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Review

Toward an understanding of cell growth and the cell division cycle of unicellular photoautotrophic cyanobacteria

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Abstract. The cell division cycle of *Synechococcus* sp. strain PCC 6301 in light is characterized by the sequential and orderly appearance of macromolecular synthesis periods. In the dark, macromolecular synthesis and cell division are severely curtailed. When dark-incubated cultures are reexposed to light, a new cell cycle is initiated. The pattern of the cell events displayed by *Synechococcus* in light and the absence of sustained growth in dark incubation conditions suggests that light-activated regulatory molecules control macromolecular synthesis and the cell division cycle. For example, ribosomal RNA synthesis is stimulated by a light-activated DNA binding factor in light but not in the dark. Light/dark conditions induce cell synchrony in *Prochlorococcus*. Distinct G1, S and G2 phases characterize cell cycles of marine *Synechococcus* and *Prochlorococcus*. Cell division in *Synechococcus elongatus* PCC 7942 and marine *Synechococcus* is controlled by circadian oscillators.

Key words. Obligate photoautotroph; cyanobacterium; *Synechococcus*; macromolecular synthesis; cell division cycle.

Introduction

Photoautotrophy is one of the four basic cellular physiologies. The others are photoheterotrophy, chemoautotrophy and chemoheterotrophy. Photoautotrophs are characterized by their ability to use light as the major source of energy, utilize CO_2 as the major source of carbon for cell growth and have evolved specific mechanisms to grow preferentially in light. In contrast, photoheterotrophs are able to grow in light or darkness in media supplemented with organic substrates such as sugars for carbon and energy sources. *Synechococcus* sp. strain PCC 6301 (formerly known as *Anacystis nidulans* 6301 or UTEX 625) is a prime example of photoautotrophic cyanobacteria. It has been of great interest in determining the basis for growth in light but not in the dark in obligate cyanobacteria [1]. It turned out that most if not all obligate photoautotrophs could utilize exogenously added organic carbon compounds in the growth medium [2]. However, medium containing organic substrates and DCMU [3′-(3,4-dichlorophenyl-)1′,1′-dimethylurea], obligate photoautotrophs are unable to grow when exposed to a light source [3]. It should be recalled that DCMU inhibits photosystem II and, thus, prevents the production of NADPH and CO_2 assimilation. Permeation of organic carbon supplied in the medium apparently occurs at low levels; however, the amount of organic substrates that are taken up would not be sufficient to support sustained growth in the dark [4]. Photoautotrophs, *Synechococcus* PCC 6301 and *Synechocystis* PCC 6308, are capable of producing energy from stored carbohydrates [5], for example through the oxidative pentose pathway. However, energy produced through metabolism of stored carbon compounds alone is apparently not sufficient to support sustained cell cycles of these photoautotrophs in the dark. Organic carbon metabolism by obligate photoautotrophs follows classical pathways as described in reviews [1, 4, 6, 7]. A detailed and lucid review [8] describes the genome and gene expression of cyanobacteria.

Fundamental questions about the cellular activities of obligate photoautotrophs in the dark are not satisfactorily answered. For example, why is it that obligate photoautotrophs do not carry out sustained cell division in the dark? If the photoautotrophs are able to produce stored energy molecules through photosynthesis, why couldn't these potential source of energy be used in the dark for cell division? What are the mechanisms that curtail cell division in the dark? What is the nature of cell maintenance or cellular activities in the dark? What initiates cell growth when light sources become available? There are also fundamental questions about the molecular biology of cell growth of obligate photoautotrophs during growth in light. What are the key cell cycle events and how are these events regulated within the cell division cycle? Attempts to answer these and other questions of cell growth and the cell division cycle of photoautotrophs will be discussed in the review.

A detailed, coherent, overall view of the cell cycle of unicellular, photoautotrophic cyanobacteria at the molecular level has not yet been published. In this regard, all basic information on cellular metabolism and cell growth characteristics must be considered in order to provide a coherent discussion of cell growth and the cell division cycle.

Understanding cell growth could come from information about the cell division cycle of unicellular cyanobacteria

Insights into the unique characteristics of cell growth and obligate photoautotrophy could come from investigations on the cell division cycle of unicellular cyanobacteria. The cell division cycle involves the production of basic cellular structures (ribosomes, genomes, internal and external membranes, cell wall and cell division septa that make up the cell, and the genetic regulatory program that coordinates the orderly appearance of macromolecules that make up cellular structures. In this view, the characteristics of cell growth would be the manifestations of the genes that coordinate the initiation of major cell cycle events. There are, of course, numerous key enzymes that occur at specific times during the cell cycle that are involved in major cellular processes such as nitrogen fixation, photosynthesis and other cellular functions. However, the enzymes involved in these processes are not directly involved in the production of cellular structures. In this regard, review of these enzymes and the cellular functions are considered to be beyond the scope of the intended aims of the review. However, a relatively small number of studies have been done in determining the consensus view on the growth characteristics of photoautotrophs in light and dark conditions (see table 1 for examples). A smaller number of studies was done in characterizing the cell division cycle of unicellular cyanobacteria, and much of the basic work (to be described later in detail) was done on *Synechococcus* sp. strain PCC 6301. There are advantages to studying a standard strain [26]. This approach has been successful in describing in detail cell division cycles of selected heterotrophic bacteria [26], yeasts and the development of bacterial viruses [26]. Note that *Synechococcus* sp. strains PCC 6301 and *Synechococcus elongatus* PCC 7942 share similar genetic information [27, 28], and therefore results described in this review with respect to *Synechococcus* PCC 6301 or PCC 7942 would most likely be applicable to both strains. Other unicellular, obligate photoautotrophs, such as *Synechocystis* sp. strain PCC 6308, could share the same growth characteristics as *Synechococcus* PCC 6301 and PCC 7492. Because the physiology of photoheterotrophs is basically different compared with photoautotrophs, the characteristics of the cell division cycle of photoheterotrophs such as *Synechocystis* sp. strain PCC 6308 would be different from the obligate photoautrophic strain. Therefore, reviews of the cell division cycle of photoheterotrophs will be limited unless the characteristics and the mechanisms in the control of the cell cycle of photoheterotrophs are common to both physiologic groups and advance our understanding of the cell division cycle.

Effects of physical parameters on exponential growth

In order to characterize the cell cycle of photoautotrophic cyanobacteria, cultures should be grown in conditions that produce balanced growth. However, it should be noted that growth rates of *Synechococcus* can vary depending on temperature, light intensity, quality of light, CO_2 concentration, composition of inorganic compounds of the medium, pH, culture volume/flask ratio, strain differences, cell density and possibly other factors. Table 1 describes examples of growth conditions used by investigators and the growth rates that were obtained. The table clearly shows that a wide range of growth rates are obtained in response to the variety of conditions utilized. However, the significant parameters of growth conditions appear to be intensity of fixed light fluence rate, cell density, CO_2 and adequate aeration. In large batch cultures shading becomes a significant factor as cell density increases [30]. Continuous cultures controlled by cell density, realized under a range of light intensities for instance, could allow precise measures of the parameters of balanced growth. Unfortunately, continuous cultures were not commonly used. An acceptable compromise is to grow cells at low cell density ($\sim 2-8 \times 10^7$ cells per milliliter) or dilute the suspensions so as to keep cell density within a minimal and a maximal value.

Growth rates can be increased as the incubation temperature is increased, while other conditions of growth para-

Table 1. Growth Conditions and Growth rates in Exponential Cultures.

Culture condition[a, b]	Temperature	Light intensity	Doubling time[c]	Genus or strain	References
Batch Cultures					
Medium C; culture volume, 8 L	38 °C	2500 lux	7 h	PCC 6301	9
Medium BG 11 Culture volume not provided	37 °C	64.4 µE · m^{-2} · s^{-1}	13.6 h	PCC 6301	10
Medium Dm Medium/flask volume ratio:1/10	32–38 °C	moderate: 3.2 J · m^{-2} · s^{-1}	8–4 h	PCC 6301	11, 12
CO_2 in air; culture rotated at 280 rpm	32–38 °C	high: 4.8 J · m^{-2} · s^{-1}	4–2 h		
Medium BG 11 or C					
Culture grown in 1-l vessels	38 °C	(see text)	2 h	PCC 6301	13
Culture in 1-l fermentation vessel	34 °C	75 W lamp	*3 h		14
Light source 12–14 cm from vessels	38 °C	(2) 75 W lamps	*2.7 h		15
Medium Cg 10 Culture grown in 60-ml flat medicine bottle	30 °C	0.54 mW · Cm^{-2}	5 h	Sy*nechococcus***	16
Medium Cg 10, culture vol. 5.5 l	30 °C	670 µE · m^{-2} · s^{-1}	3 h	PCC 6908	17
Medium C, culture volume not provided 2.5% CO_2 in air	38 °C	200 µE · m^{-2} · s^{-1}	*3.5 h	PCC 6301	18
Medium C, culture volume, 100 ml	32 °C	15,000 lux	6 h	*Anacystis****	19
	26 °C	15,000 lux	12 h		19
	39 °C	15,000 lux	2.5 h		20
Medium f/2 enriched with seawater semi continuous culture	25 °C	22.4 to 174 µE · m^{-2} · s^{-1}	11 to 55 h	*Synechococcus* WH8101	21
Medium PCR-S11	20 °C	daylight fluorescent bulbs, 5 and 57 µMol.m^{-2} s^{-1}	29, 35 h	*Prochlorococcus* CCMD 1375 CCMD 1378	22
Continuous culture					
Medium C, 2-l volume 5% CO_2: 95% N_2	39 °C	300 W Fluoresc. + tungsten bulbs	*28–3.5 h	PCC 6301	23, 24

