Molecular Plant • Volume 5 • Number 1 • Pages 1–13 • January 2012 REVIEW ARTICLE

Emerging Perspectives on the Mechanisms, Regulation, and Distribution of Light Color Acclimation in Cyanobacteria

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ABSTRACT Chromatic acclimation (CA) provides many cyanobacteria with the ability to tailor the properties of their light-harvesting antennae to the spectral distribution of ambient light. CA was originally discovered as a result of its dramatic cellular phenotype in red and green light. However, discoveries over the past decade have revealed that many pairs of light colors, ranging from blue to infrared, can trigger CA responses. The capacity to undergo CA is widespread geographically, occurs in most habitats around the world, and is found within all major cyanobacterial groups. In addition, many other cellular activities have been found to be under CA control, resulting in distinct physiological and morphological states for cells under different light-color conditions. Several types of CA appear to be the result of convergent evolution, where different strategies are used to achieve the final goal of optimizing light-harvesting antenna composition to maximize photon capture. The regulation of CA has been found to occur primarily at the level of RNA abundance. The CA-regulatory pathways uncovered thus far are two-component systems that use phytochrome-class photoreceptors with sensor-kinase domains to control response regulators that function as transcription factors. However, there is also at least one CA-regulatory pathway that operates at the post-transcriptional level. It is becoming increasingly clear that large numbers of cyanobacterial species have the capacity to acclimate to a wide variety of light colors through the use of a range of different CA processes.

Key words: Chromatic adaptation; phycobilisome; cyanobacteria; light regulation; gene regulation; signal transduction; light harvesting; phenotypic plasticity.

INTRODUCTION

Cyanobacteria comprise a phylogenetically cohesive group of gram-negative prokaryotes capable of oxygenic photosynthesis (Stanier and Cohen-Bazire, 1977; Woese, 1987), generally characterized by the presence of chlorophyll *a* and accessory pigments called phycobiliproteins. They are one of the oldest groups of bacteria, dating back to the Pre-Cambrian by some estimates (Schopf, 2002) to as much as 3.5 billion years ago, and their influence on our planet has been considerable. Unlike other photosynthetic bacteria, during the 'light reactions', they use both Photosystem I (PSI) and II (PSII) to extract and transfer electrons from water molecules to electron acceptors and generate oxygen as a by-product. They are also unique in their ability to fix both carbon and nitrogen from the atmosphere under aerobic conditions, which, along with their tolerance of a wide range of environments, has allowed colonization of many of the most extreme biotopes on Earth. From oligotrophic oceans (Paerl, 2000) to the arid Antarctic, subtropical deserts (Wynn-Williams, 2000), and the hot springs of Yellowstone (Ward and Castenholzh, 2000), cyanobacteria have adapted to occupy and expand the boundaries of the biosphere.

As photoautotrophic organisms, the rates of photosynthesis and growth of cyanobacteria are directly affected by the physical parameters of the environment, particularly light. Since this resource can vary in terms of both quality (color) and amount (intensity), sensing and adequately responding to light is a key attribute of their eco-physiological versatility.

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doi: 10.1093/mp/ssr054, Advance Access publication 19 July 2011
Received 7 February 2011; accepted 31 May 2011

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At the cellular level, the cyanobacterial photosynthetic apparatus and its light-harvesting antennae, called phycobilisomes (PBS), are intimately attuned to ambient light conditions. In large part, by controlling the size, composition, number, and location of PBS, these organisms have perfected the fine balance between maximizing the absorption of light for photosynthesis and minimizing the accumulation of excess energy in their reaction centers, which can lead to photoinhibition (damage to PSII reaction centers).

Cyanobacteria have adopted a number of photosynthetic strategies to help them cope with changes in their light environment. The different light absorption properties of the two photosystems and their associated light-harvesting proteins require frequent balancing of the input excitation energy between the two reaction centers. Such adjustments can act on a very short timescale (seconds to minutes) and these are called state transitions. Preferential excitation of PSII leads to state 2, in which excess energy is channeled to PSI, while over excitation of PSI leads to state 1, during which energy is redistributed to PSII. State transitions in plants are accomplished through phosphorylation and redistribution of the light-harvesting complex II within thylakoid membranes (Rochaix, 2007; Kargul and Barber, 2008). In cyanobacteria, this process is not as well understood, although several lines of evidence suggest that PBS are capable of transferring excess energy to PSI during state 2 and that *rpaC*, a cyanobacteria-specific gene, is essential for this response (Fujita et al., 1994; Bhaya et al., 2000; Mullineaux and Emlyn-Jones, 2005). Structural components of the PBS have been shown to be required for state transitions (Kondo et al., 2009). In addition, cyanobacteria undergoing CA have been found to exist in different states, depending on the ambient light color (Campbell, 1996). This will be discussed further below. In addition, although cyanobacterial species are usually specialized to a particular light-irradiance niche, they can acclimate to a range of irradiances above and below the level required for maximal rates of photosynthesis through a process termed photoacclimation, the subject of a number of reviews (Wyman and Fay, 1987; Anderson et al., 1995; MacIntyre et al., 2002; Walters, 2005). They also are able to deal with excess light energy through the use of a number of photoprotection mechanisms, which have also been reviewed recently (Kirilovsky, 2007; Bailey and Grossman, 2008; Kirilovsky, 2010). Lastly, cyanobacteria are capable of sensing and responding to light color, and this review will focus on these long-term acclimation responses of cyanobacteria to changes in ambient light color. This process, which occurs in a wide range of species and many different habitats, involves shifts in PBS composition via the induction of specific genes and the coordination of these changes with many additional aspects of cellular physiology and morphology.

