Trends in Microbiology

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Review

A Hard Day's Night: Cyanobacteria in Diel Cycles

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Cyanobacteria are photosynthetic prokaryotes that are influential in global geochemistry and are promising candidates for industrial applications. Because the livelihood of cyanobacteria is directly dependent upon light, a comprehensive understanding of metabolism in these organisms requires taking into account the effects of day–night transitions and circadian regulation. These events synchronize intracellular processes with the solar day. Accordingly, metabolism is controlled and structured differently in cyanobacteria than in heterotrophic bacteria. Thus, the approaches applied to engineering heterotrophic bacteria will need to be revised for the cyanobacterial chassis. Here, we summarize important findings related to diurnal metabolism in cyanobacteria and present open questions in the field.

Highlights

A cyanobacterium integrates signals from the environment and from an internal circadian clock to orchestrate diurnal physiology.

Large datasets from genomic, proteomic, and metabolomic analyses have elucidated daytime and night-time programs that cyanobacterial cells employ during diurnal growth.

A critical aspect of metabolism in the dark is the production of NADPH by the oxidative pentose phosphate pathway when photosynthesis is inactive, which drives the suppression of potentially lethal reactive oxygen species.

Understanding diurnal physiology in cyanobacteria may help to harness these organisms for biotechnology applications, where outdoor growth may be desirable.

An Introduction to Day–Night Cycles in Cyanobacteria

The daily fluctuation of light is a nearly universal evolutionary pressure for life on Earth. For cyanobacteria, microorganisms that rely almost exclusively on light for energy, the response to these day–night cycles is particularly wide ranging and includes the redirection of central metabolism [1,2] and sweeping changes in gene expression [3,4]. As important primary producers and progenitors (via endosymbiosis) to the other oxygen-evolving photosynthetic organisms [5], cyanobacteria and their responses to light–dark cycles (LDCs) have broad implications for understanding photosynthesis in higher organisms and for characterizing a phylum that has tremendous ecological impact and biotechnological potential [6,7]. For instance, natural diel cycles influence infection by viruses (Box 1) and also carry significant economic consequences for industrial-scale growth (see the section 'Beyond Cyanobacteria', below). However, due to practical experimental considerations, most research, and reviews to date, have focused on the unnaturally static condition of perpetual light. Recent work probing the physiology of cyanobacteria in LDCs has opened up a fresh perspective on the life cycle of this keystone bacterial phylum. In this review we consolidate current knowledge on cyanobacterial growth in LDCs by starting with the cellular functions that are important for the day and night states. Thereafter, we address the current understanding of regulatory processes that are required to coordinate the transitions between the two. Finally, we discuss how research on cyanobacterial biology in LDCs has revealed a paradigm for diurnal growth that generalizes beyond cyanobacteria.

Surviving the Day

Each day, a cyanobacterium faces the formidable task of turning inorganic carbon into the organic molecules of life via photosynthetic carbon dioxide assimilation. As the sun rises, the cell encounters numerous metabolic challenges. It must perform cellular division by binary fission while also storing energy reserves for the night, a period of photosynthetic quiescence. Daytime activities take place in the background of photosynthesis, a process vital to the cell and

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Box 1. Viral Infections and Light–Dark

The ocean and freshwater lake ecosystems teem with cyanobacteria, as well as viruses that infect them. The ocean is home to upwards of 10^{30} phage particles, and their interactions with marine life play a critical role in ecosystem dynamics [93] and biogeochemical cycles [94]. Viral particles infecting cyanobacterial cells co-opt and utilize ATP and NADPH produced by the cyanobacterium during photosynthesis. Light and photosynthesis influence the success of the phage infection, including the number of phage particles that are generated during infection and released upon lysis [95,96]. For example, in *S. elongatus*, light has a strong influence on infection by the contractile phage AS-1, and infection and light absorption are correlated, occurring in a diel pattern under LDCs [97].

Some phages carry metabolism genes that enhance host daytime metabolic flux. For instance, a phage-borne *psbA* gene, encoding the photosynthetic reaction center D1 protein, may help to maintain host photosynthetic capacity during infection. Moreover, genes of the pentose phosphate cycle (*talC*, *gnd*, *zwf*, and even the *cp12* gene that encodes the inhibitory factor of the CBBC) have been found in phages and are preferentially expressed during infection in the light [98]. At night, overall phage gene expression decreases 100-fold [99].

one that requires significant resources for efficient function. In the process, damaging reactive oxygen species (ROS) are generated as a byproduct [8]. As a consequence, cyanobacterial metabolism is carefully orchestrated in both space and time [4,9,10] (Figure 1, Key Figure).

