population lineage rather than on the individual, the advantage of PPTM to cyanobacteria becomes intelligible (*38*, *39*).

These observations indicate that PPTM may have more ancient evolutionary roots than previously appreciated; it may have evolved before multicellularity and before the evolution of eukaryotes. We were particularly struck by the observation that short versus long PPs induced counterbalancing responses in stress pathways whereby short days induced expression of genes that confer adaptive responses to cold temperatures (e.g., lipid desaturases and glycogen synthesis), whereas long days promoted expression of stress response pathways associated with light, redox, or heat stress (e.g., the stringent response, *trxB*, etc.). We compared our RNA-seq dataset with an independent dataset produced by exposing *S. elongatus* PCC 7942 cells to salinity and cold stressors for 1 or 24 hours (*40*); the genes that were upregulated after 24 hours of increased salinity or lower temperature (20°C) were also upregulated in our dataset by short days after 8 PP, and a similar correlation was seen for downregulated genes (similar changes were observed in Δ*kaiABC*, although with smaller fold changes; fig. S18). These associations lead us to propose that PPTM might have first evolved in prokaryotes from preexisting stress pathways that had originally evolved to combat acute stresses.

For obligate photoautotrophic organisms such as cyanobacteria, stresses that occur primarily in the day [bright light, UV, redox stress, hotter temperatures (*31*)] versus the night (metabolic stress, starvation) over the daily cycle are expanded or contracted as the daily PP changes (Fig. 4E). This leads to differential stress pathway activation over the annual cycle. Once a stress pathway exists, the introduction of a timekeeping mechanism to additionally regulate the stress pathway to not only respond acutely but also to anticipate regularly occurring environmental stresses is a logical selective pressure to evolve a PPTM. Although PP has been labeled as a possible stress in photosynthetic eukaryotes (*41*), we propose that PPTM may have evolved from preexisting stress response pathways. Moreover, our results raise the question of whether a circadian or a PPTM system evolved first. Although PPTM is now often associated with a circadian timekeeper, the first photoperiodic timing mechanism might have been an hourglass timer that later became linked to a preexisting circadian timer, or the hourglass PPTM timer might have itself been the ancestor of a more flexible, self-sustained circadian system (Fig. 4E). Therefore, the evolutionary sequencing between the daily circadian clock and the seasonal PPTM might be two independent timekeepers that became coupled, or the sequential evolution of one from the other (Fig. 4E).

The demonstration of PPTM in cyanobacteria has economic and global implications. Summer "blooms" of cyanobacteria in lakes and ocean can be economically devastating. These blooms have usually been interpreted to result from acute, local conditions (temperature, light intensity, nutrients), but perhaps they are also a manifestation of an anticipatory seasonal or photoperiodic response. For all organisms that respond adaptively to photoperiodic changes—now including cyanobacteria—the specter of climate change and global warming means that the relation between average temperature and

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any particular PP is rapidly shifting; cyanobacteria may be able to evolve rapidly enough to alter their anticipatory photoperiodic responses, but organisms with longer generation times that cannot change their latitudinal range may be trapped by seasonal responses that are no longer timed appropriately (*42*). Recognizing the existence of bona fide adaptive photoperiodic timing in a prokaryote opens the possibility that photoperiodism might be an evolutionarily ancient phenomenon and introduces a versatile model system to study the mechanisms and evolution of photoperiodic responses.

