Cyanobacterial viruses exhibit diurnal rhythms during infection

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As an adaptation to the daily light–dark (diel) cycle, cyanobacteria exhibit diurnal rhythms of gene expression and cell cycle. The light–dark cycle also affects the life cycle of viruses (cyanophages) that infect the unicellular picocyanobacteria *Prochlorococcus* and *Synechococcus*, which are the major primary producers in the oceans. For example, the adsorption of some cyanophages to the host cells depends on light, and the burst sizes of cyanophages are positively correlated to the length of light exposure during infection. Recent metatranscriptomic studies revealed transcriptional rhythms of field cyanophage populations. However, the underlying mechanism remains to be determined, as cyanophage laboratory cultures have not been shown to exhibit diurnal transcriptional rhythms. Here, we studied variation in infection patterns and gene expression of *Prochlorococcus* phages in laboratory culture conditions as a function of light. We found three distinct diel-dependent life history traits in dark conditions (diel traits): no adsorption (cyanophage P-HM2), adsorption but no replication (cyanophage P-SSM2), and replication (cyanophage P-SSP7). Under light–dark cycles, each cyanophage exhibited rhythmic transcript abundance, and cyanophages P-HM2 and P-SSM2 also exhibited rhythmic adsorption patterns. Finally, we show evidence to link the diurnal transcriptional rhythm of cyanophages to the photosynthetic activity of the host, thus providing a mechanistic explanation for the field observations of cyanophage transcriptional rhythms. Our study identifies that cultured viruses can exhibit diurnal rhythms during infection, which might impact cyanophage population-level dynamics in the oceans.

virus | cyanophage | cyanobacterium | diurnal rhythm | light–dark cycle

Similar to many living organisms, cyanobacteria adapt to the daily light–dark (diel) cycle by exhibiting diurnal rhythms of gene expression, metabolism, and cell cycle (1, 2). The unicellular picocyanobacteria *Prochlorococcus* and *Synechococcus* are the most abundant photosynthetic organisms on earth (3, 4) and contribute to ∼25% of marine primary production (5). Under light–dark cycles, both *Prochlorococcus* laboratory cultures (6, 7) and field populations (8) display diurnal rhythms in gene expression. Moreover, diurnal metabolic activities of *Prochlorococcus* populations were suggested to result in diurnal transcriptional rhythms of the cooccurring heterotrophic bacteria in the North Pacific Subtropical Gyre, which is likely to influence matter and energy transformation in the oceans (8).

Light affects the life cycle of viruses (cyanophages) that infect *Prochlorococcus* and *Synechococcus* (9, 10). As lytic double-stranded DNA viruses, cyanophages include three morphotypes: T4-like cyanomyoviruses, T7-like cyanopodoviruses, and cyanosiphoviruses (11–14). The majority of the cyanophages isolated from the oceans are cyanomyoviruses and cyanopodoviruses (11, 15). During the first step of infection, adsorption of some cyanophages to their host cells depends on light (16–18). The light-dependent adsorption is thought to be due to conformational changes in host receptors and/or phage tail fibers that can only be induced in the light (18). In addition, cyanophage replication depends on light, and their burst sizes are greatly reduced when host cells are infected in the light and then moved to the dark (17, 19–23). As far as we know, no cyanophage has been shown to complete the entire infection cycle in the dark. Similar to darkness, photosynthesis inhibitors can also inhibit cyanophage replication in the light (19–21), suggesting that cyanophage replication relies on the photosynthetic energy of host cells (20).

Several pieces of evidence suggest that cyanophages may exhibit diurnal infection rhythms. When *Synechococcus elongatus* PCC 7942 was infected by cyanophage AS-1 at different times of the day, phage progeny production seemed to vary (17). However, a diurnal rhythm of phage production could not be concluded, because the timing of the specific light and dark periods was not reported (17). A recent study showed that the latent period of *Synechococcus* phage S-PM2d is shorter under high light conditions, suggesting that the natural daily variation of light intensity may affect cyanophage infection dynamics (24). The influence of light on cyanophage dynamics also extends to field evidence of diel variation of cyanophage abundance (25, 26). Furthermore, recent metatranscriptomic studies showed transcriptional rhythms of field cyanophage populations (27, 28). However, cyanophage laboratory cultures have not been shown to exhibit diurnal transcriptional rhythms.