Central Carbon Metabolism

Much of cyanobacterial metabolism can be described as temporally partitioned, and generalized as anabolic during the day and catabolic at night. Daytime metabolism begins with shifting carbon flux from the oxidative pentose phosphate pathway (OPPP) to the Calvin-Benson-Bassham cycle (CBBC), and is controlled via products of the photosynthetic light reactions [11–13]. One of the critical steps in this process is inactivation of CP12, a redox-sensitive protein that is a master regulator of the CBBC [14,15]. During the night, oxidized CP12 structurally sequesters glyceraldehyde-3-phosphate dehydrogenase 2 (Gap2) and phosphoribulokinase (Prk) and inhibits the CBBC. This switch is mediated by the redox state of the cyanobacterial cell, which changes markedly depending on photosynthetic activity. At the onset of light, photosynthetic reducing equivalents are generated, reduced CP12 releases Gap2 and Prk, and CBBC activity resumes. Metabolomic analysis reveals that anabolic metabolism is upregulated during this phase of the day, including pathways related to amino acid, nucleotide, and quinone biosynthesis [1]. Upregulation of amino acid and nucleotide synthesis agrees with physiological observations that protein synthesis and DNA replication occur to a much greater extent during the day [16,17].

Energy Storage and Electron Sinks

A principal activity during the daytime is accumulation of excess photosynthate, which is stored as the glucose polymer glycogen. During growth in LDCs, glycogen accumulates during the day and serves two primary purposes: (i) as an energy-storage polymer in preparation for night [1,18–21], and (ii) as a 'regulatory valve' to assimilate excess reducing power produced under conditions of particularly high light intensity [8,22–24]. Mutations that target the glycogen biosynthesis genes *glgA*, *glgC*, or *glgP* significantly hinder the ability of cells to grow and remain viable in LDCs, highlighting the importance of glycogen storage [25–27].

Because cyanobacteria cannot rapidly turn off photosynthetic activity, conditions that temporarily impair daytime cell growth can cause a dangerous build-up in membrane redox potential [8,28,29]. The role of glycogen as a photosynthetic electron sink has been highlighted by investigations into the cyanobacterial nitrogen-deprivation response [25,30,31]. Nitrogen deficiency causes a rapid accumulation of glycogen as the downstream utilization of carbon skeletons via diverse biosynthetic processes is inhibited [30,32]. In strains unable to synthesize