PBS STRUCTURE

PBS reside on the cytoplasmic surface of the thylakoid membrane and consist of phycobiliproteins with covalently attached bilin (open-chain tetrapyrrole) chromophores and linker proteins. They increase the cross-sectional area for light capture and transfer this energy to the photosystem reaction centers. These structures are remarkably plastic, capable of adjusting to optimize photon capture rates in different environmental conditions by changing size, shape, protein and bilin composition, cellular number, and association with photosynthesis reaction centers. While a variety of PBS forms exist, the most common is hemidiscoidal. Fan-shaped in appearance, such PBS consist of an inner core and a series of outwardly extended rods (Figure 1). Significant variety exists even within this structural subtype, with species- and strain-specific differences in the size of the core, the number and length of the rods, the types and number of attached bilins, and the protein makeup of the rods themselves. Numerous reviews addressing the details of PBS composition are available (Sidler, 1994; MacColl, 1998; Adir, 2005), so only a basic introduction will be provided here. Both rods and cores are cylindrical structures composed of a series of discs of phycobiliproteins, which consist of chromophorylated alpha and beta subunits that are together called a 'monomer'. Two stacks of three monomers form a disc. Linker proteins hold the discs together, keep the rods connected to the core, and facilitate unidirectional energy transfer from the outer portions of the rods into the reaction centers. In addition, specialized linker proteins keep the core, and thus the PBS itself, associated with the thylakoid membrane. While different PBS may be composed of rods with various types of phycobiliproteins, the core-distal discs always have absorption wavelength maxima that are shorter than or equivalent to the core-proximal ones to ensure unidirectional energy transfer into the reaction center. In hemidiscoidal PBS, which are the most common and best-studied PBS form, cores consist of the phycobiliprotein allophycocyanin (AP; absorption maximum (λ_{max}) = 650 nm) and the core-proximal discs in the rods are made of phycocyanin (PC; λ_{max} = 620 nm). Depending on the species and environmental conditions, the core-distal discs may be made of either PC or phycoerythrin (PE; λ_{max} = 540 nm), as shown in Figure 2 and discussed below.

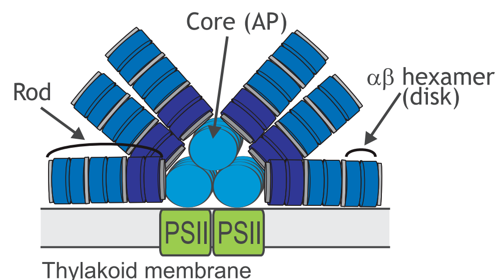

Figure 1. Face View of a Hemidiscoidal Model PBS.

The core (light blue) contains three cylinders, primarily consisting of allophycocyanin (AP), and provides attachment of the PBS to PSII. The outwardly oriented rods (dark blue and purple) are made of linker proteins (gray) and αβ hexamers of phycocyanin 1 (PC1) (purple) or phycocyanin 2 (PC2). See text for details of PC1 and PC2.

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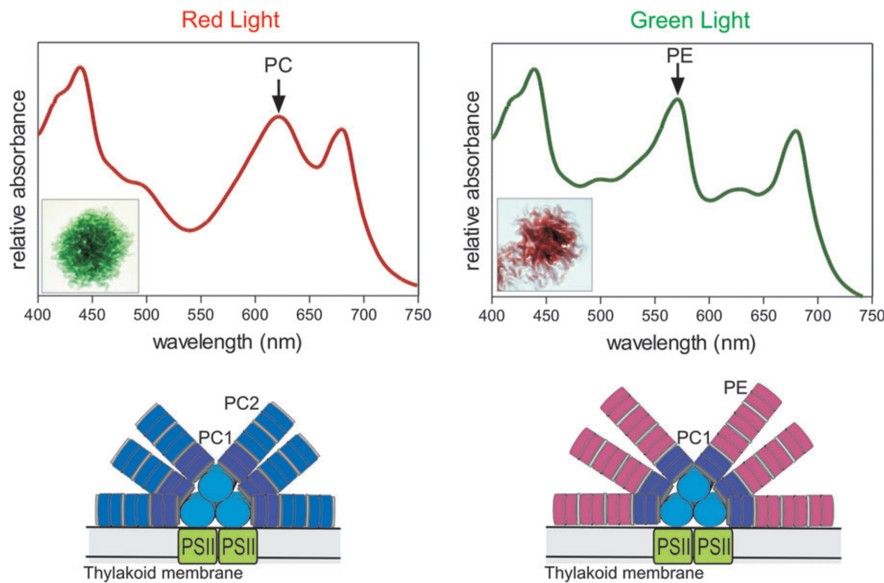

Figure 2. The Color Phenotypes and Whole-Cell Absorption Spectra of *Fremyella diplosiphon* Cells Grown in Red Light (Left) and Green Light (Right).
In red light, the blue–green pigmentation is due to accumulation of PC in the outer rods of PBS. In green light, the cells are brick red because PE accumulates in the PBS rods. Maximum PE and PC absorption peaks are indicated. Chlorophyll *a* absorption peaks are at 430 and 680 nm.

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CHROMATIC ACCLIMATION RESPONSES

Overview of Chromatic Acclimation Responses as Defined by PBS Structural Changes

In addition to light irradiance variation, cyanobacteria experience differences in light color in their natural environment. When exposed to different light colors, many species are able to adjust the composition of their PBS through a process known as chromatic acclimation or adaptation (CA) (Tandeau de Marsac, 1977, 1983; Kehoe and Gutu, 2006). Although 'adaptation' was used when this process was initially discovered, this response does not appear to involve any genetic alteration, and significant data support the premise that CA is the result of changes in gene-expression patterns. Thus, the term 'acclimation' was proposed (Kehoe and Gutu, 2006) and will be used instead of 'adaptation'. Over the years, our knowledge of the different colors of light being sensed during CA has expanded as CA-capable cyanobacteria continue to be discovered in additional environments. The original description of CA involved species that were capable of sensing red and green light (Gaiducov, 1902), but, more recently, additional species that sense blue and green, and red and infrared light have also been uncovered (Palenik, 2001; Duxbury et al., 2009). This list may expand as more CA-capable species are identified in the future.