In this study, we used cyanophage laboratory cultures to explore how the light–dark cycle affects infection dynamics and gene expression. We utilized well-characterized cyanomyoviruses P-HM1, P-HM2, and P-SSM2, and cyanopodoviruses P-SSP7 and P-GSP1 (11, 29, 30). These phages infect *Prochlorococcus*

Significance

To adapt to the daily light–dark cycle, diurnal rhythms are used by the photosynthetic cyanobacteria *Prochlorococcus* and *Synechococcus*, which are the most abundant photosynthetic organisms on earth. Field studies revealed that cyanobacterial virus (cyanophage) populations in the oceans showed transcriptional rhythms. To explore the underlying mechanism, we used cyanophage laboratory cultures to find that some showed adsorption rhythms and all showed transcriptional rhythms. We discovered that the cyanophage transcriptional rhythm is partially caused by the photosynthetic activity of host cells, explaining transcriptional rhythms of field cyanophage populations. Our study shows that cultured viruses have diurnal infection rhythms which are critical for understanding how light–dark cycles shape the interaction of cyanophages and their hosts in the oceans.

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strains MED4 (host for P-HM1, P-HM2, P-SSP7, and P-GSP1) and NATL2A (host for P-HM1, P-HM2, and P-SSM2). We first studied infection dynamics and gene expression in the dark and identified three distinct diel-dependent life history traits. Then we quantified cyanophage abundance and transcript abundance under light–dark cycles to characterize their diurnal infection rhythms. Finally, we tested whether the diurnal transcriptional rhythm of cyanophages is caused by the photosynthetic activity of host cells. Our results provide a mechanistic explanation for the field observations of cyanophage transcriptional rhythms and may have implications for both cyanophage and cyanobacterial population dynamics in the oceans.

Results

Cyanophage Replication in Dark Conditions. Before studying cyanophage infection under light–dark cycles, we first examined whether cyanophages can initiate and complete the entire infection cycle in the dark. We incubated cyanophages with unsynchronized host cells in the dark or under continuous light, and measured phage DNA copies by quantitative PCR, which provides an ∼1:1 relationship with phage particle counts (30). Following a standard method for cyanophage infection kinetics (22, 31), extracellular phage DNA copies were used to measure phage adsorption to and release from host cells, and intracellular phage DNA copies were used to measure phage replication inside host cells. We also measured the transcript abundance of representative early, middle, and late phage genes, which were categorized based on their expression times during infection (23, 31, 32).

We found three diel-dependent life history traits (diel traits) for our focal cyanophages. First, P-HM2 (Fig. 1*A*) and P-HM1 (similar to P-HM2) did not adsorb to their host cells in the dark, and thus did not replicate (*SI Appendix*, Fig. S1*A*). This light-dependent adsorption has been shown previously using *Synechococcus* phages (16–18). Consistent with its inability to adsorb in the dark, P-HM2 transcripts were not detected in the dark, while they were detected in the light (Fig. 1*D*). Second, P-SSM2 adsorbed to host cells in the dark, although fewer phages were adsorbed in the dark than in the light (Fig. 1*B*). However, P-SSM2 did not replicate in the dark (*SI Appendix*, Fig. S1*B*) and its transcripts were not detected (Fig. 1*E*), suggesting that its replication and/or gene expression are regulated by an unidentified light-sensitive mechanism. Third, P-SSP7 (Fig. 1*C* and *SI Appendix*, Fig. S1*C*) and P-GSP1 (similar to P-SSP7) adsorbed to and also replicated in host cells in the dark. Furthermore, P-SSP7 progeny was released in the dark at the same time as the cultures in the light, although the burst size was significantly smaller than in the light (Fig. 1*C*). Consistently, P-SSP7 genes were transcribed in the dark and their abundances were also lower than those in the light (Fig. 1*F*). It is notable that we have shown that some cyanophages are able to complete the entire infection cycle in the dark and are unaware of prior reports of similar dynamics.

Rhythmic Infection Patterns of Cyanophages under Light–Dark Cycles. To compare the diel infection patterns of cyanophages P-HM2, P-SSM2, and P-SSP7, we infected synchronized *Prochlorococcus* cells grown under a 14-h-light:10-h-dark cycle (*SI Appendix*, Fig. S2), where ZT (Zeitgeber Time) 0 corresponds to "lights on" and ZT 14 corresponds to "lights off." Infections were carried out at five time points (ZT 14.5, 18 on day 1, and ZT 0.5, 6, 12 on day 2) (Fig. 2). A low phage/host ratio of 0.02 was used to ensure that cyanophage production was not limited by host availability for at least 2 d after the first infection (*SI Appendix*, Fig. S3).