Key Figure

Shift Work: Snapshot of Cellular Activities across Day and Night

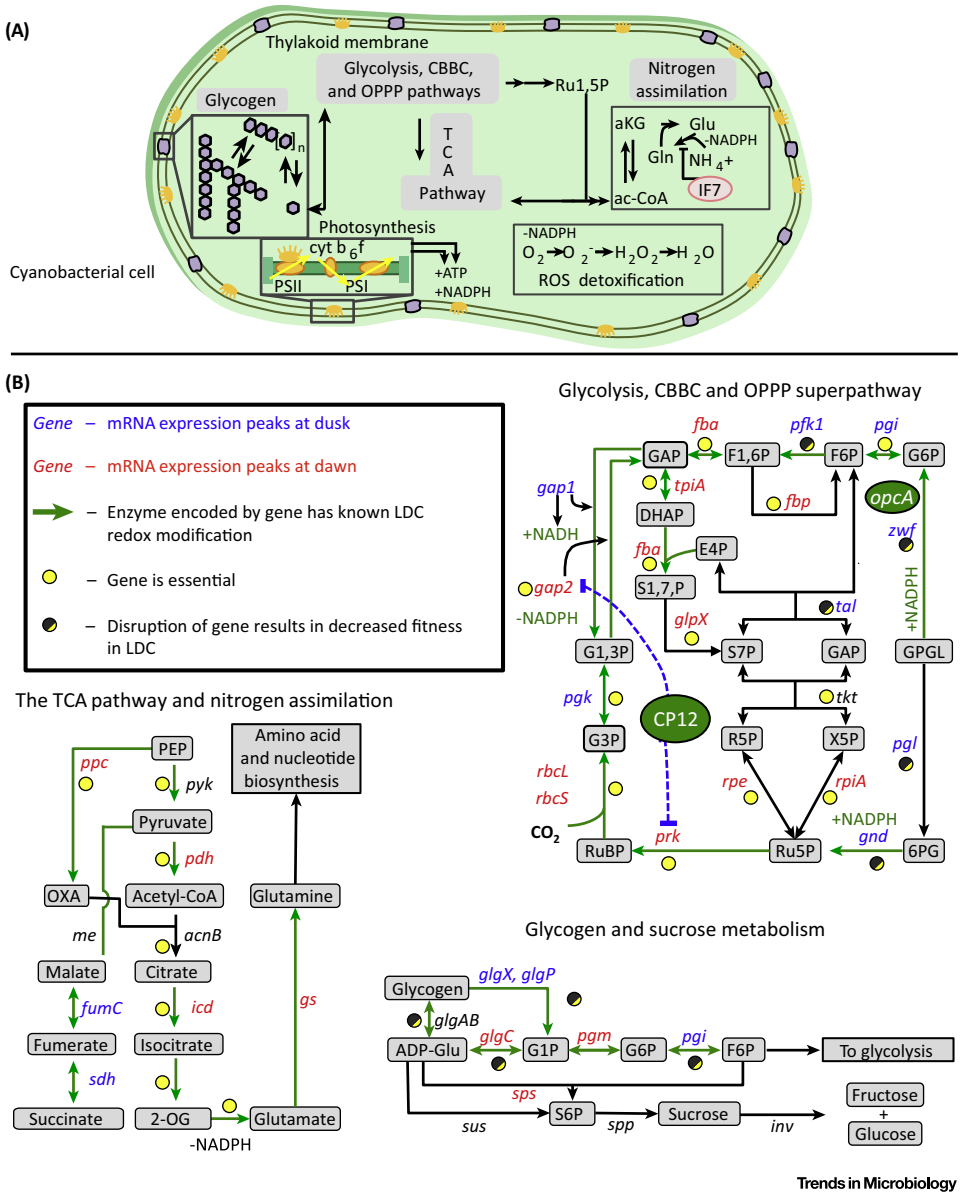

Figure 1. (A) A representation of a *Synechococcus elongatus* cell with an overview of the major metabolic pathways important for day–night physiology. (B) Reactions of glycogen metabolism, tricarboxylic acid (TCA) pathway, and central metabolism that are present in *S. elongatus* are provided in more detail. Green arrows in panel (B) indicate reactions where the respective enzyme has a detectable light/dark-dependent redox modification in *Synechocystis* sp. PCC 6803 [69]. Data about the peak expression time of circadian genes are indicated using data collected from *S. elongatus* PCC 7942 [9]. Genes colored in red peak in expression in the subjective morning, genes colored in blue peak in expression in the subjective evening, and genes colored in black have no detectable circadian rhythm in *S. elongatus*. Essential genes and genes that cause light–dark sensitive phenotypes when mutated are indicated by full yellow and half yellow–half black

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glycogen, nitrogen deprivation causes growth defects and oxidative damage at high-light intensities that otherwise do not affect wild-type (WT) cells [8,23,24]. Additionally, under nitrogen deprivation conditions, mutants unable to synthesize glycogen secrete pyruvate and tricarboxylic acid (TCA) pathway intermediates, possibly as an attempt to redirect photosynthetic output [25,30,31,33]. Overall, the buffering of cellular redox state through glycogen synthesis and degradation is an important aspect of regulating cellular physiology.

Surviving the Night

As the day ends and night begins, cyanobacteria face a drastic change of lifestyle. The past few decades of research have taught us a great deal about cyanobacterial processes that occur in the light, but little about cyanobacterial life in the dark. Although a cyanobacterium in darkness is typically viewed as being in a dormant state, the cell is not inactive, and many processes still operate dynamically. Studies on transcription, translation, and metabolism have demonstrated specific adaptive responses to darkness in *Synechococcus elongatus* sp. PCC 7942 (for a historical synopsis of this model organism see Box 2). While overall rates of these processes may be lower than in the light, or even close to zero in the case of DNA replication [34,35], they are coordinated such that the cell can conserve energy, ensuring its survival until light is available again. With photosynthesis unable to proceed in the dark, a suite of cell-wide changes occurs, ranging from shifts in ATP and reductant levels, to redirection in the flux of carbon compounds, to altered cell division activities.