Cyanobacteria containing both PE and PC and thus potentially capable of red–green CA were classified in a comprehensive study by Tandeau de Marsac and subsequent studies (Tandeau de Marsac, 1977; Bryant, 1981, 1982; Tandeau de Marsac, 1983). Group I species did not alter PC or PE levels in response to changing light colors. Group II species had higher PE levels in green light than in red light, while PC levels did not change in either light condition. The third group, called Group III, had higher PE levels in green light than in red light but also accumulated more PC in red light than in green light (Figure 2). It was a Group III species that was first noted to be capable of CA almost a century earlier (Engelmann, 1902; Gaiducov, 1902, 1903) and this response was named 'complementary chromatic adaptation' because the color of the cells was complementary to the ambient light color: the accumulation of PE makes these cells red colored in green light, while high levels of PC makes them blue–green in red light (Figure 2). The filamentous Group III species *Fremyella diplosiphon* UTEX 481 (also called *Calothrix* or *Tolypothrix* sp. PCC7601) has been used extensively for CA studies and many aspects of the photobiology and molecular biology underlying this process have been uncovered in this organism (Tandeau de Marsac, 1983; Grossman, 2003; Kehoe and Gutu, 2006). Group II species are considered to be capable of undergoing 'type 2' CA, or CA2, while Group III species undergo 'type 3' CA, or CA3. These two groups are widely distributed globally and have been found in freshwater, marine, hot springs, and soil environments (Carr, 1973; Tandeau de Marsac, 1983; Postius et al., 2001; Dufresne et al., 2008; Acinas et al., 2009; Duxbury et al., 2009).

Several additional types of CA have been uncovered more recently. Type 4 CA (CA4) is responsive to blue and green light and thus far has only been found to occur in the marine environment (Palenik, 2001). Unlike type 2 and type 3 CA, there are apparently no major changes in PBS protein composition during CA4. Instead, it appears to be the ratio of two bilin isomers that are attached to a specialized type of PE called 'PEII' in the most core-distal portions of the rods of the PBS that change (Palenik, 2001; Everroad et al., 2006). In blue light, these cells have a relatively high ratio of the blue-light-absorbing chromophore phycourobilin (PUB; λ_{max} = 495 nm) to the green-light-absorbing chromophore phycoerythrobilin (PEB; λ_{max} = 545 nm), while, in green

light, this ratio decreases significantly (Figure 3A). These changes in the PUB:PEB ratio change the color phenotype of these cells between orange and pink (Figure 3B). Another recently described type of CA appears to occur in at least one strain of *Acaryochloris marina*. PC-containing PBS increase during growth in 625 nm light and decrease in 720 nm light, suggesting that this organism has the ability to acclimate to changes in its near-infrared light environment (Duxbury et al., 2009).

Distribution and Role of CA in the Natural Environment

The ability to carry out CA is not restricted to a single branch of the cyanobacterial lineage. It occurs in all major groups of cyanobacteria and is not strongly correlated with any particular type of environment, but rather is a geographically widespread process (Carr, 1973; Tandeau de Marsac, 1983; Postius et al., 2001; Dufresne et al., 2008; Acinas et al., 2009; Duxbury et al., 2009). Despite our extensive molecular understanding of at least some forms of CA (see below), its role(s) in the natural environment has not yet been clearly elucidated. The spectral partitioning of light as it is absorbed by water is an attractive possible explanation for the existence of CA. As white light passes through water, red wavelengths are best absorbed, followed by green, then blue. Thus, CA-capable species may adjust the absorption characteristics of their PBS to match the ambient spectral distribution of light, which varies with depth. Underwater light quality is also influenced by the background turbidity due to particulates and other organic dissolved material, as well as perhaps by the vibrations of water molecules themselves (Postius et al., 2001; Stomp et al., 2007). Other field observations have shown that PE-containing cyanobacteria are prevalent in environments in which green light prevails, and cyanobacteria containing only PC are more abundant in red light-rich environments (Voros et al., 1998; Vila and Abella, 2001). However, the CA capacity of these organisms was not analyzed. Interestingly, cyanobacteria that appear to be capable of CA exist in microbial mats of hot springs (Brown et al., 2010). In this case, it is possible that CA provides a fitness advantage for a species that must cope with light that has been spectrally altered as a result of the absorption of specific wavelengths by neighboring organisms. In fact, it is likely that CA is capable of conferring a selective advantage in multiple environmental settings. CA3 has been shown experimentally to convey a fitness advantage during growth in changing light color conditions (Stomp et al., 2004), although its benefits are evident only when the light environment fluctuates on a timescale longer than the timescale required for CA-driven changes in PBS composition to occur (Li and Kehoe, 2008; Stomp et al., 2008).

The PBS composition changes that occur during CA3 have also been shown to maximize the efficiency of photon capture for photosynthesis. When *F. diplosiphon* cells are fully acclimated to and grown in red light, they have comparable chlorophyll *a* levels and exhibit photosynthesis rates similar to *F. diplosiphon* cells that have been fully acclimated to and grown in green light (Campbell, 1996). However, this study also showed that when either red or green light-acclimated cells are shifted to the opposite light color, the rate of photosynthesis drops by approximately 40%. Fluorescence, photochemical, and non-photochemical quenching measurements demonstrated that, in red light, which can be used by both PC and chlorophyll *a*, cells are in state I, with PSII exhibiting relatively high oxidation rates and low non-photochemical quenching. Conversely, green light-acclimated cells are in state II, in which some of the light absorbed by PE is channeled to drive PSI photochemistry and higher non-photochemical quenching of PSII occurs (Campbell, 1996). This 'long-term' state transition phenomenon, physiologically, is equivalent to classical state transitions, which are historically defined as occurring only on very short timescales and are controlled by redox states of the plastoquinone pool (Fujita et al., 1994; Li and Sherman, 2000).

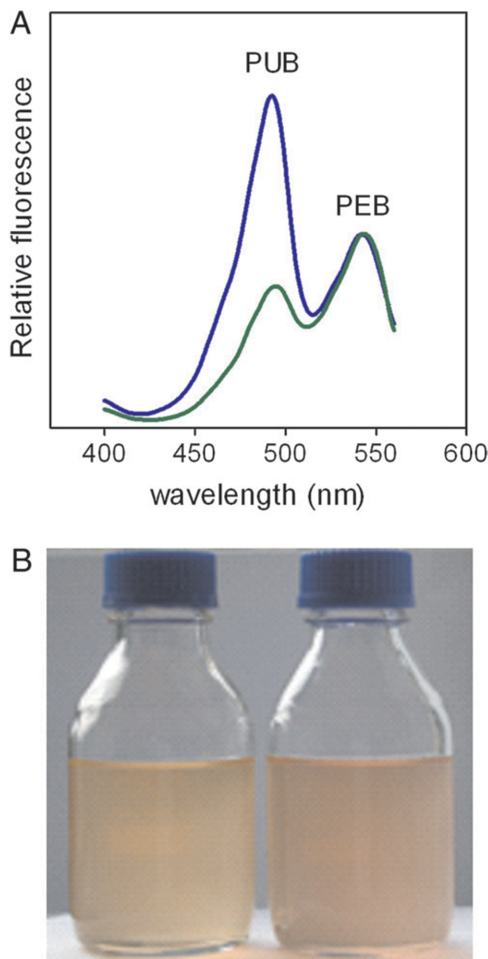

Figure 3. Fluorescence and Phenotypic Color Changes during CA4 in the Marine Unicellular Bacterium *Synechococcus* sp. RS9916.