[a] Cell densities in batch cultures range from 3×10^6 to 7×10^7 cells per ml except when noted.
[b] CO_2: 2.5–5% in air or N_2.
[c] Growth rates or doubling time reported originally in terms of *µ or *k.
** *Synechococcus* strain obtain from Dr. Kingsbury, Cornell Univ. Axenic Culture.
*** *Anacystis nidulans* Richter was from University of Gottingen and renamed as *Lauterbornia nidulans*. Defined media C, BG11and Dm can be found in [25].

meters are held constant [19]. In another growth study on *Synechococcus* [29], Arrhenius plots showed a logarithmic increase in growth rates versus the reciprocal of the incubation temperatures in the range of 28–38 °C under moderate light intensity (3.2 J m^{-2} s^{-1}) or in a range of 32–38 °C under high light intensity (4.8 J m^{-2} s^{-1}). However, beyond 38 °C at moderate light intensity, growth rate is decreased as the incubation temperature increases. According to this observation, enzymes are subjected to heat denaturation at incubation temperatures beyond 38 °C, and as a result growth rates are decreased. Because the doubling times are reduced from 12 h to 4 h at a temperature range of 28–38 °C under moderate light intensity, the concept of Q_{10} apparently is not applicable in explaining this observation. According to Campbell [32], as emphasized by Schaechter and coauthors [33], 'growth is said to be *balanced* over a time interval if, during the interval, every 'extensive' property of the system increases by the same factor. Failure to maintain balanced growth throughout an experiment makes it impossible to relate any measured quantity to the growth rate in a direct way' and on the growth characteristics of heterotrophic bacteria [34]. When cultures of *Synechococcus* were grown at 32 °C under moderate light intensity, the uptake of radioactive labels in the protein, RNA, DNA and phospholipid fractions increased exponentially at the same rate as the increase in cell number [31]. According to these results the relevant properties of balanced growth can be obtained in cells growing at incubation temperature of 32 °C and, presumably, in cultures grown in the temperature range of 28–38 °C under moderate light intensity.

In the experiments presented in table 1, light intensities, the distance of the light sources to the culture vessels and the light meter used to measure light fluences are varied. That is, radiant energy was measured by photometric, ra-

diometric and quantum methods, which measure different properties of light [35]. The photometric method utilized a photocell to determine the luminosity of specific wavelengths emitted by the light source. Luminosity is commonly reported as lux equal to l lumen m^{-2}, where 1 lumen, for example at λ 562 nm, is 1.49 mW. The radiometric method utilizes a photovoltaic cell that measures radiant energy in terms of the fluence rate in all daylight spectra. Here, W (fluence rates) is equal to Js^{-1}. The quantum method utilizes quantum sensors with appropriate filters to measure the number of photons at wavelengths from 400 to 700 nm. In this method, the fluence rate is equal to µmol m^{-2} s^{-1}. In any case, the increase in light intensity or light energy should increase the photosynthetic capacity and, consequently, increase the production of high-energy molecules that fuel the increased growth rates. Indeed, in *Synechococcus* sp. strain PCC 7942 high light intensity stimulates the transcription of *psbAII*, *psbAIII* and *psbDII* genes [36, 37]. *psbAII* and *psbAIII* code for two forms of D1 protein, and *psbDII* codes for D2 protein found in the reaction center of photosystem II. See the review by Golden [38] on light-stimulated transcription of genes associated with photosynthesis.

The variation of light fluences contributed to the differences in the growth rates presented in table 1. The intensity in the light source should alter the rates of biosynthesis as well. The increase in light intensity is basically a shift-up in nutrition [14] similar but not identical to the nutritional shift-up from poor to a richer medium in cultures of *Salmonella typhimurium* [34, 39]. Several investigators [16, 18, 24] have shown the relationships of increased growth rates and macromolecular metabolism with increased light intensities in culturing *Synechococcus* while keeping the incubation temperature constant (see table 2). In large batch culture, DNA or RNA contents per cell, cell volume and cell mass were found to increase exponentially when plotted against the arithmetic increases in growth rate values determined at high light intensities [14]. In continuous culture of *Anacystis nidulans* [24], RNA and DNA contents, reported as a percentage of dry weight, did not increase exponentially with increasing growth rates. Instead, the RNA content per cell was found to increase in a sigmoidal pattern. DNA content, on the other hand, was level up to growth rate of 0.05 h^{-1} and increased sharply to a maximum at growth rate of 0.1 h^{-1}. From 0.1 h^{-1} to 0.2 h^{-1} the DNA content/cell decreased to about 2.5 fg/cell. Binder and Chisholm [18] also reported the pattern of increase in DNA content per cell in semicontinuous culture of *Synechococcus* in conditions where growth rates were increased with respect to increases in light intensities. In their experiments, DNA content/cell reported in terms of genome equivalent per cell increased linearly when plotted against growth rates. The investigators [18, 24] did not give any reasons for the differences in the results with regard to the increase of DNA and RNA contents and, at present, the differences described here cannot to resolved from the data presented. It appears that the rates of increase of macromolecular contents are highly sensitive to the growth and cultural conditions used as well as the strains analyzed, as indicated earlier. The question remains whether growth at high light intensity can be considered as normal growth or growth adjusted to specific conditions. Further discussions on the effects of increased temperature and light intensity on the characteristics of the cell division cycle will be made in order to provide answers to this question (see below).

Effects of light and dark shifts on cellular metabolism, genome distribution and induction of synchronized growth

One of the basic characteristics of photoautotrophs is that sustained cell growth cycles do not occur in the dark. Earlier investigations indicate that metabolic processes that support cell growth cycles in light are curtailed in the dark [16, 40–43]. The experimental data suggest that chemical energy was neither produced nor consumed in the dark in sufficient levels to support cell growth cycle

Table 2. DNA and RNA contents (fg/cell) at growth rates increased by light intensity.

Culture	Growth rate	RNA	DNA	Comments	References
Exponential	0.05 h^{-1}	35	6	Nucleic acid contents	14
	0.1 h^{-1}	55	9	increase exponentially	
	0.2 h^{-1}	110	18		
Continuous	0.05 h^{-1}	20	2.25	RNA contents increase	24
	0.1 h^{-1}	~34	4.5	in sigmoidal pattern; DNA contents were level up to 0.05 h^{-1}, increase sharply to 0.1 h^{-1} then	
	0.2 h^{-1}	55	~2.5	decrease from 0.1 to 0.2 h^{-1}	
Semicontinuous	0.05 h^{-1}		3	DNA contents increase linearly	18
	0.1 h^{-1}		3.4		
	0.2 h^{-1}		4.4		

in the dark. What would be the fate of macromolecular metabolism and genome distribution in the dark? What would be the characteristics of cell growth upon reexposure of the culture to the light?

Macromolecular metabolism and cell division in the dark in *Synechococcus* and related cyanobacterial strains

Dark incubation prevents reinitiation of cell divisions and has a profound effect on the energy profile in *Synechococcus,* as indicated above. Marine phototrophs, *Prochlorococcus* sp, however, do carry out cell division in the dark (see below). Dark incubation was also used to determine the extent of macromolecular metabolism and cell division in *Synechococcus* in the dark. DNA, RNA and protein contents were increased slightly, and small increases in cell number occurred in the dark [9, 11, 44]. In another single L/D shift experiment, sizable amounts of novel RNA molecules [45] and at least a dozen protein molecules [46] were synthesized during the dark incubation period. The nature of these molecules was not determined.

In another report [10] RNA and DNA contents/cell were determined for a culture of *Synechococcus* PCC 6301 grown in medium BG 11 and subjected to a 12-h light/12-h dark schedule. The incubation temperature and light intensity were 37 °C and 64.4 $\mu E\, m^{-2}\, s^{-1}$, respectively. The growth rate of 0.06 h^{-1} (~13.6 h doubling time) in the light growth phase appears to be lower than expected. When the culture was shifted to dark condition, cell growth was arrested. Cellular RNA, 16S RNA and DNA contents increased considerably in the amounts of 10 fg/cell, 6.3 fg/cell and 3 fg/cell, respectively, during the dark incubation period.

Detailed comparison and evaluation of macromolecular metabolism and cell division cycle in the dark among strains of cyanobacteria are difficult since the growth conditions or the strains used were not the same. Further investigations should be done to clarify the basis for the apparent contradictions described above.

Genome distribution in the dark in *Synechococcus*

Genome distribution in the dark could indicate the extent of DNA metabolism during dark incubation. However, DNA and genome distribution in the dark are varied among cyanobacterial strains studied. When exponential cultures of *Synechococcus* PCC 6301 (grown at 38 °C and light intensity of 200 $\mu E\, m^{-2}\, s^{-1}$) were placed in the dark, a 'broad unimodal DNA distribution' was found which resolved into 2, 3, 4, 5 or 6, peaks per cell at the end of a 10-h period [18]. The peaks determined by flow cytometry represent chromosomes that initiated DNA replication in the light and completed during dark incubation. Cells do not divide in the dark even after chromosome replication is completed. In any case, the 'irregular' DNA distribution per cell deviated from the expected 2^n number where n = 1, 2, 3, 4 and so on. This discrepancy was explained according to one of three models proposed for *Escherichia coli* mutants [47] that implicates asynchronous rather than synchronous initiation of genome replication. The other two models, abortive initiation and selective chromosome degradation, were not entirely ruled out. It should be pointed out that the asynchronous initiation model of *recA* mutants of *E. coli* is based on cultures growing at 27 min doubling time and where doubling time is less than C + D periods.