When P-HM2 infections were initiated before (Fig. 2 *A* and *B*) or at lights on (Fig. 2*C*), the extracellular phage numbers of different infections all decreased near to ZT 2–4 on both day 2 and day 3. This rhythmic decrease in extracellular phage number at the same time of 2 d was consistent with light-dependent adsorption of P-HM2 after lights on (Fig. 1*A*), and this pattern was not observed when the infected cultures were moved to continuous light (Fig. 2*F*). Moreover, when P-HM2 infections were initiated several hours after lights on (Fig. 2 *D* and *E*), the extracellular phage numbers also decreased at around ZT 2–4 on day 3, indicating that P-HM2 infections initiated at different times of a day were synchronized under light–dark cycles. The rhythmic decrease in extracellular phage number was also seen in P-SSM2 infections under light–dark cycles (Fig. 2 *G–K* and zoom-in data in *SI Appendix*, Fig. S4) but not under continuous light (Fig. 2*L*), consistent with our observations that P-SSM2 adsorption was enhanced by light (Fig. 1*B*). Besides ZT 2–4, the extracellular phage numbers of P-HM2 and P-SSM2 infections decreased at additional time points (Fig. 2 *A–L*). This might be caused by the different initiation times of each infection and/or the different latent periods of P-HM2 and P-SSM2 (Fig. 1 *A* and *B*). In contrast to P-HM2 and P-SSM2, P-SSP7 did not show rhythmic decrease in extracellular phage number under light–dark cycles (Fig. 2 *M–Q*) and under continuous light (Fig. 2*R*),

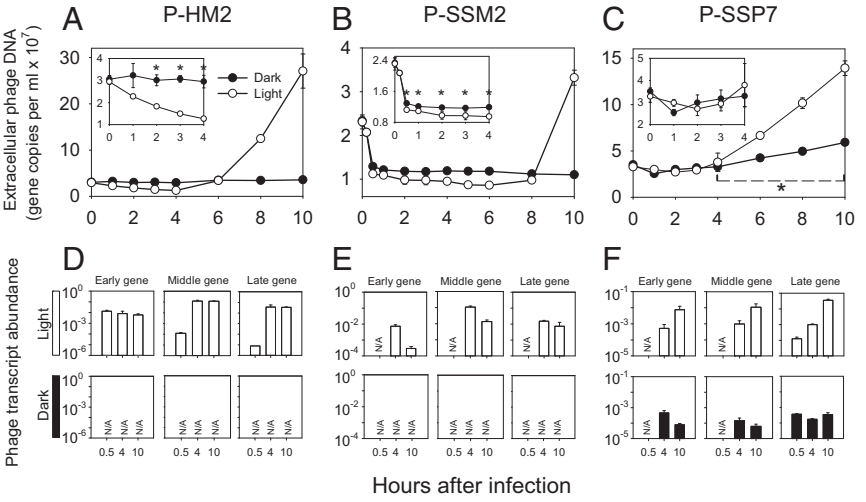

Fig. 1. Cyanophage infection and gene expression in the light or in the dark. Cyanophages P-HM2 (*A* and *D*), P-SSM2 (*B* and *E*), and P-SSP7 (*C* and *F*) were used to infect their host cells under continuous light (empty symbols) or in the dark (filled symbols). The host for P-HM2 and P-SSP7 was *Prochlorococcus* MED4 and the host for P-SSM2 was *Prochlorococcus* NATL2A. With a phage/host ratio of 0.1, extracellular phage DNA (*A–C*) was measured by qPCR. Asterisk in *C* indicates that in the dark extracellular DNA of P-SSP7 is significantly higher at 10 h than that at 4 h ($P < 0.01$, Student's *t* test). (*A–C*, *Insets*) Corresponding zoom-in data; the asterisks indicate that extracellular cyanophage DNA in the dark is significantly higher than that in the light at the same sampling time point ($P < 0.01$, Student's *t* test). With a phage/host ratio of 3, transcript abundances of phage early, middle, and late genes were measured by RT-qPCR (see *Methods* for gene names), and were normalized to those of the host *rnpB* gene (*D–F*). N/A indicates that transcripts were not detected. Error bars in *A–C* represent the range of two biological replicates and those in *D–F* represent the SD of three biological replicates. Error bars are smaller than the data point when not apparent.