(A) Fluorescence excitation spectra of RS9916 cells grown in blue light (blue line) or green light (green line) showing the change in the relative fluorescence excitation of PUB at 495 nm and PEB at 550 nm under the two light conditions.

(B) Whole-cell color differences between cells grown in blue light (left) and green light (right).

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Interestingly, recent results implicate CpcG rod-core linkers of *Synechocystis* sp. PCC 6803 in the process of state transitions, and the expression of one family member has been reported to be regulated by red and green light (Kondo et al., 2005; Katayama and Ikeuchi, 2006; Kondo et al., 2009). This suggests a possible area of study for exploring mechanisms by which 'long-term' state transitions may be maintained in CA-capable cyanobacterium.

Additional Cellular Changes Occur During CA

Although CA was originally defined by the capacity to modify the composition of PBS in response to changing light colors, many additional physiological and morphological responses that are light color regulated have been identified. In the CA3 species *F. diplosiphon*, for example, cells acclimated to red or green light have different morphologies. In red light, the filaments are significantly shorter than in green light. Also, individual cells are larger and more rounded in red light, while in green light, they are cylindrical (Bennett and Bogorad, 1973; Bogorad et al., 1983; Bordowitz and Montgomery, 2008). The shortened filament length in red-grown cells is due, at least in part, to the formation of necredia (cells undergoing programmed cell death) along the filament (Bennett and Bogorad, 1973; Bogorad, 1975). The reason for these changes is not yet known. Many cellular processes in *F. diplosiphon* are regulated by light color as well. Gas vesicle-gene expression and the development of hormogonia, which are short, motile filaments important for dispersal and survival (Rippka et al., 1979), are also regulated in part by red and green light in this species (Tandeau de Marsac et al., 1988; Damerval et al., 1991; Campbell et al., 1993). In addition, microarray and two-dimensional-protein gel electrophoresis analyses showed that at least 80–100 non-PBS proteins are CA3 regulated in *F. diplosiphon* (Stowe-Evans et al., 2004). Among these is *chlL*, encoding a subunit of the light-independent form of protochlorophyllide reductase, which catalyzes the penultimate step in chlorophyll *a* synthesis. This gene and *chlB* and *chlN*, encoding the other two subunits of this enzyme, are more highly expressed in green light than red light—a response that likely compensates for the decreased activity of the alternative form of this enzyme, PorA, which is activated by red light (Shui et al., 2009). Although these examples are from *F. diplosiphon*, CA2 and CA4 species are also very likely to have many CA-regulated cellular responses in addition to PBS biogenesis.

Molecular Basis of CA-Mediated PBS Biogenesis and the Regulatory Systems Controlling CA

Most research on the molecular mechanisms and regulation of CA has focused on CA3 in *F. diplosiphon*. However, studies examining how CA2 and CA4 operate, and how they are regulated, are now being carried out as well. These have begun to contribute to our overall understanding of the process of CA and how it is regulated at the molecular level. A summary of each of these is provided below.

Type 3 CA

Studies of the photobiology and action spectrum of CA3 were initiated over 50 years ago in two related species: *Tolypotrix tenuis* and *F. diplosiphon*. These investigations demonstrated that CA3-mediated regulation of PC and PE synthesis was photoreceptor controlled, photoreversible, and that PC accumulation was induced maximally in red light (approximately 641 nm), while the PE increase was most effectively elicited by green light (approximately 541 nm) (Hattori and Fujita, 1959; Fujita and Hattori, 1960, 1962; Diakoff and Scheibe, 1973; Haury and Bogorad, 1977; Vogelmann and Scheibe, 1978; Ohki et al., 1982). These findings suggested that a phytochrome-like photoreceptor controlled the CA3 response, even though it was maximally responsive to red and green light rather than the red and far-red light regions sensed by plant phytochromes (Bogorad, 1975; Tandeau de Marsac, 1983). Also, several studies showed that, because there was little turnover of phycobiliproteins during this response, *de novo* synthesis of PC and PE must be primarily responsible for the changes in PBS composition that occur during CA3 (Bennett and Bogorad, 1973; Ohki and Fujita, 1978; Gendel et al., 1979).

In most CA3-capable species examined, red light induces the production of a form of PC called PC2 (Bryant, 1981), which makes up the distal portions of the PBS rods (Figure 2). In green light, the distal discs of rods are instead made of PE (Figure 2). The structures shown in Figure 2 are for *F. diplosiphon* (Rosinski et al., 1981; Siegelman and Kycia, 1982), but variations of these structures exist, depending upon the CA3 species examined (Tandeau de Marsac, 1983; Ohki et al., 1985; Westermann et al., 1993; Westermann and Wehrmeyer, 1995). PC1 is present in both types of PBS, because its presence facilitates the transfer of light energy from the distal regions of the rod to the core. Two forms of PC may not exist in all cyanobacteria capable of chromatic acclimation, although this issue has not been resolved (Bryant, 1982; Tandeau de Marsac, 1983). In *F. diplosiphon*, the PBS core remains largely unaltered after switches between red and green light.