Box 2. Brief History of *S. elongatus* sp. PCC 7942

Synechococcus elongatus sp. PCC 7942 is the official name of a cyanobacterium that was isolated prior to 1973 from a local freshwater source by students taught by K.W. Floyd at California State University, San Francisco. Several samples were transferred to S.V. Shestakov of Moscow State University, whose laboratory demonstrated that one of the isolates, termed R-2, was transformable by chromosomal DNA from an antibiotic-resistant strain in their collection called *Anacystis nidulans* 602 [100]. The California isolate became known for many years as *A. nidulans* R2. In 1978 C.A.M.J.J. van den Hondel brought the strain from Moscow to the laboratory of G. van Arkel (University of Utrecht), where he was able to isolate mutants that carried the selectable transposon Tn*901* in the small endogenous plasmid of *A. nidulans* R2. This work began the era of recombinant DNA-based molecular genetics research in cyanobacteria [101]. Drs van den Hondel and van Arkel deposited the strain in the Pasteur Culture Collection, where it was given the accession number PCC 7942. A re-evaluation of the taxonomic structure of the cyanobacteria in the mid-1980s resulted in a renaming of previous *Anacystis* strains to the genus *Synechococcus*. For a period of several years, publications regarding this organism referred to it as *Synechococcus* sp. strain PCC 7942 without a species designation. A second edition of *Bergey's Manual of Systematic Bacteriology* was published in 2001 which included a section on the classification of cyanobacteria. A chapter by M. Herdman, R. Castenholz, J. Waterbury, and R. Rippka described the *Synechococcus* clade Cluster 1.1, typified by PCC 6301, which is so closely related to PCC 7942 as to be members of the same species [102]. These authors proposed the binomial *Synechococcus elongatus*, which is a name in keeping with the Botanical Code of Nomenclature. Most papers published since that date refer to the former *A. nidulans* R2 as *S. elongatus* PCC 7942. Note that the name *Synechococcus elongatus* had been used previously with reference to thermophilic cyanobacteria that are phylogenetically distant from PCC 6301 and 7942. The name *Thermosynechococcus elongatus* is now used for those thermophilic strains, but care is advisable in reading the literature to distinguish the *S. elongatus* that refers to PCC 7942 and PCC 6301, typically grown at 30°C, from the relatives of *T. elongatus*, typically grown at 45–50°C [103].

circles, respectively. Abbreviations: PBS, phycobilisome; PSII, photosystem II; cyt b_6f, cytochrome b_6f; PSI, photosystem I; Fd(red), ferredoxin (reduced); Ru1,5P, ribulose-1,5-bisphosphate; 3PG, 3-phosphoglycerate; 1,3-BPG, 1,3-bisphosphoglycerate; GAP, glyceraldehyde-3-phosphate; F6P, fructose-6-phosphate; G6P, glucose-6-phosphate; G1P, glucose-1-phosphate; 6PGL, 6-phosphogluconolactone; 6PG, 6-phosphogluconate; Ru5P, ribulose-5-phosphate; ac-CoA, acetyl-CoA; aKG, a-ketoglutarate; Glu, glutamate; Gln, glutamine; LDC, light–dark cycles; CBBC, Calvin-Benson-Bassham cycle; OPPP, oxidative pentose phosphate pathway.

Glycogen Breakdown and the OPPP

The initiation of glycogen degradation is essential for night-time survival in those cyanobacteria that are unable to utilize an external fixed carbon source [1,26]. The majority of the released glucose is shunted directly into the OPPP rather than the glycolytic pathway favored by diverse heterotrophs [11,13,36]. The primary function of the OPPP at night is to generate reducing power in the form of reduced nicotinamide adenine dinucleotide phosphate (NADPH) when the main production route during the day, photosynthesis, is not active [2,26,37]. This preference for the OPPP over glycolysis is functionally relevant in its production of NADPH over NADH. A number of enzymes in photosynthetic organisms have evolved a preference for NADPH over NADH as a reductant source, including some that are important for detoxifying ROS [38,39]. Cyanobacteria possess a variety of antioxidant and redox-buffering systems, such as enzymatic defenses involving superoxide dismutase and catalases, and nonenzymatic strategies using glutathione, peroxiredoxin, and carotenoids. Of these ROS responses, reduced glutathione-mediated reactions are critical for protection from the multiple ROS species that cells encounter. Importantly, the reduction of the disulfide in glutathione is mediated by glutathione reductase, which is dependent on NADPH. It is therefore not surprising that NADPH produced through the OPPP appears to be vital to surviving LDCs; many mutations that cause LDC sensitivity result in high oxidative-stress build-up that is not cleared in the dark [2,27,40]. Thus, carbon flux through the OPPP is one of the most critical processes for night-time survival, and inactivation of any of the three core OPPP genes *zwf*, *gap*, or *gnd* causes severely attenuated growth when cells are cultured in LDCs [27,41–43]. In contrast, the OPPP genes are dispensable when cells are grown under continuous light where NADPH is generated by photosynthesis (Figure 2). Detoxification of ROS using NADPH produced by the OPPP is a common theme in other higher organisms as well, such as in the hawk moth (see the section 'Beyond Cyanobacteria', below).