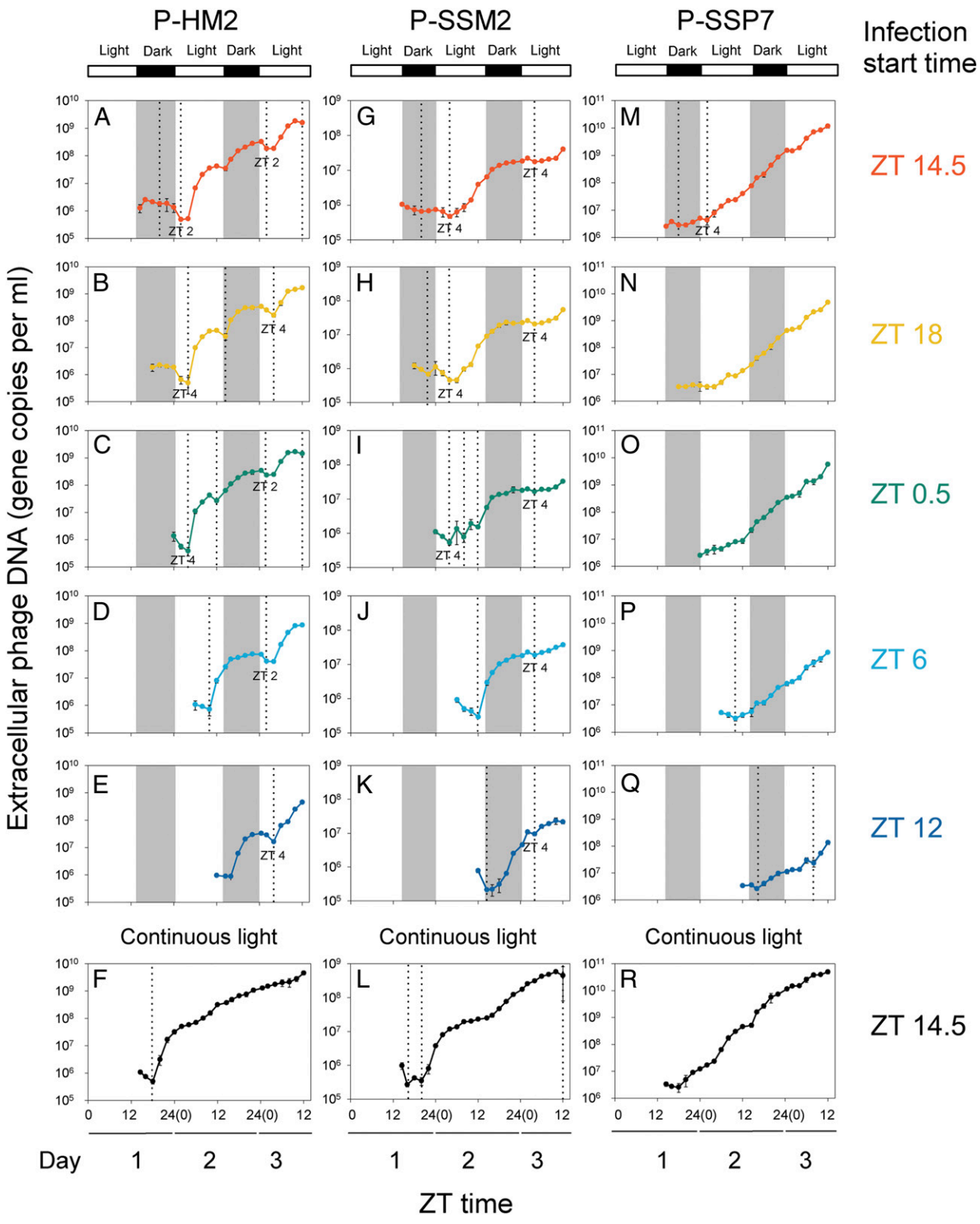

Fig. 2. Diel infection patterns of cyanophages P-HM2, P-SSM2, and P-SSP7. At a phage/host ratio of 0.02, *Prochlorococcus* MED4 was infected by P-HM2 (*A*–*F*) or P-SSP7 (*M*–*R*) and *Prochlorococcus* NATL2A was infected by P-SSM2 (*G*–*L*). Under a 14-h-light:10-h-dark cycle (lights on at ZT 0 and off at ZT 14), synchronized *Prochlorococcus* cultures were infected at ZT 14.5 (*A*, *G*, and *M*), 18 (*B*, *H*, and *N*), 0.5 (*C*, *I*, and *O*), 6 (*D*, *J*, and *P*), and 12 (*E*, *K*, and *Q*). The ZT 14.5 infections were also carried out under continuous light (*F*, *L*, and *R*). Extracellular phages were measured by qPCR. In each graph, vertical dotted lines indicate the times of phage adsorption (at least 10% decrease of extracellular phage number compared with the previous time point). Error bars represent the range of two biological replicates. In this heroic experiment, we could only handle two biological replicates for each infection, since we sampled a total of 18 infections every 2 h for 48 h.

again consistent with observations that it can replicate in the dark and its adsorption was not stimulated by light (Fig. 1*C*).