Another assessment of genome distribution was made [10] in batch cultures of *Synechococcus* PCC 6301 grown in 12-h light and 12-h dark conditions, as indicated earlier. During the first light growth period (after emerging from a 12-h dark period) the cell number increased exponentially. The number of genomes was estimated to be three per cell at the onset of the light period. At the end of the 12-h light incubation the genome number decreased to two per cell as a result of cell division. In the succeeding dark incubation period, the number of genomes increased to three. The result was explained as follows. DNA replication was initiated shortly before subjecting the culture to the dark and continued to completion during the dark incubation period. Cells did not divide during the dark incubation period. However, it is not clear how two genomes per cell in the light-grown cultures increased to three genomes per cell in the dark-incubated culture.

In marine *Synechococcus* WH8101, WH8103 and WH805 one genome per cell was found in dark-incubated cells. In *Synechococcus* WH7803, there are one to six genomes per cell as found in *Synechococcus* PCC 6301, described above [21, 48]. Asynchronous initiation of DNA replication could occur in *Synechococcus* WH7803 [48], as found in *Synechococcus* PCC 6301 [18].

In another study [31], *Synechococcus* PCC 6301 initiation of DNA replication is synchronized or partially synchronized. DNA replication, initiated in the light, could be completed in the dark (see below). The number of genomes per cell can be estimated to be two to three per cell in the dark.

As in other topics on the cell growth of photoautotrophic cyanobacteria, detailed experiments in the mode of DNA replication and the regulatory molecules that control DNA replication in the dark are needed to understand the fates and dispositions of the genome in the dark. In any case, further discussions of DNA replication in the context of the cell cycle will be described below.

Synchronized cultures of *Synechococcus* induced by L/D shifts

In a surprising coincidence, two groups of investigators [13, 31] were independently inducing and utilizing syn-

chronized cultures to generate the genetic map of *Synechococcus* (*Anacystis nidulans*) by a nongenetic recombination technique. This approach was based on the notion that a shift from light to darkness will cause all cells to arrive at a common rest point. Upon returning the cells to the light, cells were expected to grow from the common rest point in a synchronized mode, and the initiation of genome replication would be synchronized within the synchronized growth cycle. The possible establishment of a sequence of specific genes on the genome [15, 31] confirmed the notion that genome replication was synchronized and was initiated from a common point of origin. Figure 1A depicts a scheme of synchronized growth induced by this L/D induction method [11, 13, 44, 49, 50].

In other investigations [9, 10, 24] the L/D shift in growth conditions did not result in synchronized growth. In these reports a lag of about 1–3 h occurs before the cell number increases exponentially. In other investigations, L/D shifts induce cell division in a circadian time frame [51]. Explanations for the production or absence of synchronized cultures and circadian cell divisions by the L/D regime will be described in the sections that follow.

Light-activated heterotrophic growth in *Synechocystis* sp. strain PCC 6803

In contrast to obligate photoautotrophs, *Synechocystis* PCC 6803 demonstrate light-activated heterotrophic growth (LAHG) in the dark [52]. Blue light was found to be necessary for the observed LAHG. A recent article [53] indicated that 783 genes were expressed in response to light during a L/D transition period as determined by DNA microarray. The results suggest that the LAGH is a complex process and requires further experimentation to elucidate this phenomenon.

Synchronized culture of *Synechococcus* sp. strain PCC 6301 and the sequential order of macromolecular synthesis periods

Synchronized cultures are useful in studying the characteristics of cell growth, particularly when the mechanism of induction does not upset the 'normal' order of cell division cycle. A synchronized culture of *Synechococcus* sp. strain PCC 6301 is shown in figure 1A. Cell number doubling occurred at about 7 h and the second doubling at 15 h after exposure to light. Macromolecular synthesis periods occurred in sequential order within the first division period and recurred before the second cell division period [11, 29, 49], as shown in figure 2. This highly ordered sequential appearance of macromolecular synthesis periods is unique and has not been reported in other unicellular and filamentous cyanobacteria or in heterotrophic prokaryotes at this time. For example, in *E. coli*, the most intensively studied bacterial cell cycle system, major cell cycle events are (i) initiation of DNA replication, (ii) nucleoid segregation and (iii) initiation of cell division [54]. The mechanisms in the coordinate regulation of the cell cycle events in *E. coli* are not yet known [55]. In *Synechococcus* PCC 6301, the order in the appearance of the macromolecular synthesis periods is

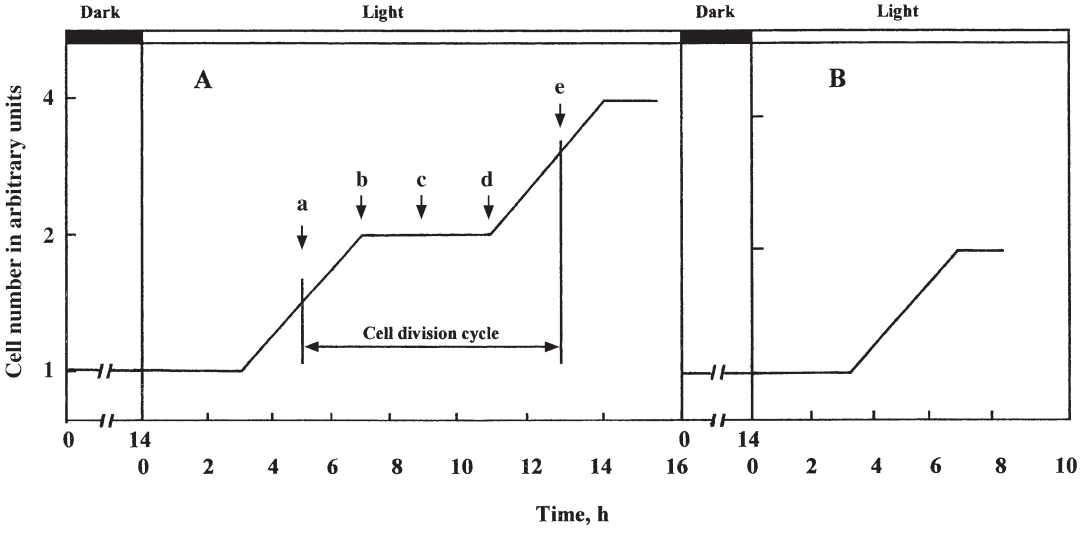

Figure 1. Synchronized growth of *Synechococcus*. (*A*) L/D shift induces synchronized growth. Growth conditions of synchronized cultures are similar to [11, 49] with temperature and light intensity adjusted to 32 °C and 3.2 J m^{-2} s^{-1}. Synchronized culture was induced by incubating exponential cultures in the dark for 12–14 h and then reexposing them to the light. The cell growth cycle is 8 h. The arrows indicate the times when samples were taken for analysis, which are described in the text. (*B*) Effect of dark incubation on synchronized cultures from samples (a, b, c, d, and e in panel *A*). In all samples, the dark incubated cultures initiated a new cell cycle upon reexposure to light.

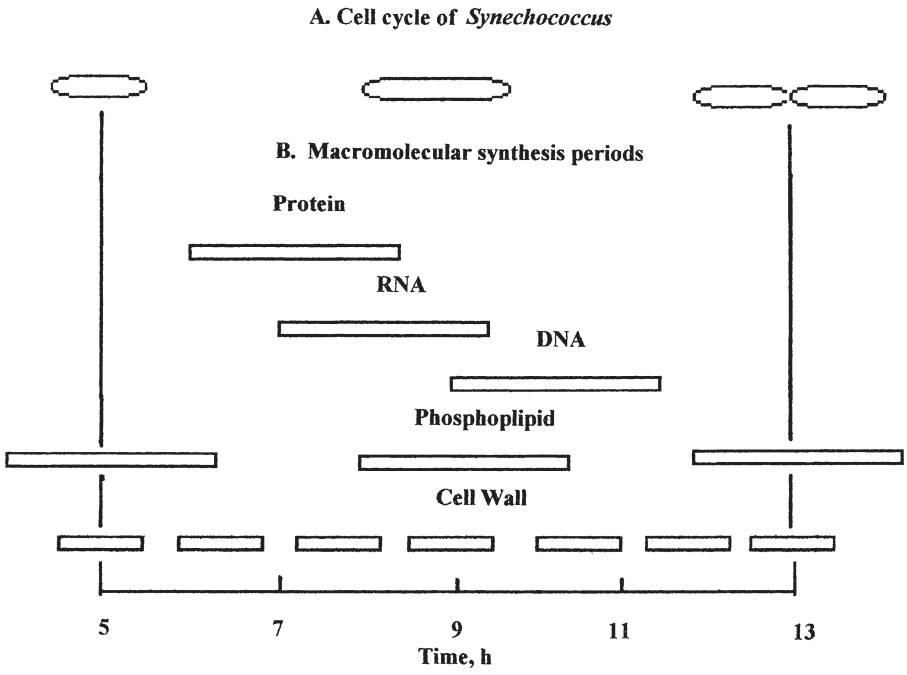

Figure 2. Macromolecular synthesis periods during the synchronized cell cycle of *Synechococcus*. (*A*) Cell division cycle as determined microscopically. (*B*) Macromolecular synthesis periods. The vertical lines indicate the midpoints of the cell division periods. Protein, RNA, DNA, phospholipid and cell wall synthesis were determined by pulse labeling with radioactive precursors or by chemical analysis. The synthesis periods were determined and reported in [11] and [29].