REFERENCES AND NOTES

1. R. J. Nelson, D. L. Denlinger, D. E. Somers, Eds., *Photoperiodism: The Biological Calendar* (Oxford Univ. Press, 2010).
2. D. S. Saunders, *Insect Clocks* (Elsevier, 2002).
3. W. W. Garner, H. A. Allard, *Mon. Weather Rev.* **48**, 415 (1920).
4. A. Taton *et al*., *Nat. Commun.* **11**, 1688 (2020).
5. M. J. Dring, in *Photobiology of Microorganisms*, P. Halldal, Ed. (Wiley, 1970), pp. 345–368.
6. T. Kondo *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **90**, 5672–5676 (1993).
7. M. Ishiura *et al*., *Science* **281**, 1519–1523 (1998).
8. M. Nakajima *et al*., *Science* **308**, 414–415 (2005).
9. C. H. Johnson, C. Zhao, Y. Xu, T. Mori, *Nat. Rev. Microbiol.* **15**, 232–242 (2017).
10. P. Flombaum *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **110**, 9824–9829 (2013).
11. E. Leypunskiy *et al*., *eLife* **6**, e23539 (2017).
12. D. S. Saunders, *J. Insect Physiol.* **12**, 569–581 (1966).
13. D. S. Saunders, *J. Insect Physiol.* **17**, 801–812 (1971).
14. D. S. Saunders, *Physiol. Entomol.* **6**, 99–116 (1981).
15. O. M. Heide, *New Phytol.* **128**, 347–362 (1994).
16. M. Krzymuski *et al*., *Plant J.* **83**, 952–961 (2015).
17. Y. Yang, C. Yin, W. Li, X. Xu, *J. Bacteriol.* **190**, 1554–1560 (2008).
18. X. Tan *et al*., *J. Bacteriol.* **193**, 2675–2683 (2011).
19. Y. Xu, T. Mori, C. H. Johnson, *EMBO J.* **19**, 3349–3357 (2000).
20. U. Nair, J. L. Ditty, H. Min, S. S. Golden, *J. Bacteriol.* **184**, 3530–3538 (2002).
21. J. S. Markson, J. R. Piechura, A. M. Puszynska, E. K. O'Shea, *Cell* **155**, 1396–1408 (2013).
22. B. T. Sherman *et al*., *Nucleic Acids Res.* **50**, W216–W221 (2022).
23. W. Huang, B. T. Sherman, R. A. Lempicki, *Nat. Protoc.* **4**, 44–57 (2009).
24. M. J. Eriksson, A. K. Clarke, *J. Bacteriol.* **178**, 4839–4846 (1996).
25. J. Porankiewicz, A. K. Clarke, *J. Bacteriol.* **179**, 5111–5117 (1997).
26. F. Khajepour, S. A. Hosseini, R. Ghorbani Nasrabadi, G. Markou, *Appl. Biochem. Biotechnol.* **176**, 2279–2289 (2015).
27. V. Krynická *et al*., *Plant Commun.* **4**, 100502 (2023).
28. M. J. Mallén-Ponce, M. J. Huertas, F. J. Florencio, *Antioxidants* **11**, 654 (2022).
29. H. Rajaram, A. K. Chaurasia, S. K. Apte, *Microbiology* **160**, 647–658 (2014).
30. R. D. Hood, S. A. Higgins, A. Flamholz, R. J. Nichols, D. F. Savage, *Proc. Natl. Acad. Sci. U.S.A.* **113**, E4867–E4876 (2016).
31. D. G. Welkie *et al*., *Trends Microbiol.* **27**, 231–242 (2019).
32. M. Gründel, R. Scheunemann, W. Lockau, Y. Zilliges, *Microbiology* **158**, 3032–3043 (2012).
33. T. Mohammadi *et al*., *EMBO J.* **30**, 1425–1432 (2011).
34. T. Mohammadi *et al*., *J. Biol. Chem.* **289**, 14707–14718 (2014).
35. I. Alados, I. Foyo-Moreno, L. Alados-Arboledas, *Agric. For. Meteorol.* **78**, 121–131 (1996).
36. M. L. Jabbur, C. Zhao, C. H. Johnson, in *Circadian Rhythms in Bacteria and Microbiomes*, C. H. Johnson, M. J. Rust, Eds. (Springer, 2021), pp. 111–135.
37. R. A. Hut, S. Paolucci, R. Dor, C. P. Kyriacou, S. Daan, *Proc. Biol. Sci.* **280**, 20130433 (2013).
38. C. H. Johnson, S. S. Golden, T. Kondo, *Trends Microbiol.* **6**, 407–410 (1998).
39. B. R. Levin, C. T. Bergstrom, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 6981–6985 (2000).
40. K. Billis, M. Billini, H. J. Tripp, N. C. Kyrpides, K. Mavromatis, *PLOS ONE* **9**, e109738 (2014).
41. S. Nitschke, A. Cortleven, T. Schmülling, *Trends Plant Sci.* **22**, 913–916 (2017).
42. M. L. Jabbur, C. H. Johnson, *Front. Physiol.* **12**, 815847 (2022).

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SUPPLEMENTARY MATERIALS

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MDAR Reproducibility Checklist

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