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Adenylate cyclase in *Arthrospira platensis* responds to light through transcription

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ABSTRACT

Cyclic 3′,5′ adenosine monophosphate (cAMP) is a ubiquitous signaling molecule, but its role in higher plants was in doubt due to its very low concentration. In this study we wanted to look at the flux of cAMP in response to light in algae, considered to be the more primitive form of photosynthetic organisms. While it did not fluctuate very much in the tested green algae, in the cyanobacterium *Arthrospira platensis* its level was closely linked to exposure to light. The expression from cyaC, the major isoform of adenylate cyclase was strongly influenced by exposure of the cells to light. There was about 300 fold enhancement of cyaC transcripts in cells exposed to light compared to the transcripts in cells in the dark. Although post-translational regulation of adenylate cyclase activity has been widely known, our studies suggest that transcriptional control could also be an important aspect of its regulation in *A. platensis*.

1. Introduction

One of the most primitive needs of all organisms is for their adaptation to the daily and seasonal changes in light intensities. This question has been studied in many organisms as different forms of circadian rhythms. In the cyanobacterium *Synechococcus* PCC 7942 cell division, among other physiological processes, is under circadian regulation [1]. Similarly in the protozoon *Euglina gracilis* cell division is tightly regulated by circadian changes so that the organism is able to orchestrate growth on a daily basis. cAMP was identified to be the mediator for this process, the concentration of which oscillated with a periodicity of about 12 h, but by a process which was not influenced by changing light conditions [2]. In *Synechococcus elongate,* use of non-optimal codons allowed the organism to survive better at low temperature than if they were to be encoded by optimal codons [3].

To photosynthetic organisms light is not only a cue to the changing diurnal and seasonal cycle but also the very source from which energy is captured and consolidated at times of availability. Besides assimilating energy directly from light, organisms also require to regulate other physiological processes for optimal metabolic integration. Although light sensing phytochromes have been studied as key regulators of response to daily and seasonal changes, their presence in other organisms such as bacteria and fungi [4,5] suggest their primacy as a light response regulator.

cAMP was the first signaling molecule to be identified [6] and regulates diverse metabolic functions such as glycogen metabolism, ion channel activation, cardiac output [7]. Although its regulatory role in photosynthetic organisms, particularly in higher plants was debated [8] more recent experiments indicate its ubiquitous role as a signaling molecule [9]. cAMP concentration is subjected to stringent regulation by synthesis and breakdown [10]. We wanted to inquire the status of this secondary signaling molecule which could be an important aspect of adaptation to light by algae. Although eubacteria have only one adenylate cyclase [11,12] most other organisms have multiple genes for adenylate cyclase. Cyanobacteria have genes coding for both the archetypal membrane bound as well as cytoplasmic forms of adenylate cyclases [13,14]. In the genome of *Arthrospira* 22 genes have been identified as possible adenylate cyclases [15]. In this paper we report the light associated expression of CyaC, one of the major contributors to cAMP levels in the cyanobacterium *A. platensis*.

2. Materials and methods

2.1. Cells and growth condition

Arthrospira (Spirulina) platensis was obtained from Central Food

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Technological Research Institute, Mysore, and grown in Zarrouk's medium [16]. The green algae *Chlorella pyrenoidosa* and *Scenedesmus* sp. were isolated from a local pond by growing on BG11 medium solidified with 1% agar, and characterized by morphological features and 18 S rDNA sequence analysis. The algae were maintained in respective liquid media at 28° C in a room illuminated at 16:8 h light – dark cycle.

2.2. Experimental procedures

The algae were exposed to natural light – indirect, dissipated sunlight, or artificial light in a room illuminated with fluorescent lamp at 60 μmol photons m^{-2} s^{-1} for the experiments. In many of the experiments, the cells were pre-incubated in a dark room (wrapped in aluminum foil) at least for 30 h before shifting to light for experimentation.

2.3. cAMP determination

The algal cells were harvested by centrifugation at 5000 rpm for 10 min, washed twice in water, and stored at −20 °C. The cellular contents were extracted following the procedure of Gordillo et al. [17]. Briefly, the cells were ruptured using liquid nitrogen, extracted with 3.5% perchloric acid, neutralized with K_2CO_3 and ammonia, centrifuged at 10000 g at 4° C for 15 min cAMP from the supernatant was quantified by HPLC analysis (Agilent LC 1120) using anion exchange column (Nucedur HILIC). The chromatogram was developed at room temperature with a 70:30 mixture of acetonitrile, ammonium acetate (100 mM, pH 5.3).