fixed and not altered even in synchronized cultures growing at doubling times of 6 and 4 h [11]. This schedule of cell events strongly indicates involvement of a coordinated genetic regulatory system. Does the synchronized culture depict the normal order of cell growth cycle in *Synechococcus*? It could be argued that the data to support this scheme of cell growth were obtained from synchronized cultures and may not represent the normal cell division cycle. That is, the L/D-induced synchronized growth is an artifact resulting from the induction method. Nevertheless, obligate phototrophs grow in an environment where light-to-dark conditions are normal occurrences and most likely grow in a synchronized cell growth pattern [11]. Interestingly, the conditions of the natural environment cannot be duplicated in the laboratory, and the growth conditions used in the laboratory can be considered as an artificial simulation of the conditions in nature. So the question about the normal order of cell growth cycle in photoautotrophs cannot be easily answered. In any case, the characteristics of synchronized growth more nearly represent the growth of these cells in the natural environment as in *Prochlorococcus* [56], and the ordered appearance of the macromolecular synthesis periods is the expression of a uniquely coordinated genetic regulatory system. It is likely that there is a common genetic regulatory system of cell growth cycle that controls the ordered and sequential appearance of synchronized cultures in contrast to the notion that several genetic regulatory systems exist which are expressed according to the growth conditions imposed on the culture. The cell growth cycle as described in figure 2 is of great interest since the cell growth cycle is unique among prokaryotes so far investigated, and the mechanisms that regulate the order of cell events must also be unique. The sequential and orderly appearance of macromolecular synthesis periods under coordinate regulation in *Synechococcus* PCC 6301 will lead to the orderly formation of basic cellular structures such as ribosomes, genomes, membranes, cell wall and cell septa during the cell developmental cycle. The characteristics of the synthesis periods as well as the cell division periods of the cell cycle are described below.

It should be pointed out that the concept of balanced growth cannot be applied to synchronized cultures. However, the exponential cultures that were used to obtain synchronized culture displayed the characteristics of balanced growth. In any case, cell numbers and macromolecular contents should double within the cell division periods of synchronized cultures.

There are a growing number of studies performed on the cell cycle of marine cyanobacteria. These studies represent the current views on the cell cycle of marine cyanobacteria in contrast to studies done on fresh water *Synechococcus*. For continuity, organization and historical development in coverage, the cell cycle of the marine species be will described in depth below.

Cell division periods

The cell division cycle in a synchronized culture can be demarcated as a period between the midpoints of two consecutive cell division periods, as shown in figure 1 A. (The midpoints indicated here refer to the time when 50% of the cells have divided). The cell division periods of synchronized cultures can be shown to occur for 12 generations (4 days) when the cultures are diluted, so that the density of cell number was maintained at $2-6 \times 10^7$ cells per ml [unpublished results]. The following sections will describe the cellular events that are involved in the cell developmental cycle determined from synchronized cultures of *Synechococcus* sp. strain PCC 6301.

Protein synthesis

Bulk protein synthesis begins about 1 h after cell septum formation. The time period of protein synthesis is about 3 h in an 8-h doubling time [11]. The protein content doubles by the end of the cell division period. Although the molecular species of the protein has not been experimentally identified, ribosomal protein (r-protein) would most likely be synthesized during the protein synthesis period. Analyses of r-protein gene clusters of cyanobacteria were recently reported, and the results show that proteins involved in transcription, translation and other miscellaneous proteins are also encoded [57–60]. The synthesis of ribosomal proteins together with transcription and translation factors indicates an effective and well-coordinated production of the translational apparatus. Furthermore, the analysis of the r-protein gene clusters suggests a possible regulatory scheme that controls the expression of the r-proteins. Meng and coauthors [57] described a *rps* gene cluster containing genes for r-proteins S12 and S7 and genes coding for EF-G and EF-Tu. The order of genes in the *rps* gene cluster is similar to the structural order of the *str rps* operon of *E. coli*. Sequence similarities were found between *E. coli* and *Synechococcus* with respect to S7 r-protein binding regions located in the middle of 16S rRNA as well as the 5′ end of the S7 mRNA. These sequence similarities suggest that, as in *E. coli,* S7 could function as a feedback regulatory factor in controlling the translation of the messenger RNA (mRNA) that contains the encoded sequences of S7 and other genes of the gene cluster in *Synechococcus*. Other r-protein genes such as the gene encoding S1 [61], the S14 gene cluster [59] and L3 gene cluster [60] were also analyzed in *Synechococcus*. In *Synechocystis* sp. strain PCC 6803 [62] and *Spirulina platensis* [58], the S2 r-protein operon contains *rpsB* and *tsf* similar to the S2 r-protein operon of *E. coli* [63]. All r-proteins are most probably synthesized during the protein synthesis period. rRNA, as described below, is synthesized about an hour after the protein synthesis period is initiated. The sequence in the appearance of r-proteins and ribosomal RNA (rRNA) would be consistent with a scheme of ribosome assembly in the manner described in *E. coli* [64]. The times of synthesis of other major proteins such as phycobilisome proteins are not known. Some amounts of membrane proteins and proteins that make up the photosynthetic apparatus can be made during the protein synthesis period which coincides with the burst of phospholipids synthesized during mid-cell cycle. Synthesis of the reaction center proteins also obeys other regulatory pathways, since it can be induced by high light intensity or exposure to specific wavelengths [38].

RNA synthesis

Bulk RNA synthesis is detected about 1 h after initiation of the protein synthesis period. The RNA synthesis period is about 3 h and the RNA content doubles at the end of the period [11]. On the other hand, Herdman and coauthors [13] found that RNA synthesis occurred at mid-cell cycle and continued to the end of the cell cycle. The differences in the appearance of RNA synthesis periods are most likely due to the growth conditions and the method of inducing synchronized cultures (see above). The underlying basis for the differences is not presently known. The bulk of the RNA molecules synthesized are the ribosomal and transfer RNA (tRNA) molecules [65]. Two rRNA operons (*rrnA* and *rrnB*) were cloned [66] and located on the physical maps of *Synechococcus* PCC 6301 [67] and in the photoheterotroph, *Synechocystis* PCC 6803 [68, 69]. Interestingly, the locations of *rrn*A and *rrn*B on the genomes of *Synechococcus* and *Synechocystis* are not the same. rRNA synthesis by light-activated transcription factor will be described below.

Determination of mRNA transcripts involved in the control of major cell cycle events has yet to be done. Recently, a global mRNA transcription pattern in the control of cell cycle events during the synchronized growth of *Caulobacter crescentus* was analyzed using a DNA microarray system [70]. The application of the microarray analysis of the synchronized culture of *Synechococcus* could identify the crucial mRNA transcripts that are involved in the control of the macromolecular synthesis periods shown in figure 2.

DNA synthesis

The periodic and synchronized synthesis of DNA was first reported independently by two groups of workers [13, 31]. Many other reports [11, 15, 50] have substantiated the periodic nature of DNA synthesis in synchronized cultures of *Synechococcus*. The DNA content doubles by the end of the cell division cycle [11]. During the DNA synthesis period, genome synthesis is synchronized and DNA replication is initiated at a specific origin of replication (*oriC*). This is supported by the construction of a temporal genetic map,

which could be achieved only if synchronized genome replication from a specific *ori*C site took place [15, 31]. Two genomes per cell were found in *Synechococcus* PCC 6301 [31]. In cells that have more than two genomes, asynchronous initiation of genome replication can occur (see above). There are gaps in DNA synthesis before and after cell septum formation [11, 50]. In the terminology of Cooper and Helmstetter [71], the letter D denotes the gap in DNA synthesis, and C represents DNA synthesis in *E. coli*. Using these designations, the gap-synthesis-gap pattern in *Synechococcus* can be referred to as D_1-C-D_2 phases and resembles the G_1-S-G_2 sequence in the cell cycle of eukaryotes. The D_1-C-D_2 phases are also found in synchronized cultures growing at doubling times of 6 and 4 h at growth temperatures of 35 and 38 °C, respectively [11]. In *E. coli* there is one D period; the order of the events during the cell cycle is C, D and cell septum formation. However, in slowly growing cultures of *E. coli* gaps, before and after genome replication, may occur [72–75].

Very little is known about the control of DNA replication in *Synechococcus* PCC 6301. In the marine cyanobacterium *Synechococcus* WH 8101 cell mass could play a key role in regulating the timing of chromosome replication [76]. On the other hand, information on the initiation of DNA replication in other organisms has advanced [77]. DnaA initiates replication by binding to DnaA boxes in eubacteria [78], which are commonly located upstream of *dnaA*. Surprisingly, DnaA boxes were not found in cloned *dna*A region of *Synechocystis* sp strain PCC 6803 and were not evident in nucleotide searches of the complete nucleotide sequence [62] of the same strain [79]. Moreover, mutant strains that have inactive *dna*A are able to grow in standard growth conditions [80]. The results indicate that *dnaA* cannot be involved in the initiation of DNA replication in *Synechocystis* sp. strain PCC 6803 and suggest that other initiators must exist. Interestingly, in *Synechococcus* sp. strain PCC 7942 [81] a cluster of 11 DnaA boxes was found upstream of *dnaN* (which codes for the β subunit of DNA polymerase). In *Prochlorococcus marinus* CCMP 1375, DnaA boxes were not found in a cloned segment containing *dna*A [82], in contrast to other eubacteria [78]. In *Synechococcus* PCC 7942, *dnaN* was found to be expressed rhythmically, suggesting that DNA replication could be under circadian control [81]. The results indicate that the genetic elements that regulate DNA replication as well as other macromolecules such as rRNA synthesis (see below) in photoautotrophs are expected to be quite different, in contrast to heterotrophs, despite the existence of common elements in the synthesis of these macromolecules. These differences are most likely manifestations resulting from differences in the coordinate regulation of cell events between photoautotrophs and other bacteria. Obviously, future investigations must be done to resolve the complex mechanisms that are involved in coordinating the initiation of DNA replication during progress of the cell cycle in cyanobacteria.