Consistent with a paradigm of the OPPP as the primary catabolic route for stored carbon, a genome-wide transposon mutagenesis screen in *S. elongatus* showed that enzymes of the

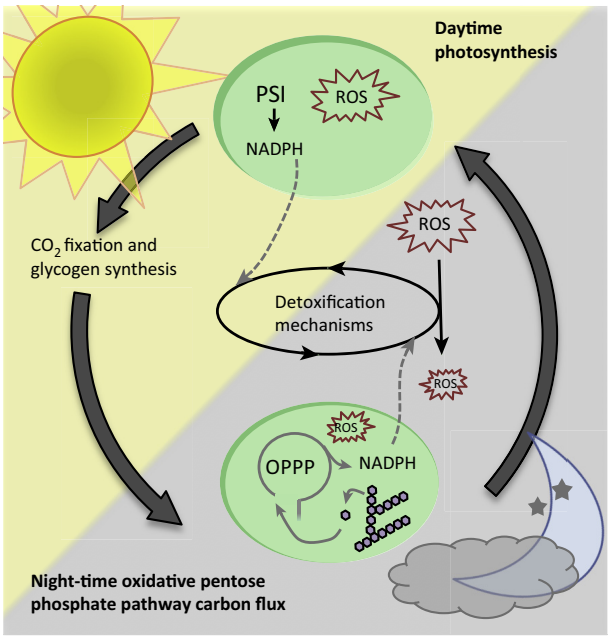

Figure 2. Detoxifying Reactive Oxygen Species (ROS) in the Diurnal World

For a Figure360 author presentation of Figure 2, see the figure legend at https://doi.org/10.1016/j.tim.2018.11.002

High rates of photosynthetic metabolism in light generate damaging ROS. During the day, NADPH produced via photosynthesis can aid in clearing such molecules. At night, ROS production ceases, and the remaining ROS are cleared by night-time metabolism. Here, detoxification can be aided by NADPH produced through degradation of glycogen by the oxidative pentose phosphate pathway (OPPP). Abbreviation: PSI, photosystem I.

regenerative phase of the canonical TCA cycle are not essential for viability under either continuous light or in LDC conditions [27]. Metabolic flux studies utilizing ^{13}C isotopic tracing in *Synechocystis* sp. PCC 6803 show that carbon primarily cycles within the OPPP even when that species grows heterotrophically [12,44]. These data reveal a fundamental difference in the orchestration of carbon-processing pathways between cyanobacteria and most heterotrophs. While many of the reactions that are usually part of a TCA cycle have been shown to be nonessential in *S. elongatus*, the first three steps leading to 2-oxoglutarate (2-OG) production are mediated by essential genes. It is likely that intermediates produced from other TCA-cycle precursor metabolites can be generated via alternative reactions, but 2-OG is the only known precursor for nitrogen assimilation into glutamine and glutamate in cyanobacteria. Production of 2-OG via the TCA reactions and subsequent nitrogen assimilation strongly influence NADPH reductant balance, because glutamine and glutamate biosynthesis require considerable reductant input via reactions that preferentially utilize NADPH over NADH [45]. Thus, nitrogen assimilation via 2-OG is inhibited in the dark, in part due to the induction of glutamine synthase inactivating factor (IF7) ([46]; see also Table S1 in the supplemental information online).

Transcription and Translation

Several specific enzymatic reactions important for LDCs have been discussed, but an additional layer of regulation should be taken into account: the transcription and translation of the genes that encode these enzymes, and many others, is dynamic across the day–night cycle. Generally, gene expression decreases in the dark, with a few exceptions [3,47,48]. Of the genes that are expressed upon dark exposure, some can be classified as being induced by darkness independent of the time of day or circadian phase [49], while the expression profile of others in darkness relies on a functioning circadian clock [3].

It is clear that circadian regulation provides a fitness benefit to cyanobacteria when cells are in sync with the environmental LDC [50]. Yet, there is considerable overlap between genes found to be induced during dark exposure at night-time [3], those induced by dark exposure during the day [49], and those induced by dim light during the late afternoon [51] (Table S1). Although many of these genes encode either proteins of unknown function or are annotated simply as *dig* (for dark-induced gene), some gene annotations provide clues to important functions during dark exposure. For example, a protein with high sequence similarity to CP12 (*synpcc7942_0252*) [14], a regulator of ribosomal status *hpf* (*lrtA; synpcc7942_2352*) [49], and a probable chaperone protein (*hspA, synpcc7942-0241*) suggest that redox regulation and control of protein synthesis and degradation are key processes in the dark.