Transcriptional Rhythms of Cyanophages under Light–Dark Cycles. To study the expression patterns of cyanophage genes under light–dark cycles, we quantified the transcript abundance of cyanophage late genes, which were transcribed during the late stage of a lytic cycle and might be responsible for phage particle formation (23, 31, 32). When P-HM2 (Fig. 3*A*) and P-SSM2 (Fig. 3*B*) infections were initiated on day 1, their transcript abundances both increased in the light and then decreased near to lights off, which were observed for 2 d under light–dark cycles. Similarly, P-SSP7 transcript abundance increased in the light on day 1, decreased in the dark, and increased again on day 2 after lights on (Fig. 3*C*). After lights off on day 2, P-SSP7 transcript abundance flattened (Fig. 3*C*), and host population abundance collapsed after 2 d of infection (*SI Appendix*, Fig. S5*C*). The decrease of cyanophage transcripts in the dark is consistent with our dark infections showing that P-HM2 and P-SSM2 transcripts were not detected (Fig. 1 *D* and *E*) while P-SSP7 transcript abundance was much lower in the dark than those in the light (Fig. 1*F*). In addition, our previous transcriptomic study also showed that P-HM2 transcript abundance decreased after the infected cultures were transferred from the light to the dark (23).

It has been hypothesized that the diel transcriptional activity of cyanophages could result from the host cell cycle or the light–dark cycle (27). We set out to disentangle the host cell cycle from the light–dark cycle. It has been well established that light–dark synchronized cyanobacteria can use the KaiABC circadian clock to maintain the rhythms in gene expression and cell cycle for several days under continuous light (33). Although *Prochlorococcus* does not have a robust circadian clock due to deletion of the *kaiA* gene (34, 35), synchronized *Prochlorococcus* cells can maintain the cell-cycle rhythm for at least 1 d after being transferred to continuous light (34, 36). Similar to previous studies (34, 36), we transferred synchronized *Prochlorococcus* cultures from light–dark cycles to continuous light and found that cell division occurred at subjective night (*SI Appendix*, Fig. S5 *D–F*). Despite similar cell-cycle phases of the host cultures (*SI Appendix*, Figs. S2 and S5), transcript abundances of P-HM2, P-SSM2, and P-SSP7 decreased dramatically in the dark on day 1 (Fig. 3 *A–C*), while they did not decrease at subjective night under continuous light (Fig. 3 *D–F*). Therefore, our results suggested that the transcriptional rhythm of cyanophages can be uncoupled from the host cell cycle. Consistent with our results, a previous study found that cyanophage AS-1 infection of *S. elongatus* PCC 7942 was not affected by deletions in the host circadian clock genes, without which *Synechococcus* cells lose circadian rhythms and cannot maintain synchronized cell cycle (17).

Effect of Host Photosynthesis on Cyanophage Transcription. Since maximal cyanophage replication relies on continued photosynthesis (21), we hypothesized that the decrease of cyanophage transcripts in the dark may be due to a lack of photosynthetic activity of the host cells. To test this hypothesis, we added the photosynthesis inhibitor DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) under continuous light to see whether it can mimic the effect of darkness in reducing cyanophage transcripts. We infected synchronized *Prochlorococcus* MED4 cells with P-HM2 at lights on and kept the infected cells under continuous light. At 14 h after infection, when subjective night started, we added DCMU to the infected cultures and found that it inhibited P-HM2 replication under continuous light (Fig. 4*A*), similar to the study using cyanophage P-SSP7 (21). DCMU also reduced the transcript abundance of P-HM2 under continuous light (Fig. 4*B*), suggesting that cyanophage transcription is regulated by photosynthetic activity of the host cells. Consistent with the function of DCMU to partially inhibit the photosynthetic electron transport chain (from photosystem II to the plastoquinone pool), DCMU only reduced P-HM2 transcript abundance by 58.62% at 24 h (Fig. 4*B*), while darkness caused a 99.71% reduction (Fig. 3*A*). Thus, the transcriptional rhythms of cyanophage genes we observed under light–dark cycles (Fig. 3) could be caused by diel photosynthetic activity of the host cells, which is high during the daytime and low at night.

Discussion

In this study, we discovered three diel-dependent life history traits of cultured cyanophages that infect the ubiquitous marine cyanobacterium *Prochlorococcus* (Fig. 1). Our diel infection experiments further showed that cyanophages exhibited diurnal rhythms in adsorption (P-HM2 and P-SSM2) (Fig. 2) and transcript abundance (P-HM2, P-SSM2, and P-SSP7) (Fig. 3). It should be noted that P-HM2, P-SSM2, and P-SSP7 all produced more progeny phages under continuous light than under continuous dark (Fig. 1 *A–C*), and therefore they were all expected