The time of replication of plasmid DNA [83] has not been investigated.

Phospholipid synthesis

The organization and composition of cytoplasmic and thylakoid membranes of cyanobacterial strains are complex [84, 85]. The uptake of ^{32}P in the phospholipid fraction occurs essentially during mid-cell cycle and during cell division periods [11]. It is interesting to note that a surge of oxygen production occurs during the mid-cell cycle [86]. It is tempting to predict that much of the increased phospholipid synthesis during this period represents the synthesis of thylakoid membrane. Significant increases in the components of the photosystems should occur as well. Periphery cell membrane synthesis could occur throughout the cell cycle and be coordinated with the cell wall synthesis. However, the sizeable uptake in the label during the cell division phase could represent septa membrane formation. In any case, much work needs to be done to determine synthesis and the control of membrane complexes.

Peptidoglycan synthesis

The uptake of radioactive *N*-acetylglucosamine occurs in short periodic pulses throughout the cell cycle [29]. It is not clear whether the cell wall increases occur periodically as part of a grand regulatory scheme in the cell developmental cycle or whether cell wall synthesis is coordinated in some manner with the production of macromolecules and supramolecular structures such as ribosomes and genomes. On the other hand, the rate of cell wall synthesis slows down as the cell density of the culture increases [29]. As a result, cell size is decreased. The mechanism of coordinating cell wall synthesis during development of the cell is complex and remains to be described in detail.

Cell septum formation

In rod-shaped cyanobacteria, such as *Synechococcus,* cell septum formation bisects along the length of the cell. However, in *Synechocystis* sp. strain PCC 6803, cell division septa can occur at right angles on agar plates such that tetrads are formed. Cell division septum in *Synechococcus* PCC 6301 can be seen under light microscope from about 1.5 h after completion of genome replication in an average cell in a synchronized culture growing at 8 h doubling time [11]. The time for initiation and completion of the cell division septum should be less than 4 h [31]. There are chemically induced mutants (*sna* or *fil* mutants) of *Synechococcus* PCC 6301 [87–91] and ma-

rine cyanobacterium, *Agmenellum quadruplicatum* [92–95]. Filamentous mutants (*flm*) of *Synechococcus* PCC 7942 were generated by insertional inactivation [96]. This latter method has an added advantage since the inactivated gene can be mapped by restriction endonucleases. There are many causes for the filamentous phenotypes, but none of the genes that are responsible for the filamentous phenotypes and their roles in the formation of the cell division septum have been identified. However, in *E. coli*, nine cell division proteins are presently identified that are required for cell division [97]. Mutations in *ftsA*, *ftsQ*, *ftsI* and *ftsZ* genes are responsible for the filamentation phenotype and were obtained as '*f*ilamentous *t*hermo *s*ensitive' or *fts* mutants. While *sna* are unable to form cell septa, wild-type cells growing normally may not always form cell septa at the end of the cell cycle as observed under light microscope [unpublished observations]. During the cell division period, a minority of cells is found with one cell division septum at about a distance of one quarter of cell length from a terminal end. The daughter cells are about the size of newborn cells and are much larger than minicells. In addition, cells with three division septa can be seen in a field containing a majority of cells with one division septum that bisects the cell during the cell division period of synchronized cultures. Therefore, termination of the cell cycle cannot always be demarcated by cell septum formation events. In this case, the cell cycle can be considered as a growth cycle that involves the formation of cellular structures such that at the end of the cell cycle, the cell has formed the vital cellular structures that are necessary for the formation of two independent cells. This interpretation implies that DNA replication can be uncoupled to septum formation in a fraction of normally growing cells.

The coordination between cell septum formation and other cell cycle events is poorly understood in prokaryotes. However, significant progress has been made in heterotrophic bacteria. In *E. coli*, Fts*Z* protein [91] is the first to appear at the septum formation site followed by the sequential binding of cell division proteins, Fts and ZipA, in the septosome assembly process [98]. In *Caulobacter*, CtrA has been found to bind to a site near the replication origin and prevent initiation of chromosome replication and *ftsA* expression [99–101]. When the time for formation of cell septum arises, Clp, a protease [102] degrades CtrA, and a new round of septum formation is initiated. The investigation in septum formation involving cell division proteins in cyanobacteria has barely begun. However, recent accomplishments can serve as a basis for future studies on cell septum formation. Nucleotide sequences of several cell division genes (*ftsH,Y,Z*), septum site-determining genes (*minC,D,E*), cell division inhibitor (*sulA*) and ORF of cell division cycle protein of *Synechocystis* PCC 6803 have been reported (see [62] for further details) In addition, fts*Z* was cloned and sequenced in *Anabaena* PCC 7120 [103, 104]. Cell division gene clusters containing *ftsZ* and *ftsQ* were analyzed in *Prochlorococcus* sp. strain PCC 9511 [105].

In *Synechococcus* PCC 6301, the initiation of DNA replication has been reported to be coupled to cell division septum formation [106]. This is consistent with earlier finding that the addition of chloroamphenicol early in the cell division cycle was found to arrest septum formation in *Synechococcus* [17]. DnaA initiates DNA replication in heterotrophic bacteria. In *Prochlorococcus* PCC 9511, maximal mRNA levels of *dnaA* and *ftsZ* appear sequentially during the period of DNA replication, suggesting that replication and cell division are coordinated [105]. It would be of great interest to determine the regulatory factors that coordinates the synthesis of DnaA and Fts proteins in cyanobacteria.

In *Synechocystis* PCC 6803, the loci of genes in the synthesis of macromolecules such as tRNA, rRNA, DNA, cell division septum as well as other genes was presented in the sequenced genome [62]. The entire nucleotide sequence of *Synechocystis* PCC 6803 is provided in Cyanobase (http://www. kazusa.or.jp/cyano). This work is monumental and is obviously a great reference source for investigating the genes responsible for the formation of macromolecules. An equally monumental task is to identify the genes that initiate and coordinate the cell events described in figure 2. For example, the elements that coordinate DNA synthesis – cell division septum formation and r-protein – rRNA transcription (see below) are key to understanding the orderly progression of cell cycle events. These regulatory genes are often cryptic genes and cannot be identified by analyzing sequenced genomes.

However, there are molecular and genetic techniques that can be used to determined the putative regulatory genes. The potentially most useful method is the DNA microarray system [70, 107]. In *Caulobacter*, DNA microarray technology in conjunction with in vivo genomic binding site analysis, CtrA was found to regulate at least 95 genes involved in cell cycle events [108]. For example, CtrA regulates initiation of DNA replication, cell division and cell wall metabolism. In cyanobacteria, regulatory mutants could be obtained by classical mutagenesis, newer methods of mutagenesis such as gene-transfer-based methods [109] and insertional inactivation of genes [96]. Other genetic tools, such as site-directed mutagenesis, reporter genes for measuring gene expression [110] and differential display techniques for screening regulated gene expression by employing HIP (highly iterated palindrome) elements [111], could be used. Complete genome sequence of *Synechocystis*, and of other unicellular cyanobacteria, *Synechococcus*, *Prochlorococcus* and *Gloeobacter*, in progress, could be used for designing experiments to identify cell cycle regulatory genes. DNA-binding techniques could also be used to identify and isolate DNA-binding proteins that are involved in regulating cell events such as rRNA syn-

thesis [65] (to be described in detail below). Conditional lethal mutants such as heat-sensitive mutants of *E. coli* could be used to verify and provide in vivo evidence for the existence of genes involved in coordinating the synthesis of cellular structures within the cell cycle such as cell division septum formation [91] and perhaps in other cell events such as DNA, rRNA, and r-protein synthesis.

Finally, it should be pointed out that the cell division cycle of *Synechococcus* summarized in figure 2 can serve as a basis for investigations that support, refute, modify or redefine the tenets of the proposed model of cell division cycle. However, very few studies are currently done to take on this challenge in order to further delineate or redefine the 'normal' order of cell division cycle of *Synechococcus*. In any case, synchronized cultures could be used to determine the effects of increased incubation temperature, high light intensity and dark incubation on the cell cycle. These studies described in the following sections provide basic information in elucidating the regulatory mechanisms that control highly ordered cell cycle events.

Combined effects of increased temperature and light intensity on synchronized cultures

Growth rates of exponential cultures can be increased when the incubation temperature or light intensity is increased as described earlier. When both incubation temperature and light intensity are increased, doubling times can be increased dramatically [29]. For example, the doubling time of an exponential culture at 32 °C exposed to moderate light intensity (3.2 J^{-1} s^{-1}) is 8 h. At an incubation temperature of 38 °C and higher light intensity (4.8 J^{-1} s^{-1}), the doubling time is 2 h. The combined effects of temperature and light intensity should influence the characteristics of synchronized culture as well.