Studies of the molecular basis of these pigmentation changes showed that changes in PBS composition during CA3 was controlled at the RNA level in *F. diplosiphon*. The expression of *cpcB1A1*, encoding PC1, is not significantly different during growth in red versus green light, consistent with the presence of this protein in both light conditions (Conley et al., 1986, 1988; Houmard et al., 1988; Mazel et al., 1988). The mRNA produced from the operon encoding PC2 (*cpcB2A2-H2I2D2*, hereafter called *cpc2*), which is significantly higher in red light than in green light (Conley et al., 1985; Lomax et al., 1987; Conley et al., 1988), is controlled primarily at the level of transcription (Oelmüller et al., 1988a; Casey and Grossman, 1994). The induction of *cpc2* mRNA expression is relatively rapid in red light, reaching a relatively steady state in 2 h, which is similar to the rate of its decline in non-inducing conditions (Oelmüller et al., 1988a). The alpha and beta subunits of PE are encoded by *cpeBA* (Mazel et al., 1986) and the PE

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linkers are encoded by the first three genes of the *cpeCDESTR* (*cpeC*) operon (Federspiel and Grossman, 1990; Federspiel and Scott, 1992). mRNA levels from the *cpeBA* and *cpeC* operons increase rapidly and reach a maximum 4–8 h after transfer from red to green light (Federspiel and Grossman, 1990). In addition, the genes encoding the chromophore-synthesis enzymes (Frankenberg et al., 2001) are regulated by CA3 in *F. diplosiphon*. The expression of *pcyA*, which encodes an oxidoreductase that produces phycocyanobilin, the bilin that is attached to PC, is approximately fivefold higher in red light than in green light (Alvey et al., 2007), while *pebAB*, the operon that encodes two additional oxidoreductases that produce PEB for attachment to PE, is more highly expressed in green light than in red light (Alvey et al., 2003).

CA3 in *F. diplosiphon* is controlled by two light-responsive regulatory systems. The Rca (regulator for complementary chromatic adaptation) (Sobczyk et al., 1994) system activates *cpc2* and *pcyA*, and represses *cpeC* expression in red light (Li et al., 2008), while the Cgi (control of green light induction) (Kehoe and Gutu, 2006) system has no detectable effect on *cpc2* or *pcyA* expression and only represses *cpeC* expression in red light (Seib and Kehoe, 2002; Alvey et al., 2003; Li and Kehoe, 2008) (Figure 4). An interesting feature of this regulatory system is that the final gene in the *cpeC* operon, *cpeR*,

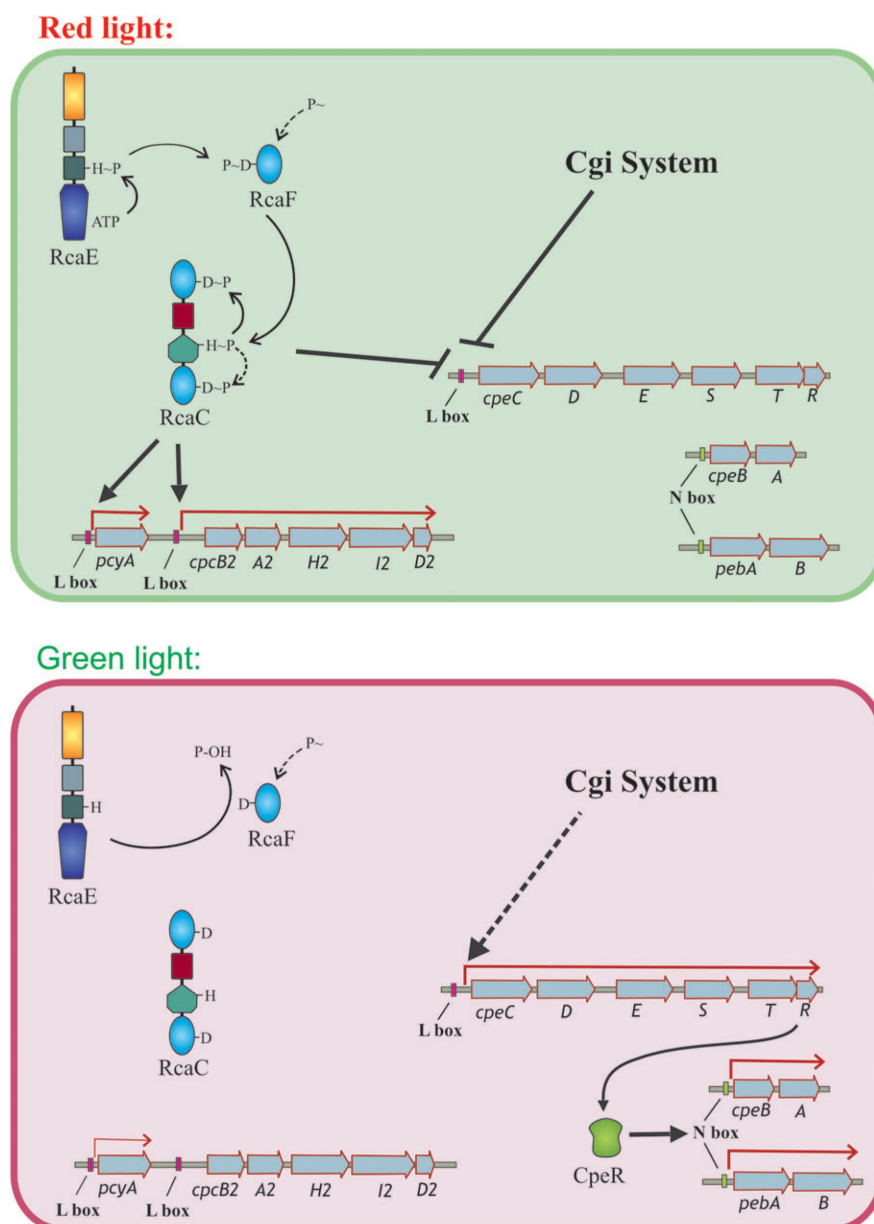

Figure 4. Current Model of the Asymmetric Regulation of PBS Genes during CA.

In red light, the Rca system appears to be phosphorylated due to the kinase activity of RcaE, which leads to enhanced binding of more highly phosphorylated RcaC to L-boxes. This leads to positive regulation of the *cpc2* and *pcyA* genes and negative regulation of *cpeCDESTR* expression. In green light, RcaE apparently acts as phosphatase, which biases the Rca system towards a dephosphorylated state and decreases RcaC binding activity. The red-light-induced genes are not activated, whereas the *cpeCDESTR* operon is expressed at a high level, which, through the production of CpeR, leads to the positive regulation of other green-light-induced genes (*cpeBA* and *pebAB*). Although not shown, the *chl* genes are also known to be under the control of the Rca and Cgi systems (Stowe-Evans et al., 2004; Shui et al., 2009). RcaF, and perhaps RcaC, may be phosphorylated to a certain extent by other cellular sources independently of CCA. The Cgi system contributes to CA3 regulation by further repressing the expression of *cpeCDESTR* in red light. In green light, the Cgi system may also slightly enhance the expression of this operon (indicated by the dashed line).