Examination of the protein landscape tells a different, less dramatic story. Ansong *et al*. [52] showed that only 4% of proteins in *Synechococcus* sp. PCC 7002 change in abundance between light and dark. This trend is also seen in other cyanobacteria; for example, few proteins were found to have >twofold change across light and dark phases in *Cyanothece*, a N_2-fixing cyanobacterium [53]. However, while the overall abundance of protein is relatively constant, the rate of translation of proteins has been observed to decrease in the dark [17,49], and post-translational modifications and intracellular localizations may act as strong regulatory components at the protein level in LDCs.

Orchestration of Cell Physiology under Light–Dark Cycles

The many metabolic changes that occur during the day–night transition are managed through a complex network of interconnected regulatory processes. The majority of the genome in *S. elongatus* is differentially transcribed in LDCs. A number of factors drive both transcriptional and protein levels, including: circadian clock output, chromosome topology, signaling nucleotides, and changing concentrations of metabolites, such as NADPH and ATP (Figure 3).

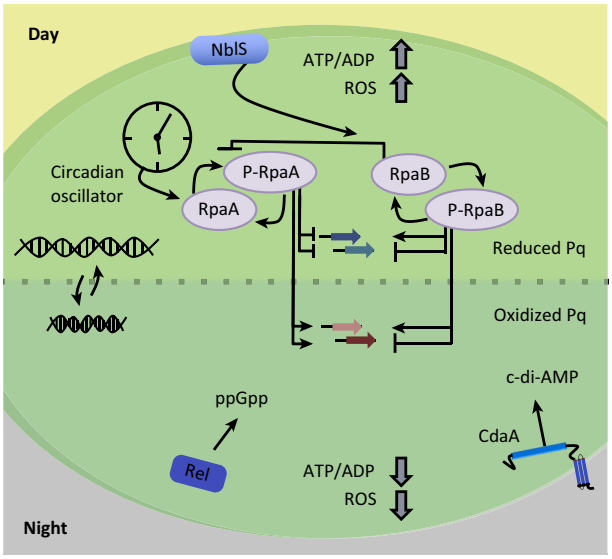

Figure 3. Getting the Message: Signaling Pathways Important for the Day–Night Transition. The signaling pathways effective during the day-to-night transition consist of environmental sensing via RpaB and its cognate histidine protein kinase NblS, circadian status via RpaA, intracellular redox and energy status through changes in concentrations of NADPH and ATP, and redox state of the plastoquinone (Pq) pools, chromosome compaction, and nucleotide signaling molecules such as ppGpp and cyclic-di-AMP (synthesized by RelA and CdaA, respectively).

Transcription Factors

While multiple transcription factors affect transcription in cyanobacteria, two with wide-ranging influence stand out. The response regulators RpaA and RpaB are master transcription factors in cyanobacteria that act as control hubs of LDC physiology. RpaA provides the key output mechanism to convey temporal information from the circadian clock, and is responsible for the clock-dependent regulation of hundreds of genes [54,55].

The link between the circadian clock – whose oscillator comprises proteins KaiA, KaiB, and KaiC – and activity of the transcription factor RpaA lies with two histidine protein kinases, SasA and CikA, which engage with the oscillator complex at different times of day [56]. Association of SasA or CikA with the oscillator stimulates an activity that either phosphorylates RpaA (SasA) or dephosphorylates RpaA (CikA) [57]. Phosphorylated RpaA (P-RpaA) activates genes important for night-time metabolism, and in the absence of RpaA (or KaiA [27]) the clock is locked into a daytime transcriptional regime that leads to metabolic imbalance, increased oxidative stress, and death in LDCs [2,27,54,58]. In contrast, a strain that is locked in a 'night-time' mode through elimination of KaiC experiences constitutive expression of the OPPP and other night-time metabolic pathways. This situation is generally permissive for growth in LDCs, although the lack of a timing mechanism exacts a fitness cost that can be observed when WT and KaiC-null strains are grown together in competition [59].

Less investigated is the activity of RpaB. It acts as a light-responsive regulator of gene expression that is independent of the clock [60,61]. Manipulating RpaB expression level or phosphorylation state affects growth in LDCs, and its output overlaps with that of RpaA in ways that are not yet understood [62]. While RpaA acts as the sole output signal of the clock, RpaB feeds in signals of environmental light status to the cell, and integration of both signals is important for fitness in LDCs.