A commonly observed synchronous growth occurs when cultures grown at 32 °C under moderate light intensity are induced by L/D shifts (fig. 3A). When exponential cultures (grown at temperature and light intensity of 32 °C and 4.8 J^{-1} s^{-1}) are incubated in the dark for 12–16 h and then shifted to light at the same temperature and light

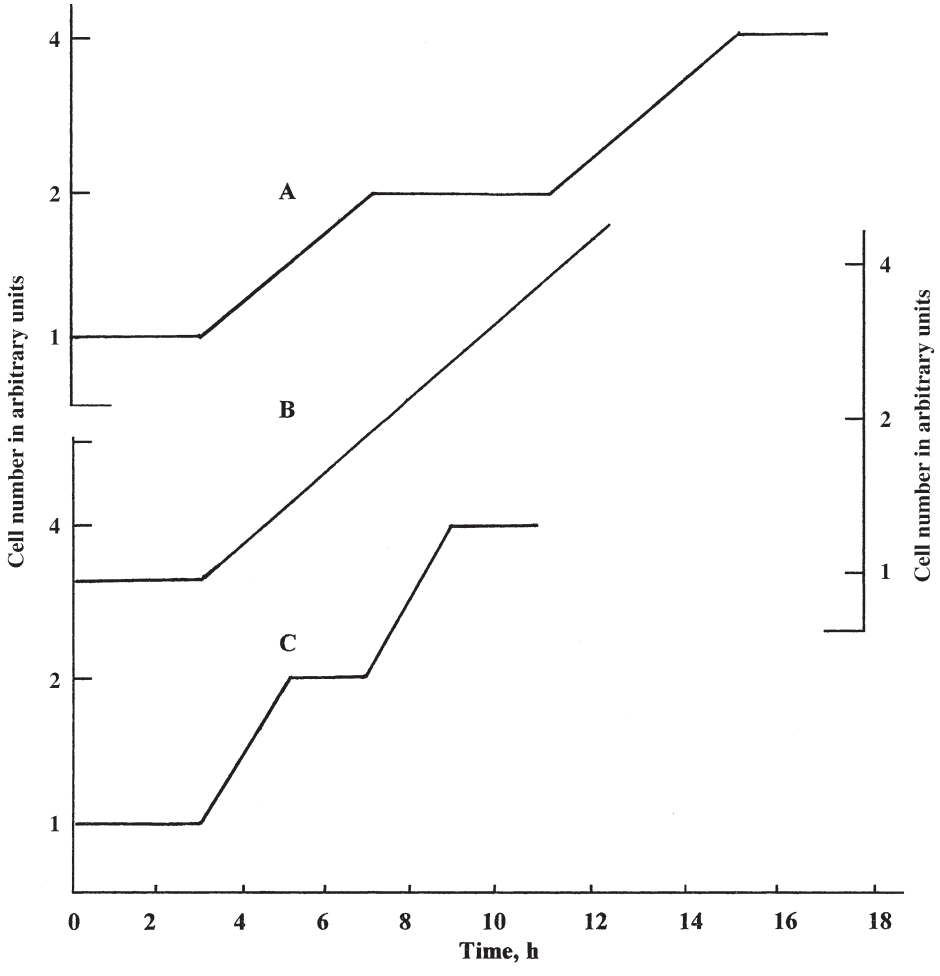

Figure 3. The characteristics of synchronized growth in various conditions of temperature and light intensity. (*A*) Synchronized growth at 32 °C and moderate light intensity (3.2 J m^{-2} s^{-1}). Doubling time, 8 h. (*B*) Growth at 32 °C and high light intensity (4.8 J m^{-2} s^{-1}). Doubling time, 4 h. See text for explanation of growth. (*C*) Synchronized growth at 38 °C and moderate light intensity. Doubling time, 4 h.

conditions, cell numbers increase exponentially after a 3-h lag [29]. The usual stepwise growth was not observed (fig. 3B). The doubling time is 4 h rather than 8 h. However, the macromolecular syntheses occurred in a periodic and sequential order; complete rounds of macromolecular synthesis periods occur within the 4-h doubling time. This unusual growth pattern can be explained [29] as follows. The rates of macromolecular synthesis are increased at the higher temperature and light intensity. However, increasing light intensity may not increase the rate of cell septum/cell wall formation. Note that when the temperature is raised to 38 °C at moderate light intensity, the rate of cell division is increased and a stepwise growth is obtained, as shown in figure 3C. Note also that the doubling time is 4 h. A possible reason for this observation is that the rate of exports of the peptidoglycan building blocks, particularly during the cell septa formation period, responds to the temperature of incubation but may not increase as light intensity is increased. As the cell septum/cell wall synthesis period of one cell cycle comes to an end, it appears that the next round of cell septum/cell wall forming period commences. Consequently, a phase devoid of cell division that normally occurs during the interdivision period is not observed (fig. 3B). There are several implications that arise as a result of this growth behavior. It appears that when the L/D shift is applied to exponential cultures (containing low cell numbers per milliliter) grown at high light intensities, the cultures may not show the stepwise growth that characterizes synchronized growth as described earlier [10, 21, 51]. Thus, when attempting to synchronize cultures by the L/D shift method, the behavior of macromolecular synthesis could be a more meaningful criterion of synchronized growth, particularly when high light intensity is applied to cultures containing low cell numbers per milliliter. Obviously, the effects of high light intensity of the cell growth cycle is a very complicated and will require critical experiments to confirm or modify the interpretations made above. In any case, the results described here provide possible bases for understanding the effects of temperature and light on cell growth that are reported in the literature and described in various sections of the text.

Effects of darkness on growth and macromolecular synthesis on synchronized culture

Dark incubation dramatically affects the growth and metabolism of photoautotrophs. However, dark incubation imposed on exponential cultures elicited different responses, as indicated earlier. The reasons for the contradictory responses are difficult to explain, since very few studies have been done in evaluating this problem. It turns out that synchronized cultures can be used to evaluate the effects of dark incubation on growth and macromolecular synthesis. That is, synchronization can amplify the characteristics of macromolecular synthesis in the dark. Herdman and Carr [112] utilized the L/D regimen-CO_2 deprivation method to induce cell synchrony. When samples of synchronized cells of *Synechococcus* were taken during mid-DNA synthesis period and placed in the dark, the cells completed DNA replication but did not divide, that is genome replication and cell septum formation were uncoupled temporarily in the dark. When the cells were returned to the light, the cells were able to perform one synchronous division, but thereafter the cells grew exponentially at a lower rate. The reason for the loss of sustained synchronized growth is not known.

A similar but more detailed study was done to characterize cellular events in the dark [44]. The growth conditions are given in table 1 and in [11]. When samples of synchronized culture (induced by 14-h dark incubation) were taken at hourly intervals during the synchronized cell cycle and placed in the dark, cell septum formation was curtailed whatever the time of sampling (see fig. 1A, which shows an example of the sampling schedules during synchronized growth). Bulk protein, RNA and DNA synthesis were significantly modified during the dark incubation period. When the ongoing synthesis of these macromolecules was in the last stages of their respective synthesis period in the light, small increases in macromolecular contents were observed during the dark incubation period. For example, increases of 3.9% in protein content and 10% in RNA content were found during the dark incubation period in samples that were undergoing the respective synthesis periods. The increases in protein and RNA contents in the dark represent residual synthesis, but new initiations of the macromolecules did not occur.

DNA metabolism in the dark appears complex. In samples of synchronized culture [44] that were undergoing the early stages of genome synthesis in light, DNA content per cell showed an initial increase of 10% within 2 h after transfer to the dark environment. However, a decrease of 15% of DNA content/cell was detected in the next hour of darkness. Thereafter, DNA content remained the same throughout the remaining 12-h incubation period. In samples of synchronized culture that were undergoing the late stages of DNA synthesis, DNA contents increased approximately 15%. The data were interpreted as follows. When the genome replication is in the early stages of synthesis, genome replication would be aborted. However, in cultures that are near the end of DNA synthesis, genome replication would continue to completion. These findings lead to the question of what happens to the replicating DNA molecule that is not completed in the dark. One possible explanation can be inferred from pulse-labeling experiments. In this analysis, cells were pulse-labeled with ^{32}P at 1-h intervals during the DNA synthesis period of synchronized culture. The hourly labeled samples were

then incubated in the dark for 12 h. It was found that ^{32}P-labeled DNA decreased (~15% in the radioactively labeled DNA fraction) in the dark in cells that were at the early stages of DNA replication [44]. The decrease in labeled DNA results if the newly duplicated DNA is cut out, released and digested [44]. Nucleotides from the DNA digest can be used for DNA repair in the dark or serve as nucleotide pools for the synthesis of DNA upon reexposure to light. In cells that are at the late stage of DNA synthesis, uptake of the ^{32}P in the dark suggests that genome replication is completed in the dark. It should be pointed out that (whether DNA replication was aborted or completed in the dark) cells reinitiated DNA replication 2 h after transfer from dark to light conditions [49].

According to Marino and Asato [44], cell cycles are aborted when synchronized cells at any stage of the cell cycle in the light are transferred to the darkened environment. That is, dark incubation prevents appearance of the scheduled sequence of macromolecular synthesis that is found in synchronized cultures grown in the light. What would happen when these dark-incubated cells are returned to light growth conditions? It turns out that cells did not continue or resume their cell growth cycle. Instead, whatever the stages reached before transfer to the dark, the cells reinitiated a new synchronized cell growth cycle upon return to the light (see fig. 1B). Appearance of the macromolecular synthesis periods corresponds to that of a 'standard' synchronized culture during the reinitiated synchronized cell cycle in the light [49].