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encodes an activator that is required for the expression of *cpeBA* and *pebAB* (Cobley et al., 2002; Seib and Kehoe, 2002). This provides the *cpeC* operon with a central role in the CA3 regulation of *cpeBA* and *pebAB*, and both the Rca and Cgi systems regulate these two operons by controlling *cpeC* expression. Specifically how CpeR controls *cpeBA* and *pebAB* expression is not yet clear, although their promoters both contain a short, direct repeat sequence called the N-box (Kehoe and Gutu, 2006) that is bound by a protein called RcaA/PepB (Schmidt-Goff and Federspiel, 1993; Sobczyk et al., 1993; Alvey et al., 2003). While no functional evidence exists for their role in CA3 regulation, the N-box and RcaA/PepB are good candidates for the CA3 elements that link *cpeC*, *cpeBA*, and *pebAB* expression. It is not yet known whether or not CpeR, RcaA, and PepB are the same protein.

Analysis of the Rca pathway has provided insights into its composition and how it regulates PBS production during CA3. It is controlled by RcaE, a phytochrome-class photoreceptor containing a histidine-kinase domain, and two response regulators, RcaF and RcaC (Figure 4). RcaE was the first phytochrome-class photoreceptor discovered in bacteria (Kehoe and Grossman, 1996; Terauchi et al., 2004). The N-terminal half of RcaE contains a GAF domain similar to chromophore-binding domains of plant phytochromes, adjacent to a PAS domain. In the C-terminal region, the histidine-kinase module is present, containing the dimerization-histidine-phosphotransfer and ATP binding-catalytic domains. Similar to plant phytochromes, a cysteine within the GAF domain covalently binds a bilin chromophore *in vivo*, but, unlike the plant photoreceptors, which respond maximally to red and far-red light, RcaE appears to be green-red responsive (Terauchi et al., 2004). The type of bilin bound to RcaE has not yet been reported, nor has green–red photoreversibility of the purified protein been demonstrated. An *rcaE*-null mutant is phenotypically black in all light conditions due to the intermediate level of expression of the *cpc2, pcyA, pebAB, cpeC,* and *cpeBA* operons, which has been proposed to be due to non-light color-regulated phosphorylation of RcaF and RcaC by other cellular sources (Kehoe and Grossman, 1996; Terauchi et al., 2004). The response regulator RcaF is encoded immediately downstream of *rcaE* and consists of a single receiver domain containing a conserved aspartate residue that is typically the site of phosphorylation. *rcaF* mutants are phenotypically red in both red and green light due to the lack of *cpc2* expression and high-level production of *cpeC*, *cpeBA*, and *pebAB* (Kehoe and Grossman, 1997; Alvey et al., 2003). RcaC is a large, complex response regulator consisting of two receiver domains that are located at the N- and C-termini, an OmpR/PhoB-class-DNA binding domain, and a histidine-phosphotransfer domain. As with *rcaF* mutants, *rcaC* mutants are red in all light colors due to the absence of *cpc2* RNA and high-level expression of the genes required for the production of PE-containing PBS (Chiang et al., 1992; Kehoe and Grossman, 1997).

Several lines of evidence provide clues about phosphoryl group movement in the Rca pathway during CA3. The genetic data described in the previous paragraph, along with site-directed mutational analyses of the probable phosphorylation site histidines and aspartates within RcaE, RcaF, and RcaC, suggest that the Rca system is more phosphorylated in red light than in green light (Li and Kehoe, 2005). After RcaE autophosphorylation, phosphoryl group transfer to the aspartate of RcaF has been proposed, followed by transfer to the histidine of the histidine phosphotransfer domain of RcaC, and finally to the aspartate of the N-terminal receiver module of RcaC. The conserved aspartate within the C-terminal-receiver domain of RcaC is essentially not required for the CA3 response and may act as an input site for another signal transduction system, although this has not yet been shown (Li and Kehoe, 2005). RcaE has been proposed to act as a kinase in red light and a phosphatase in green light, based on the phenotypes of different classes of *rcaE* mutants (Figure 4) (Kehoe and Grossman, 1997; Terauchi et al., 2004).

In addition to the apparent effect of its phosphorylation state, the activity of the Rca system is significantly influenced by the cellular level of RcaC. *F. diplosiphon* produces five to six times more RcaC protein in red light than in green light (Li and Kehoe, 2005, 2008). This differs from the photoreceptor RcaE, which is present at equal levels in both red and green light (Terauchi et al., 2004). Part of this change occurs at the RNA level and these changes in RcaC levels are required, but not sufficient, for a normal CA3 response. This RcaC abundance change depends on the presence of the same conserved histidine and aspartate residues necessary for the CA3 response (Li and Kehoe, 2008). Thus, it appears that the combination of RcaC abundance changes and modification of RcaC by phosphorylation is needed to provide the dynamic range required for proper transcriptional control of highly expressed, RcaC-regulated genes.

In red light, RcaC activates the transcription of *cpc2* and *pcyA* and represses *cpeC* transcription by binding to the L-box—a seven base-pair-direct repeat located at –35 (relative to transcription start) within the *cpc2* promoter and in the inverse orientation at –78 of *cpeC* (Alvey et al., 2007; Bezy and Kehoe, 2010). In green light, the Rca system no longer activates *cpc2* or *pcyA*. In addition, *cpeC* is no longer repressed, leading to its expression and, as a result of the production of CpeR, the expression of *cpeBA* and *pebAB* (Cobley et al., 2002; Seib and Kehoe, 2002) (Figure 4). The operation of this system explains the phenotypes of *rcaF* and *rcaC* mutants well. In these lines, the lack of RcaC or its activation would lead to the persistent inability to activate the production of PC2 and to inactivate the synthesis of PE, resulting in the light-independent red coloration observed for these mutants.

RcaD is a protein that has been proposed to coordinate the expression of several operons whose activity is increased by red light. It consists of domains with similarity to E2 proteins of papillomavirus and the DNA polymerase sliding clamp. RcaD was found to protect two regions of the *cpc2* promoter from DNase I treatment, binding that appears to be phosphorylation dependent (Sobczyk et al., 1994). Although the *cpc2* promoter

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regions to which RcaD binds are not needed to maintain the fully acclimated red light state during CA (Casey and Grossman, 1994; Li et al., 2008), it has been proposed that RcaD may regulate the expression of red light-activated genes only during the transient period of early acclimation, before PBS have become fully adjusted to red light (Noubir et al., 2002).