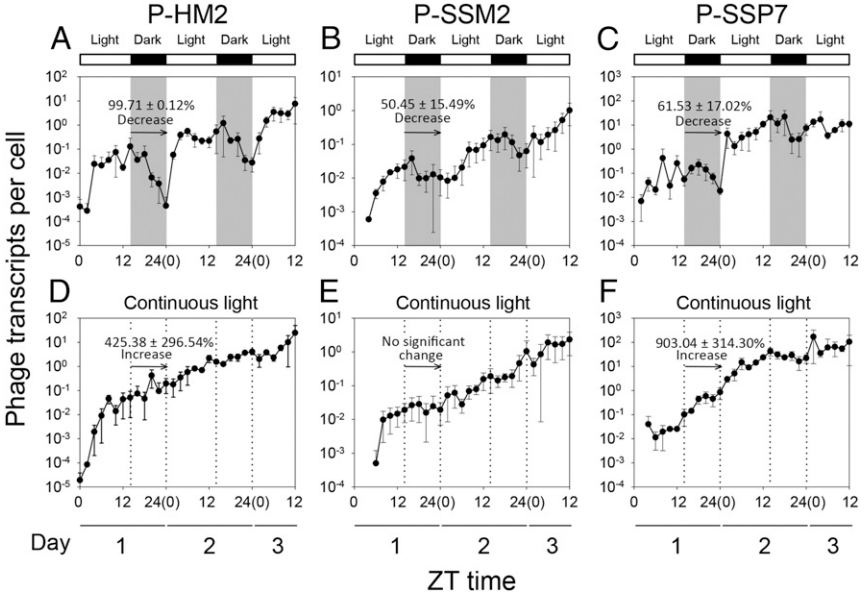

Fig. 3. Diel transcript abundance of cyanophages P-HM2, P-SSM2, and P-SSP7. *Prochlorococcus* MED4 was infected by P-HM2 (*A* and *D*) or P-SSP7 (*C* and *F*), and *Prochlorococcus* NATL2A was infected by P-SSM2 (*B* and *E*) (phage/host ratio 0.02). Infections were initiated at ZT 0.5 under a 14-h-light:10-h-dark cycle, and were kept under light–dark cycles (*A–C*) or under continuous light (*D–F*). Absolute transcript numbers of cyanophage late genes were quantified (*gp22* for P-HM2 and P-SSM2, and *gp10* for P-SSP7). Data shown are transcripts per cell and reveal similar trends as those of transcripts per ml. Data points are not shown when phage transcript abundance was under the detection limit at the beginning of infection. Error bars represent the SD of three biological replicates. In each figure, the transcript abundance at the end of the dark period on day 1 is compared with that at the beginning of the dark period, and a percentage change is shown. For this calculation, we did not use the peak transcript abundance, since the peak time varied in different infections.

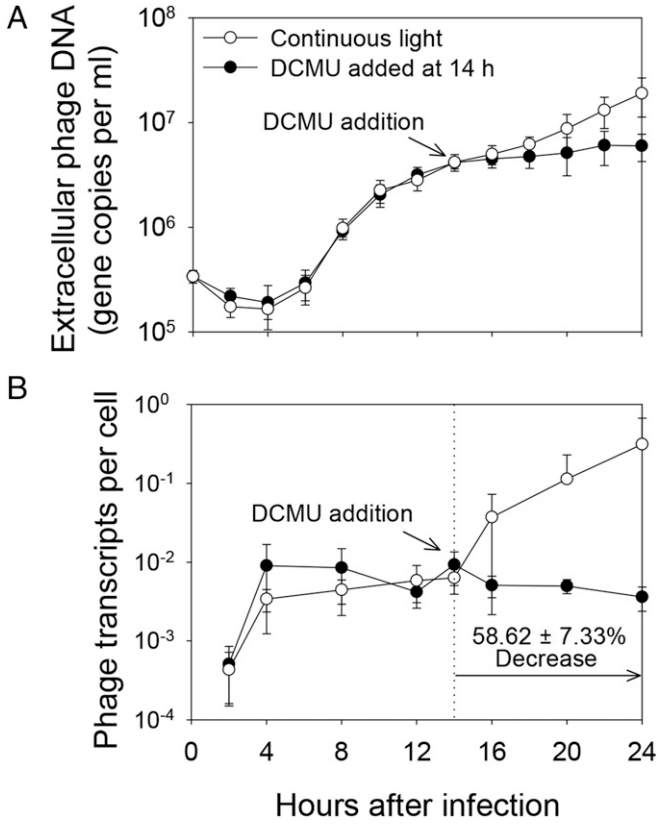

Fig. 4. Transcript abundance of cyanophage P-HM2 after DCMU addition under continuous light. Light–dark synchronized *Prochlorococcus* MED4 cells were infected by P-HM2 (phage/host ratio 0.02) at lights on and were kept under continuous light. Arrows indicate that DCMU was added to aliquots of the infected cultures at 14 h after infection [final concentration 50 μM, as used previously (21)]. Extracellular phage DNA was measured by qPCR (*A*) and absolute transcript numbers of cyanophage late gene *gp22* were quantified (*B*). Error bars represent the SD of three biological replicates. The transcript abundance at 14 h is compared with that at 24 h and a percentage change is shown in *B*.