There are several crucial questions that must be asked at this point. First, why couldn't *Synechococcus* reinitiate and synthesize macromolecules in the dark, at the same rate or at a slower rate, since the cells have the capability of carrying out ATP synthesis in the dark? Second, what is the mechanism in aborting cell cycle events in the dark? The answers to these questions could explain (i) the mechanism of induction of synchronized cell growth by L/D induction methods, (ii) the mechanism responsible for obligate phototrophy and (iii) the mechanisms underlying the light-regulated cell cycle events of *Synechococcus*. In an attempt to address these questions, a model of the regulation of the cell growth cycle is described below.

A model of the regulation of cell division cycle of *Synechococcus*

The major characteristics of the cell division cycle in cultures growing at 8-h doubling times at 32 °C under moderate light intensity can be outlined as follows. In light, as the cell growth cycle is initiated, macromolecules – protein, RNA, DNA, cell membrane, cell wall and cell septum – are synthesized in a highly coordinated and sequentially ordered schedule. When cells are placed in the dark, cell division stops. Macromolecular synthesis is curtailed or stopped, and the cell growth cycle is aborted. When the cells are returned to the light, a new cell cycle is initiated. The behavior of cells in light and dark conditions indicates that light plays a major role in regulation of cell cycle events. These characteristics of the cell cycle suggested the constructing a working model that is consistent with the coordinated regulation of cell cycle events [49]. Three major constructs of the model were described in [49].

1) The temporal and sequential order of macromolecular synthesis is coordinated by a cascade of sequentially expressed regulatory (or initiator) molecules; that is, a regulator of a cell cycle event would lead to the production of another regulatory molecule that initiates the next cell cycle event (see fig. 4). The role of specific sigma factors

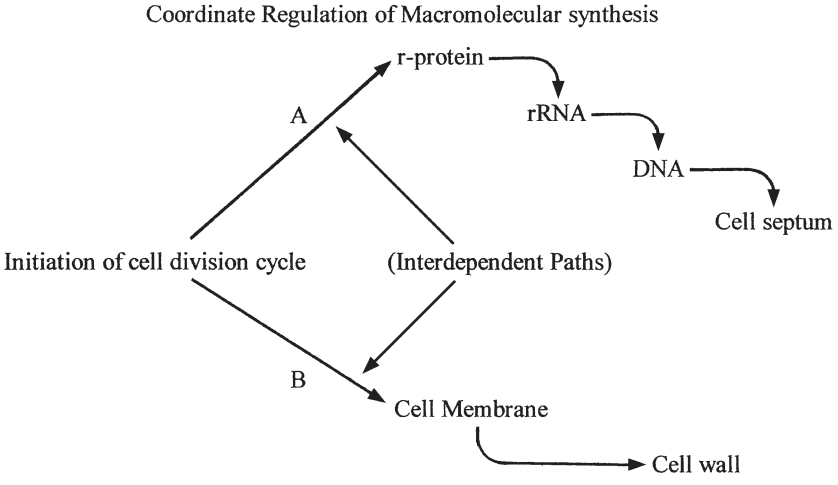

Figure 4. A model of coordinate regulation of macromolecular synthesis. The initiation of the cell cycle leads to two interdependent paths. Path A involves the coordinate regulation of r-protein, rRNA, DNA and cell septum synthesis. Path B involves the coordinate regulation of cell membrane and peripheral cell wall synthesis.

in coordinating the sequential expression of genes or operons might constitute key ancillary processes. The expression of at least some of them might be light dependent. This scheme of coordinate regulation is based on the finding that the schedule of macromolecular synthesis is not continued in cells that are incubated in the dark [49]. There could be at least two interdependent paths in the cell growth cycle. Path A is responsible for the sequential appearances of r-proteins, RNA and DNA, and culminates in cell septum formation. Path B involves the coordinate regulation of cell membrane and cell wall synthesis. The mechanisms that are involved in the interaction of paths A and B are not known.

2) The initiation of cell cycle events could be stimulated by light-activated molecules with possible involvement of photoreceptors as initial intermediates between light and the regulators. In the dark, the regulatory molecules become inactive, and initiation of all cell cycle events is curtailed. A report on the regulation of rRNA synthesis in *Synechococcus* by light-activated effector molecules [65] exemplifies the involvement of light in the control of gene expression (see below) predicted by the model.

3) The quantity of macromolecules per cell must also be regulated, and the quantity of each class of macromolecules is controlled independently, possibly through some kind of feedback loop or by autogenous regulation. In *Synechococcus*, S7 protein [57] or a similar r-protein, such as S2, could assume a role as autogenous regulator in the translational control of r-protein synthesis and transcription of rRNA, as predicted by the model. As seen earlier, synchronized cells that have completed the synthesis period of any macromolecule in the light, neither initiate synthesis of that macromolecule nor divide when transferred to the dark environment. When transferred back to light, these cells produce limited amounts of the specific macromolecules [49]. In this scheme, the macromolecular content of a cell would subsequently be adjusted to the normal level in the succeeding cell cycle. The mechanism for this adjustment is presently not known. It is possible that the basic features of cell cycle model could exist in other photoautotrophic cyanobacteria, although some modification of scheme would be necessary.

Light-activated transcription of rRNA synthesis and its implications in the regulation of cell cycle events

The *rrn* operons *rrn*A and *rrn*B of *Synechococcus* were cloned [113] and have been located on the physical map [67]. The organization of the *rrnA* operon in *Synechococcus* PCC 6301 was determined to be in the order 16S rRNA, $tRNA^{Ile}$, $tRNA^{Ala}$, 23S rRNA, 5S rRNA, and its sequence was subsequently determined [114–116]. The map of the upstream regions of the *rrn*A operon is shown in figure 5 A. Upstream of promoter P1 (UP in fig. 5 A), base sequences of –61 to –40 [65] share 50% sequence identity with the UP element (α-subunit binding site of the RNA polymerase, RNAP) of *rrnB* of *E. coli* P1 [117]. A G_6 tract is found immediately before the –35 hexamer of P1 [65]. In the leader region, six stem-and-loop structures are predicted which are thought to represent elements involved in antitermination signals [116]. Within the first stem and loop structure, sequences similar to box B of *E. coli* [118] can be found, but elements of box A and box C are not present [65]. The possible roles of the UP element, G_6 stretch and the stem-and-loop structures in the control of rRNA transcription are not presently known.

The in vivo synthesis of rRNA occurs at about the second hour of an 8-h cell cycle or an h after the protein synthesis period has been initiated [11]. rRNA synthesis stops abruptly when a light-grown cell culture is placed in the dark. In in vitro transcription analyses, 670-nt run-off transcripts were found [116] to be initiated from the (+1) transcription start site shown in figure 5 A. Two other run-

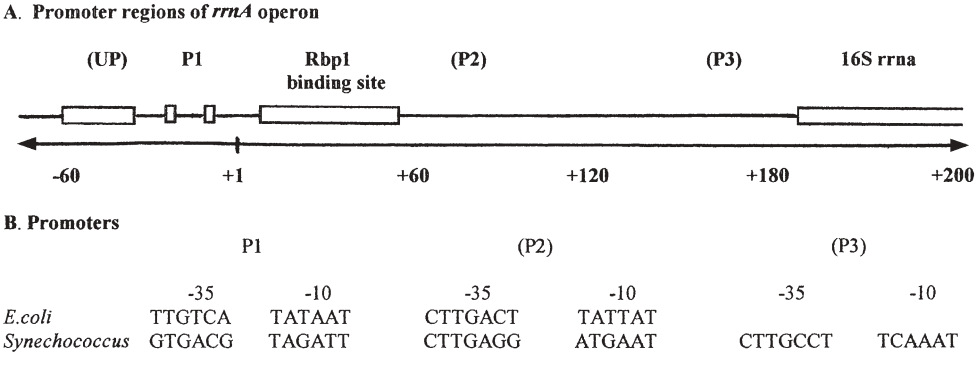

Figure 5. Regulatory regions of *rrnA* operon in *Synechococcus*. (*A*) Physical structure of the regulatory regions of *rrnA* operon. (UP), possible binding site for the α subunit of RNA polymerase as reported for *E. coli* [117]; P1, (P2), (P3) indicate promoters of the *rrnA* operon. Rbp1, binding protein. Values below the physical map indicate (–) and (+) bases relative to the +1 transcription start site of *rrnA* operon. (*B*) The P1 promoter of *Synechococcus* was reported in [113]; (P2) and (P3) are putative promoters as described in the text.

off transcripts were found with no promoters and transcription start sites identified. These transcripts could be transcribed from two putative promoters, P2 and P3 (see fig. 5B). There is, however, no information on the actual activities of these putative P2 and P3 promoters during cell growth of *Synechococcu*s. The occurrence of putative P3 of *rrnA* is unique to *Synechococcus*.

Note that only two *rrn* operons have been identified in *Synechococcus* compared with seven rrn operons in *E. coli*. In *E. coli* rRNA synthesis from P2 of *rrn*B is increased when the growth rates are increased by nutritional shift-up procedures [119, 120]. *Synechococcus* must have an efficient system to produce the required amount of rRNA from two *rrn* operons. It is possible that the putative promoters P2 and P3 are enlisted to increase rRNA transcription during the time when ribosomes are assembled.