The Rca system has also been shown to regulate non-PBS responses in *F. diplosiphon*. The CA3 response of *chlLN* and *chlB* gene expression is Rca controlled and, although the mechanism is not known, these genes also require CpeR for their expression, similar to *cpeBA* and *pebAB* (Stowe-Evans et al., 2004; Shui et al., 2009). In addition, the CA3-regulated changes in morphology appear to be largely dependent on RcaE, although a gene encoding one component in this response, *tonB*, is CA3 regulated but not controlled via the Rca system (Shui et al., 2009; Pattanaik and Montgomery, 2010). More work is needed to elucidate the molecular basis of this photoregulatory response.

Much less is understood about the Cgi system than the Rca pathway. The existence of this pathway was first proposed during pigment analyses of PE chromophore attachment (*cpeYZ*) mutants, based on the observation that some photoregulation of PE continued in the absence of *rcaC* (Kahn et al., 1997). Thus far, the Cgi system is known to control only the CA3 response of the *cpeC*, *cpeBA*, and *pebAB* operons, although many additional genes may be under its control (Seib and Kehoe, 2002; Alvey et al., 2003; Li and Kehoe, 2005). Based on transcript abundance analyses in various *rca* mutant backgrounds, the Cgi pathway is a significant contributor to the light color response in *F. diplosiphon*, controlling approximately one-third of the 9–10-fold change in CA3-mediated expression for these genes. This system acts by repressing *cpeC* expression during growth in red light, apparently through a post-transcriptional mechanism that requires a sequence capable of forming a stem-loop within the 5′ leader region of *cpeC* (Bezy and Kehoe, submitted). Thus, for PBS genes that are up-regulated in green light, the Cgi and Rca systems work together to jointly repress *cpeC* expression, thus blocking the synthesis of the CpeR activator and the production of RNAs from the genes it controls. There have been no reports on the isolation of Cgi pathway components thus far.

Type 2 CA

The physiology and regulation of the changes in PBS composition that occur during CA2 have been studied in many cyanobacterial species. An action spectrum, using *Synechocystis* sp. PCC 6701, demonstrated that the accumulation of PE is most effectively controlled by red and green light (Tandeau de Marsac et al., 1980). PBS structural changes have been elucidated in a number of type 2 species. In general, the rods of PBS from green-light-grown cells are one disc longer than those from red-light-grown cells, and the PBS composition changes during CA2 always occur at the core-distal end of the rods (Tandeau de Marsac, 1983). Changes in both PE abundance and *cpeBA* mRNA levels in red versus green light were shown for *Synechocystis* sp. strain BO 8402 (Neuschaefer-Rube et al., 2002). In *Nostoc punctiforme*, quantitative changes in PE accumulation have been measured in cells grown in red versus green light (Wolf and Schussler, 2005) and CA2 regulation of the RNA levels of three genes involved in PBS biogenesis, *cpeC*, *cpcG2*, and *cpeR1*, has been demonstrated (Hirose et al., 2010). The CA2-mediated expression of these genes is regulated by a two-component system that is controlled by the sensor histidine kinase CcaS, a cyanobacterial phytochrome-class photoreceptor that is red light–green light photoreversible. CcaS has strong sequence similarity to RcaE from *F. diplosiphon*. The response regulator of this system is CcaR, which is capable of binding to the DNA region upstream of *cpeC* in *N. punctiforme* and has strong sequence similarity to RcaC from *F. diplosiphon* (Hirose et al., 2010).

Type 4 CA

An action spectrum has not yet been conducted for CA4. However, the initial analysis of this response demonstrated that changes in the relative abundances of PUB and PEB in a wide range of marine *Synechococcus* species could be achieved with green and blue light, but not by changes in the intensity of a white-light source or different nitrogen sources (Palenik, 2001). Subsequent research on two *Synechococcus* strains isolated from the Gulf of Mexico suggested that CA4 does not involve the production of different phycobiliproteins during the acclimation process, as occurs for CA3, but rather the differential chromophorylation of the alpha subunit of PEII, one of the two types of PE found in these species (Everroad et al., 2006). PEII, which is located at the core-distal regions of the rods, consists of alpha and beta subunits called MpeA and MpeB, and these researchers suggested that the replacement of chromophores attached to MpeA is the molecular basis of CA4. MpeA has been proposed to have three PUBs attached in blue light and one PUB and two PEBs attached in green light. The differential attachment of these two isomers was proposed to occur through the action of one or more phycobilin lyases or lyase/isomerases whose activity or level(s) is controlled by CA4. Neither these enzymes nor any CA4 regulatory components have been identified to date. However, an in-depth analysis of the genomes of many marine *Synechococcus* strains, some capable of CA4 and many that are not, has uncovered genes that may be involved directly or indirectly in this acclimation process. These were identified based on the correlation between the presence of these genes in a particular genome and the physiological capacity of the organism containing that genome to undergo CA4 (Six et al., 2007; Dufresne et al., 2008). Although 12 or 13 predicted proteins with homology or relatedness to known lyases were found to be present in strains capable of CA4, these workers suggested that MpeV, MpeU, and MpeZ might be the best candidates for involvement in CA4. The only possible photoreceptor identified using this approach was AplA, which was identified in *F. diplosiphon* as a member of a new class of cyanobacterial photoreceptors of unknown function (Montgomery et al., 2004).

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Evolution of CA Systems

An initial photobiological study correctly concluded that CA3 in *F. diplosiphon* was regulated either by two separate systems or by a single sensory system that controlled PC and PE production through different downstream components (Oelmüller et al., 1988b). While it is now clear that two separate photosensory systems indeed control CA3, Nature has introduced a twist into this scheme, since the Rca pathway controls both red- and green-light-expressed genes, while the other, the Cgi system, appears to regulate only green-light-expressed genes.