Chromosome Topology

One striking cellular change in cyanobacteria that occurs over a daily cycle, and is circadian-controlled, is that of chromosome topology. The extent of chromosome or plasmid compaction

in *S. elongatus* varies depending on the time of day, being compact and highly supercoiled at some times and relatively relaxed at others [9,63–65]. When supercoiling is relaxed by the addition of an inhibitor of DNA gyrase, changes in gene expression patterns are observed. Expression of genes that are normally expressed when the genome is in a highly supercoiled state decrease, and those normally expressed when the genome is relaxed increase, upon the addition of the inhibitor, consistent with expectations for the time-of-day peak expression of a given gene and the topological state. While mostly correlative, this relationship between DNA compaction and supercoiling with transcriptional outputs suggests another possible mechanism for global regulation. The details of how genome organization, compaction, and accessibility to interacting proteins may impact gene expression, protein localization, and cell division [16,66,67] is an area ripe for further study.

Cellular Energy Levels

Shifts in ATP/ADP and NADPH/$NADP^+$ ratios unavoidably occur in LDCs due to temporal division of photosynthesis and catabolism. The dynamics of ATP changes in LDCs have been measured by many researchers over decades, but results vary. In *S. elongatus* ATP levels were reported to fall precipitously within the first 2 min after a shift to darkness and then recover to near pre-dark levels within 20–60 min [48,68]. More recently, Rust *et al*. made similar measurements, but over a longer duration of dark exposure. They found that, despite this quick recovery, ATP levels gradually decrease overall during dark exposure, reaching ~50% of the pre-dark level and remaining low until light is reintroduced, after which the ATP concentration rapidly recovers [69]. The physiological implication of these changes is likely to be global for the cell. For instance, ATP concentration directly affects the status of the circadian oscillator and its stimulation of the RpaA kinase, SasA [69].

Cellular Redox

Cellular redox homeostasis is of critical importance for growth in LDCs [52,70,71]. Mutants that are unable to funnel carbon metabolites through the OPPP produce an insufficient amount of NADPH at night [2]. This deficit results in diminished ability to detoxify cellular ROS accumulated during the day, which requires NADPH reducing equivalents [2,8,72]. Thus, limiting night-time NADPH production by compromising OPPP activity likely has severe redox consequences for broad metabolic and regulatory systems in photoautotrophic microorganisms.

One common theme between cyanobacteria and plant chloroplasts is the redox-dependent regulation of many proteins [73]. Although both photosynthetic reactions and the OPPP generate NADPH, reductant levels drop in the dark as the photosynthetic reactions cease [14,68]. Compounding this redox change, NADH levels rise in the dark, leading to a further decrease in the NADPH/NADH ratio [14]. Overall, the concentrations of NADPH and $NADP^+$ vary to an even greater extent than that of ATP over the course of LDCs, and have been correlated with changes in the transcription of genes that encode enzymes of photosynthetic and metabolic homeostasis processes [71]. In addition, many metabolic enzymes are redox-modified. As LDCs drive the changes in oxidation state that control these modifications, the subsequent enzymatic activities also follow suit [14,52,70,73–76] (Figure 1).

In the absence of notable changes in protein levels, redox modifications of metabolic enzymes likely play a major role in dictating metabolic flux when cells are in the dark. Indeed, many of the critical enzymes required for survival in darkness are redox modified, including Zwf, Gnd, GlgC, GlgA, GlgP, and enzymes that regulate nitrogen assimilation [70,77,78]. Redox regulation directly mediates the critical shift between CBBC and OPPP activity at light-to-dark transitions through the direct inhibition of the CBBC enzymes Gap2 and Prk by CP12 in a redox-controlled

and light-dependent manner [14,15]. Moreover, numerous redox-active proteins and small molecules – thioredoxins, ferredoxins, peroxiredoxins, and glutathione – can directly modify target protein thiols [77–81] and impact enzymatic activity [52,70]. While many questions remain regarding the mechanisms of redox regulation during growth under LDCs, the maintenance of redox homeostasis is unquestionably important for cyanobacterial metabolic processes at night.