to show rhythmic phage production under light–dark cycles. However, in our experiments we could not compare phage production in the light and in the dark, since it is not currently feasible to measure the number of infected cells at different times of a day. Two recent metatranscriptomic studies found that aggregated cyanophage transcript abundance in the oceans peaked at around sunset and decreased at night (27, 28). Thus, the transcriptional rhythm we found using cyanophage laboratory cultures (Fig. 3 *A*–*C*) provides a potential mechanistic explanation for the aggregate transcriptional activity of natural cyanophage populations (27, 28). In addition, the natural daily light intensity could also affect the peak time of cyanophage transcript abundance in the oceans, since high light can shorten the latent period of cyanophages (24). Diurnal rhythms have been shown for all three domains of life (37), and our study provides an example of diurnal infection rhythms of cultured viruses.

The molecular mechanisms for the three diel-dependent life history traits seem to be specific to cyanobacteria, since these diel traits have not been found in phages infecting heterotrophic bacteria. Light-dependent adsorption of P-HM2 (Fig. 1*A*) might be due to light-sensitive host receptors and/or phage tail fibers (18), and this process does not rely on photosynthesis of the host cells, as shown using photosynthesis inhibitors in the cyanomyovirus S-PM2 (18). On the other hand, cyanophage transcription seems to be regulated by the photosynthetic activity of host cells, since darkness and the photosynthesis inhibitor DCMU can both reduce cyanophage transcript abundance (Figs. 3 and 4). The photosynthetic electron transport chain has been shown in cyanobacteria to regulate gene expression through the thiol redox state (38), and hence cyanophage genes could be regulated by similar mechanisms. It is noteworthy that darkness shows a greater degree of transcriptional reduction on P-SSM2 than on P-SSP7. While P-SSM2 transcripts were not detected in the dark (Fig. 1*E*), P-SSP7 transcripts could still be detected (Fig. 1*F*), which may explain why P-SSM2 cannot replicate in the dark (Fig. 1*B*) and P-SSP7 can replicate (Fig. 1*C*).

Our results suggest that different diel traits could affect cyanophage fitness under natural light–dark cycles. P-HM2–like phages are unable to infect host cells at night. However, they may achieve a larger burst size by initiating infection during the daytime when host cells are metabolically more active. On the other hand, P-SSP7–like phages infect host cells at every opportunity, although at night cellular resources for phage replication are lower than during the daytime. This trait may confer P-SSP7–like phages a fitness advantage if cyanophages are competing for a limited number of host cells. Interestingly, P-SSM2–like phages seem to have the combined advantages of P-HM2 and P-SSP7—they do not miss the chance to adsorb to a host cell at night and fully utilize the host's resources by replicating only during the daytime. However, there might be tradeoffs associated with this trait; otherwise, P-SSM2–like phages should have replaced phages with other traits. Future studies are needed to understand how different diel traits affect the fitness of cyanophages, which in turn might also affect the population dynamics of *Prochlorococcus* cells.

Methods

Culture Conditions. Axenic *Prochlorococcus* strains were grown in Port Shelter (Hong Kong) seawater–based Pro99 medium (39). Batch cultures were incubated at 23 °C in continuous light (25 μmol quanta $m^{-2}{\cdot}s^{-1}$) or a 14-h-light:10-h-dark cycle (35 μmol quanta $m^{-2}{\cdot}s^{-1}$ in the light period). Cultures were acclimated in the same condition for at least 3 mo before they were used for the infection experiments.

Flow Cytometry and Cell-Cycle Analysis. *Prochlorococcus* cells were preserved by mixing 100 μl culture with 2 μl 50% glutaraldehyde to a final concentration of 1% and were stored at −80 °C. Cells were enumerated using a BD FACSCalibur flow cytometer with CellQuestPro software. We followed a published protocol to determine the percentage of cells in each cell cycle stage (6). Briefly, *Prochlorococcus* cells were stained with the DNA stain SYBR Green (Invitrogen) and flow cytometry data were analyzed with the ModFit LT software (version 3.2, Verity Software House). ModFit LT built DNA histograms of cells in G1, S, and G2/M phases, which contain one, one to two, and two copies of genomic DNA, respectively (40). Bimodal DNA distribution was modeled and quantified by ModFit LT, with two Gaussian peaks for cells in G1 and G2/M phases, and a rectangle for S-phase cells.