How commonly are the Rca and Cgi regulatory systems used in CA3-capable cyanobacteria? Although this cannot be answered for the Cgi pathway, since it has not yet been sufficiently characterized, genome sequence information is available from enough CA3 species to begin to address this question for the Rca system. Highly conserved, similarly oriented L-boxes are present upstream of PBS genes in all three of the CA3 species for which sequences are available (Alvey et al., 2007; Bezy and Kehoe, 2010), and also are present at similar locations within the incomplete draft genome of a recently described hot springs cyanobacterium that may be capable of CA3 (Brown et al., 2010). Genes encoding homologs of the Rca components are also present in all of the available sequenced genomes of CA3-capable species, so the Rca system appears to be a widely used control pathway for CA3. Because the species carrying these genomes are in different branches of the cyanobacterial 16S rRNA tree and originate from diverse environments (marine, freshwater, and hot springs), it is most likely that the ability to undergo CA3 has been spread by extensive lateral gene transfer, although it is also possible that this capability was simply lost non-uniformly. A detailed study of L-box structure and function demonstrated that the sequences of these elements are highly conserved across species and that this conservation is required for high-level activation of gene expression (Bezy and Kehoe, 2010). Taken together, these results suggest that the portion of the CA3 system that is controlled by the Rca pathway has either recently spread throughout cyanobacteria and/or that natural selection maintains the high sequence conservation between L-boxes from different species, perhaps because of the need for continued high-level expression of genes that encode very abundant light-harvesting proteins.

In *F. diplosiphon*, the Rca system has subjugated the activator CpeR and made it part of the CA3 regulatory system. But many species that are not capable of any form of CA still contain CpeR and N-boxes. In fact, all cyanobacteria that produce PE have been found to contain at least one *cpeR* gene (Cobley et al., 2002), so the role of CpeR in controlling genes involved in PE production seems to be widespread and is certainly not always linked to CA, as it is in *F. diplosiphon*. It is also likely that CpeR has been widely integrated into the CA control of green-light-expressed genes, since N-boxes have been identified upstream of the *cpeBA* operon in both a CA2-capable *Synechocystis* species and a *Pseudanabaena* strain capable of CA3 (Neuschaefer-Rube et al., 2002). Biochemical and functional studies are needed to test the hypothesis that the function of CpeR is to coordinate the expression of multiple genes whose increased activity is green-light dependent.

The discovery of the Cgi system in *F. diplosiphon* led to the hypothesis that CA2 species use only the Cgi system to highly express genes in green light, while CA3 species also contain the Rca system, which both further represses these genes in red light and activates genes involved in the production of PC (Kehoe and Gutu, 2006; Kehoe, 2010). Currently, this hypothesis cannot be tested by examining CA2 species, since the components that make up the Cgi system in *F. diplosiphon* are not known. Another approach that was taken to begin to test this hypothesis was to identify the components controlling the regulation of *cpeC* gene expression in the CA2-capable species *N. punctiforme*. This was accomplished by testing the role of CcaSR, a phytochrome-class photoreceptor-based two-component system, in the CA2 response (Hirose et al., 2010). In *N. punctiforme*, the genes encoding these components are adjacent to *cpeC*. This system was capable of sensing red and green light and controlling the CA2 response of *cpeC* in this species. These researchers proposed that this pathway is phosphorylated in green light, resulting in *cpeC* up-regulation through enhanced binding of the response regulator to its promoter region. This light regulation is the inverse of the proposed mechanism of the Rca system in *F. diplosiphon*, where the transcriptional repression of the *cpeC* operon in red light has been proposed to be the result of the phosphorylation of this system (Li and Kehoe, 2005; Kehoe and Gutu, 2006; Li et al., 2008). This led to the proposal that the Cgi system in CA3-capable species simply consists of an RcaE-like photoreceptor controlling a two-component system whose light color regulation is at the transcriptional level and whose operation is complementary to the Rca system (Hirose et al., 2008). However, recent work on the Cgi system in *F. diplosiphon* has shown that it operates post-transcriptionally through the 5′ leader sequence of *cpeC* (Bezy and Kehoe, submitted). Taken together, these data demonstrate that the hypothesis that the Cgi and the CA2 regulatory systems are related (Kehoe and Gutu, 2006; Kehoe, 2010) is not correct, and establish that the CcaSR and the Cgi systems are different in at least the final steps of these pathways. This does not eliminate the possibility that components related to CcaSR are acting upstream of the post-transcriptional regulatory step within the Cgi system. But these results do suggest that, unlike the apparently widespread use of the Rca system for CA3 control of red-light up-regulated genes, there may be some variation in the CA control mechanisms for genes that are up-regulated by green light. As the components of the Cgi pathway in *F. diplosiphon* are discovered, it will be interesting to examine the virtual proteomes of other CA3 species for such components and to compare them to the CcaSR two-component system that controls gene expression during CA2 in *N. punctiforme*.

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SUMMARY

Recent advances in the study of CA in several cyanobacterial species have begun to uncover the diversity of these responses and their regulation. The capacity to undergo CA is present in a wide range of species and environments and is likely to make a significant contribution globally to maximizing the efficiency of photon capture for photosynthesis. Our current understanding of the various types of CA suggest that, although they all maximize the efficiency of ambient light capture, in many cases, they appear to be the result of convergent evolution. For example, the blue–green CA4 response does not involve any detectable changes in PBS rod proteins, while red–green CA2 and CA3 responses do. In addition, some of the signal-transduction pathways controlling these responses, such as the Rca system, may be broadly employed, while the use of others, such as the CcaSR and Cgi systems controlling green-light-expressed genes during CA2 and CA3 in *N. punctiforme* and *F. diplosiphon*, may be less widespread. The future should expand our knowledge concerning these relationships and further cement our understanding of how frequently CA3 species use the Rca and Cgi systems and CA2 species use the CcaSR pathway. We predict that, as more is understood about this fascinating, colorful process, even more variations in CA responses and their modes of regulation will become apparent. Some may be quite subtle and others pronounced. It will be an enormous challenge to determine whether these variations have arisen and been employed by chance, or whether each of the differences that is uncovered represents a finely tuned form of CA that provides a selective advantage to the species that employs it in a very specific environmental setting.

FUNDING

This work was supported by funding from the National Science Foundation under Grant No. MCB-1029414 to D.M.K.

ACKNOWLEDGMENTS

The authors wish to thank the members of the Kehoe laboratory for many helpful discussions. No conflict of interest declared.

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