2.4. Adenylate cyclase estimation

Adenylate cyclase was extracted from the algae as per Hindermann and Parish [18]. Cultures were harvested as above and homogenized in a buffer containing 0.5 M sorbitol, 2.5% Ficoll, 2 mM dithiothreitol, 1 mM EDTA in 50 mM TrisHCl, pH 7.4 with 0.3 mm sterile glass beads, filtered through nylon fiber filter, centrifuged at 2000 g for 5 min, and the supernatants were analyzed for enzyme activity as per Yang and Epstein [19]. Briefly, a solution containing TrisHCl, pH 7.0 (10 mM), $MgCl_2$ (2 mM), ATP (1.5 mM), phosphocreatinine (0.5 mM) and NaF (0.5 mM) was incubated with the cell extract at 37 °C for 60 min, the reaction terminated by keeping in a boiling water bath for 1 min, and the cAMP content in the reaction mixture determined as described above. The protein content of the extracts was determined by Bradford method [20].

2.5. Transcript analysis

Total RNA was isolated from algal cultures as per Pathak and Lochab procedure [21]. Washed cells were lysed by freezing in liquid nitrogen, extracted with phenol, chloroform and amyl alcohol, precipitated with 4.0 M LiCl, washed with ethanol and dissolved in water containing 0.1% diethyl pyrocarbonate. The primers used for transcript analyses are listed in Table 1. Semi-quantitative transcript analysis was done following cDNA synthesis using gene specific primers and MMLV reverse transcriptase (New England Biolabs) and polymerase chain reaction using Taq polymerase (Thermo Fisher Scientific) for 15 to 30 cycles. The DNA concentration in the reaction product was quantified using a nanophotometer (Implen Gmbh) and by separating in agarose gels by electrophoresis and visualization. Quantitative transcript analysis was done in a Real Time PCR (Applied Biosystems StepOne Plus) using SYBR Green Master Mix (Takara). 16 S rRNA was used as the internal control for normalization of expression. Relative expression was determined as:

$$R = 2^{-(\Delta Ct\ sample\ -\Delta Ct\ control)}$$

Table 1
Primers used for transcript analyses.

Primer	Sequence	Amplicon size
cyaA-FP	AACGGATTTCTCCCCAAGAT	143 bp
cyaA-RP	ATCATCTGGACTCCGACCAA	
cyaC-FP	CGAAGCCCCCGAATTGATGA	182 bp
cyaC-RP	CTTCAATGCAGCCGGCCCAC	
cyaG-FP	CCTGAAGCGGATTTTATGGA	145 bp
cyaG-RP	AGGCTTCACTGAGGCGTAAA	
16S rRNA-FP	CAAGCGTTATCCGGAATGAT	173 bp
16S rRNA-RP	GTTCTTCCCGATATCTACGC	

3. Results and discussion

3.1. cAMP flux in algae

We wanted to determine how primitive organisms, exemplified by algae, both eukaryotic and prokaryotic, might respond to a cyclic external signal such as light by looking at the status of a secondary signaling molecule. The intracellular concentration of cAMP in algae incubated in dark for 36 h followed by shifting to diffused sunlight illuminated light-dark cycle was measured (Fig. 1). Among the cells incubated in the dark the concentration of cAMP in *Scenedesmus* sp. was the highest, about two fold higher than that found in *Chlorella pyrenoidosa* and about five fold higher compared to the cyanobacterium *Arthrospira platensis*. It is not unusual that the signaling molecules can show wide variations. For example, the role of cAMP in vascular plants as a signaling molecule was in debate due to the very low levels [8], although more recent studies showed that cAMP was involved in pollen growth and phototaxis [9]. Barott et al. [22] report a 1000 fold difference in cAMP concentration between corals and their surrounding symbiotic protozoons.

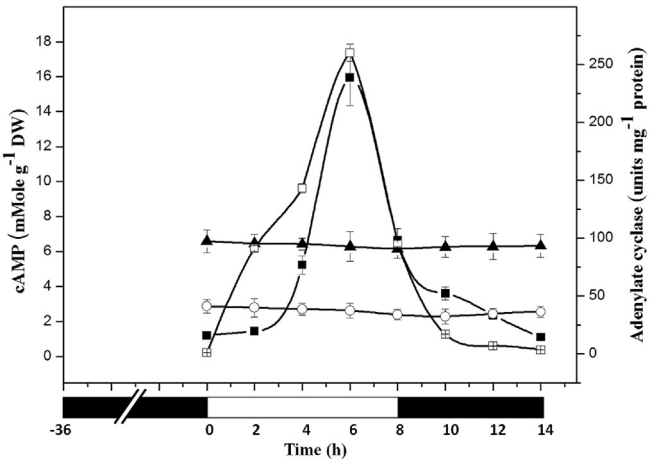

Fig. 1. Intracellular concentration of cAMP and adenylate cyclase in algae shifted to natural lighting condition. Cultures of *Arthrospira platensis* (filled squares), *Chlorella pyrenoidosa* (open circles) and *Scenedesmus* sp. (filled triangles) were kept in kept in the dark for 36 h prior to shifting to dispersed ambient sunlight at 8 a.m. (0 h) and exposed to the natural lighting till shifting back to a darkroom at 4 p.m. (8 h). cAMP content in all algae and adenylate cyclase in *A. platensis* (open squares) in periodically collected samples is plotted against time.