Signaling Nucleotides

Evidence is accumulating that signaling nucleotides act as intracellular messengers of LDCs. Levels of cAMP, c-di-AMP, c-di-GMP, and ppGpp are all light-dependent in cyanobacteria [40,49,82,83]. ppGpp, in particular, is a potent effector of transcription in *S. elongatus* that is synthesized after a light-to-dark transition, and is critical for maintaining fitness during dark-induced stress [49]. Viability in cells unable to synthesize ppGpp is impaired after exposure to darkness, although the mechanisms behind this phenotype are not yet known.

c-di-AMP, a newly discovered signaling nucleotide in cyanobacteria [40,84], is also important for survival of *S. elongatus* during darkness. Inactivation of its cyclase, *cdaA*, leads to increased oxidative stress and decreased survival of the night periods of LDCs [40]. c-di-AMP and ppGpp levels are linked in Firmicutes [85,86], bringing up the possibility that their activity is coordinated in cyanobacteria grown in LDCs; however, this potential connection remains unexplored.

Beyond Cyanobacteria

The need for metabolic shifts as an adaptation to diel cycles is also a dominating force for plants and eukaryotic algae, and influences global biogeochemical cycles such as CO_2 balance. In plants, constant adjustments to physiology in response to changes in light quality, intensity, and duration are made through the use of photoreceptors [87]. Changes in the photoperiod of LDCs cue plants to undergo different phases of growth, development, and metabolism. In *Arabidopsis*, for example, darkness elicits the expression of over 80 genes that code for functions involved in photosystem II inhibition, starch degradation, chloroplastic translation inhibition, and redox regulation, similar to what we observe in cyanobacteria [88].

The responses of plants to darkness and LDCs are also important from an economic and agricultural point of view. Post-harvest storage of green leafy vegetables, such as kale and cabbage, in LDCs results in significantly improved appearance and health value of crops compared to constant-condition controls due to increased tissue integrity, chlorophyll content, and levels of glucosinolates [89,90].

Moreover, photosynthetic organisms are not alone in struggling with oxidative stress during LDCs, and some of the preventative mechanisms they utilize may be conserved in heterotrophs. For instance, hovering flight in nectarivores is an immensely energetic endeavor that comes with high metabolic turnover that generates ROS. In hawkmoths, like cyanobacteria, this oxidative stress is likely detoxified by the activity of the OPPP during rest, which, by producing NADPH, maintains sufficient quantities of reduced glutathione to act as an antioxidant [91] (Figure 2). This strategy for oxidative stress management after intense exercise may be of importance in other animals as well [92].

Concluding Remarks

Lessons from the genetically tractable and evolutionarily ancient cyanobacteria can educate us on the metabolic strategies that have evolved to enable organisms to deal with LDC stress, a phenomenon that is difficult to study in many other phyla. Insights from cyanobacteria can also

Outstanding Questions

Increased ROS has been correlated with growth defects in all of the LDC-sensitive mutants where it has been assayed. However, is this truly a causal relationship? Is ROS the dominant stress that must be mitigated in LDCs, and what are the important pathways in resisting this stress?

How can the direct response to LDCs be perturbed in a controlled way (through mutant or condition) to enable observations, similar to the manipulations that have proven so fundamental to research on the circadian clock?

While transcript and protein data can provide insight into metabolism during LDCs, we currently do not have a good picture of what the total metabolome looks like as cells transition between day and night growth phases. Is the flux of carbon and accumulation of metabolites indicative of a restorative process or an active process to prevent light-induced redox stress?

What differences are detected in physiological responses when cells are exposed to abrupt (square-wave) versus sinusoidal LDC (more like that found in nature)?

While the activities of the circadian clock output transcription factor RpaA when it is in its phosphorylated active state have been detailed as being important for LDC viability, what is its role, if any, when it is in its 'inactive' unphosphorylated state during the day?

Cyanobacteria are pervasive across latitudinal space and must contend with large seasonal variations in day and night lengths, but how the cyanobacterial clock functions when driven by LDCs of different photoperiods, as would be present in different seasons, is still a relatively unexplored area in the field.

aide in developing strategies to harness photosynthetic organisms for real-world industrial, biotech, and agricultural applications.

In this review, we have attempted to condense much of the knowledge that now exists on the cyanobacterial response to LDCs. There remain, though, many areas for growth in this respect (see Outstanding Questions). Many variables, such as protein and metabolite levels, appear surprisingly constant in LDCs on average. However, this balance seems possible only in the context of large shifts in transcription, primary metabolism, and glycogen levels that occur during the day. These shifts, in turn, are caused by large alterations in ratios of electron carriers, redox poise, nucleotide signaling, and chromosome structure, all compelled by signals from the internal circadian clock and external environment. Thus, maintaining physiological balance in a world of potentially jarring LDCs is an exhaustive task that requires a vigilant sensing of external and internally generated signals, and appropriate cellular responses.

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Appendix A Supplementary Information

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