Quantification of Cyanophages. To set the phage/host ratio for an infection experiment, total phage particles were collected on a 0.02-μm Whatman Anodisc filter, stained with 5× SYBR gold (Molecular Probes), and counted under an epifluorescence microscope (41, 42). At least five discrete fields on a filter were photographed using the SPOT Advanced Imaging software and fluorescent dots representing phage particles were counted manually.

During infection, extracellular phage DNA was quantified using a quantitative PCR (qPCR) method (31). Briefly, infected *Prochlorococcus* cultures were filtered through 0.2-μm polycarbonate filters in a 96-well filter plate (Pall). Filtrates containing extracellular phage particles were diluted 100-fold in dH_2O and were then used as templates for qPCR reactions in a 384-well plate. A qPCR reaction contained 4.6 μL template, 0.2 μL forward primer (10 μM), 0.2 μL reverse primer (10 μM), and 5 μL iTaq Universal SYBR Green Supermix. The LightCycler 480 Real-Time PCR System (Roche Diagnostics) was used for thermal cycling, which consisted of an initial activation step of 5 min at 95 °C, 45 amplification cycles of 10 s at 95 °C and 60 s at 60 °C, and a melting curve analysis at the end. The number of cyanophages in each well was quantified using a standard curve generated from phage particles that were enumerated by epifluorescence microscopy. The qPCR primers are listed in *SI Appendix*, Table S1.

Cyanophage Adsorption in the Light and in the Dark. Phage adsorption experiments were carried out using *Prochlorococcus* strains MED4 and NATL2A, which have been used extensively to study phage infection kinetics (21, 32, 43, 44). *Prochlorococcus* cells were acclimated under continuous light for at least 3 mo. After midlog cultures were mixed with cyanophages at a phage/host ratio of 0.1 in the dark, aliquots were moved to an incubator with continuous light while aliquots were kept in the dark throughout the whole experiment (in a box wrapped with aluminum foil in the same incubator). Cultures kept in the dark were sampled in a dark room with a dim red light on, which does not affect the adsorption of cyanophages to their host cells (18) (Fig. 1*A*). At each sampling time point, 100 μL culture was filtered through a 0.2-μm filter, and extracellular phages in the filtrate were quantified by qPCR.

Infection of Synchronized *Prochlorococcus* Cells under Light–Dark Cycles. *Prochlorococcus* cells were acclimated under light–dark cycles for at least 3 mo and were synchronized, as confirmed by flow cytometry (*SI Appendix*, Fig. S2). Midlog cells were infected at different times of a light–dark cycle at a phage/host ratio of 0.02.

Quantification of Cyanophage Transcripts. In Fig. 1 *D–F*, unsynchronized *Prochlorococcus* cells were infected by cyanophages at a phage/host ratio of 3. In Fig. 3, synchronized *Prochlorococcus* cells were infected at a phage/host ratio of 0.02. A low phage/host ratio was used in Fig. 3 because we wanted to follow the infection process for 3 d. At this ratio, phage transcripts were below the detection limit at the beginning of infection. At each time point, 1.5 mL infected culture was collected by centrifugation (20,000 × *g* for 10 min at 4 °C). Cell pellets were resuspended in 55 μL autoclaved seawater. After resuspension, 5 μL was used for cell counting and the rest was stored at −80 °C for RNA extraction. Following our previous protocols (32, 43), total RNA was extracted using a ZR RNA MiniPrep kit (Zymo Research), residual DNA was removed by a Turbo DNA-free kit (Ambion), and RNA was reverse transcribed with an iScript cDNA Synthesis kit (Bio-Rad). Using iTaq Universal SYBR Green Supermix (Bio-Rad), cDNA copies were quantified with 0.5 μM forward and 0.5 μM reverse primers (*SI Appendix*, Table S2). In Fig. 1 *D–F*, the relative transcript abundances of early, middle, and late phage genes were normalized to that of the host *rnpB* gene (43). For phage P-HM2, the early, middle, and late genes are PHM2_063, *gp32*, and *gp22*, respectively. For phage P-SSM2, the early, middle, and late genes are *cobS*, *psbA*, and *gp22*, respectively. For phage P-SSP7, the early, middle, and late genes are *int*, *gp2.5*, and *gp10*, respectively. We chose these genes based on their expression times in previous studies (23, 31, 32). In Fig. 3, we added a spike-in RNA (transcribed from the FLuc plasmid, NEB) into the resuspended cells before RNA extraction as we described previously (32). Absolute transcript abundance of cyanophage late genes was determined by normalizing to the amount of spike-in RNA (32).

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