The in vitro transcription of the *rrnA* operon is modulated by a transcription factor referred to as Rbp1 [65]. The binding site of Rbp1 is located downstream of promoter P1 of the *rrnA* operon. In vitro transcription assays show that Rbp1 permits the synthesis of rRNA from the transcription start site, +1, in light but not in the dark. That is, light activates Rbp1 to bind to the binding site and stimulate transcription of *rrnA* operon; in the dark, Rbp1 decays to an inactive form or is degraded by proteases.

The production of ribosomes in *Synechococcus* must involve two basic factors: (i) rRNA synthesis is controlled such that initiation and synthesis occur in light, but initiation of rRNA synthesis does not occur in the dark [44]; (ii) r-protein synthesis must be coordinated with rRNA synthesis. Taken together, (i) and (ii) suggest a scheme that involves initiation and transcription factors in which one or both factors are light activated in order to coordinate the transcription of r-protein and rRNA molecules. Several possible mechanisms can be considered. Two heuristic schemes are presented here, but they do not exclude other models.

1) Rbp1 as a coordinating factor. In this scheme, *rbp1* is an integral part of *rps* operons such as S7 or S2. *rbp*1 would be transcribed and translated along with the r-protein. In light conditions Rbp1 protein becomes activated and binds to its binding site on the of *rrnA* operon. Acting alone or together with other proteins, the light-activated Rpb1stimulates transcription of *rrnA* operon. It is possible that sigma factors or photoreceptors in the light-activated transcription of *rrnA* (and possibly *rrnB*) are also involved.

2) r-protein as the coordination protein. In *E. coli* r-proteins such as S2 serves as an autogenous translation regulator of S2 operon [121–123]. In the S2 operon of *E. coli* [123], *Spirulina* [58] and *Synechocystis* PCC 6803 (Cyanobase accession No. SII1260, 1722953-1722987 complement) the 5′ ends of *rpsB* mRNA and 16S rRNA have similar nucleotide sequences which can form similar spatial or secondary structures that act as binding sites for r-proteins. The possibility exists that S2 r-protein can bind to the 5′ regions of S2 mRNA, 16S rRNA [124, 125] and the 16S rRNA gene as well. In mechanism (2), S2 r-protein, produced during the protein synthesis period, would bind to the 5′ end of the 16S RNA gene, and Rbp1would bind to Rpb1 binding site. The binding of these proteins and possibly other ancillary transcription factors would form a complex that initiates *rrnA* transcription in light.

rrnA and *rrnB* have been located on the physical maps of photoheterotroph, *Synechocystis* sp. strain PCC 6803 [68]. The regulatory regions P1, P2, P3 and the Rpb1 binding site of *rrn*A have not been found in the sequenced genomes of *Synechocystis* PCC 6803 [62] or *Anabaena* sp. strain PCC 7120 [126] from the nucleotide similarity sequence searches through BLASTn (http://ncbi.nlm.nih.gov/Blast/gi/389181) and Cyanobase (http://www.kazusa.or.jp/cyano).

In *Synechococcus*, initiation of cell division cycle synthesis of r-protein, DNA, and perhaps cell wall and cell membrane syntheses might be controlled by light-activated regulatory factors, as shown in the path A scheme of figure 4 and described by a model above. The putative light-activated regulatory factors in any of the operons associated with the initiation of r-protein, rRNA or DNA synthesis should play major roles in regulating the cell cycle events of this organism and other cyanobacteria in light, and curtailing the progress of the cell cycle in the dark. For examples, in *Synechocystis* sp. strain PCC 6803, transcription of *dna*A is light dependent [80].

There are a large number of reports on the effect of light on the transcription on a variety of genes. For example, the *nif* gene of *Synechococcus* PCC 8801 (strain RF-1) is transcribed in the dark but not in light [127]. This finding is consistent with the experimental evidence that nitrogen fixation in a marine *Synechococcus* strain is found to fix N_2 in the dark, while O_2 production occurs in light [128]. In S*ynechococcus* sp. strain PCC 8801, *rbc*L is transcribed in light but not in the dark [127]. This result is consistent with the report that CO_2 fixation in *Synechococcus* sp. strain PCC 6301 is not detected in the dark. On the other hand, Tan and coauthors [129] reported that light repressed the transcription of *irtA*. Interestingly, the transcription of *irtA* occurs in the dark, but the translation of the *irtA* transcript occurs in light [130]. However, the expression of these genes described above are involved in basic biochemical processes. They may not be directly involved in the production of macromolecules that form the major cellular structures that occur during the cell growth cycle, as described previously. The most challenging task that remain is determining the regulatory factors that coordinate the sequential appearance of macromolecular synthesis periods in photoautotrophs. Rbp1appears to be one of these regulatory factors.

Cell division as an expression of circadian rhythm

Circadian rhythm describes physiological or cellular activities that are controlled by intrinsic or endogenous timing devices and occur in about a 24-h cycle. In cyanobacteria the activities can occur after subjecting an exponential culture to an entrainment protocol, usually a 12-h L/D cycle, or by a temperature shift regime [131]. The properties of circadian activities are (i) entrainability by L/D or temperature shifts, (ii) persistent cycling of the activity in constant conditions, (iii) phase shiftability or resetting, (iv) temperature compensation, that is a similar period of the circadian activity at different temperatures of incubation. See reviews [132–134], which describe circadian rhythmic events in cyanobacteria in detail.

It is of great interest that circadian rhythm in cell division was found in *Synechococcus*, since the occurrence of circadian cell division in prokaryotes was not thought to be possible. In a marine *Synechococcu*s sp. strain, WH7803, persistent cell divisions were indeed found to occur in 24-h cycles in continuous light after imposing a 12-h Light/12-h dark regime to exponential cultures [135]. The circadian appearances of cell divisions exhibited temperature compensation, that is the circadian rhythms of cell division occur at temperatures of 22, 20 or 16 °C. Remarkably, the generation times during continuous light conditions were determined to be 55, 135 or 200 h in the growth temperatures given above. The experimental data indicate that the occurrence of cell divisions was phased by a circadian timing device. However, the number of cells increased by 90 and 35 % during each successive circadian cell division event, indicating that not all cells divided during the circadian cell division period.

In another report [51], circadian gating of cell division of the cyanobacterium *Synechococcus elongatus* PCC 7942 has been described in detail. The cultures were grown in 1-l bottles, aerated by bubbling air and mixed by stirring. The incubation temperature and light intensity were 30 °C and 125 μE m^{-2} s^{-1}, respectively. The cultures were diluted at about 30 ml/h/500 ml culture volume to ensure that the cell density did not increase beyond 4.5×10^7 cells/ml during the course of the investigation. Under growth conditions, the doubling times were 10.9–11.8 h. In a series of experiments, cultures of AmC149, a luciferase reporter strain of *Synechococcus* PCC 7942 containing *Ppsb*A::*luxAB* transcriptional fusion, were entrained by 12-h L/D cycle and subsequently exposed to continuous light for 5 days. Luminescence as the result of *pbsA* gene expression occurred rhythmically in a 24-h time frame. Sharp increases in cell numbers occurred within the first 12-h period in light and recurred at 24-h intervals thereafter. The intrinsic or endogenous elements of the circadian oscillator was proposed as a mechanism that was able to slow or bypass cell division periods such that the cell divisions were mainly expressed at ~24-h intervals even though the doubling time was 11.8 h (see oscilloid model below). However, certain other observations should not be overlooked in order to provide an objective evaluation of the data. For example, cell numbers increased exponentially, and when the cumulative increase in cell number was calculated (by considering the dilution factor), the number of cells per milliliter increased by about 6.5 times (about 2.4 doublings) within an 18–20-h period. DNA contents determined at 6- or 9-h intervals by cytometric measurements showed a heterogeneous population with an average genome number of 3.9–5.4 per cell during the 2.5 days of incubation in continuous light. Analysis of DNA synthesis data suggested that DNA synthesis was initiated and proceeded at a constant rate even though the cells were dividing in a circadian time frame. Apparently, DNA replication was uncoupled to cell division during the circadian cell division episode, and the rate of cell division was slightly faster that the rate of DNA replication. Cell divisions slowed or were arrested after dividing for about 18 h. A pause in cell division could be controlled by circadian oscillator and, thereby, enable DNA replication to catch up with cell division.

A recent model (oscilloid model) is proposed to account for the circadian cell division and global regulation of circadian gene expression [136]. The major genetic elements of the oscilloid model are the *kaiABC* cluster [137] and *sasA* [138]. The promoters of *kaiA* and *kaiBC* are rhythmically active, and upon transcription of the gene clusters, mRNA transcripts of *kaiA* and *kaiBC* become rhythmically abundant [137, 139]. The control and timely coordination of *sasA* and *kaiABC* expressions are not clarified at present. KaiC has two major functions : (i) it represses *kaiBC* by negative feedback when *kaiB*C is overexpressed [137], and (ii) it regulates oscillation (condensation/and or supercoiling) of the genome in order to regulate global gene expression [140]. KaiC, a member of the RecA/DnaB or recombinase/helicase family [141], could bind to a DNA site and in some way cause genome oscillation. But KaiC does not act alone. Functional proteins KaiA, KaiB, KaiC and SasA form a multimeric complex that rhythmically regulates genome oscillations [138]. SasA and CikA, a phytochrome-like protein [142], are thought to serve as light intensity compensators that allow circadian cell division even in dim light but not in the dark. The oscillating chromosome regulates the rhythmic cell division or rhythmic gene expression.

Other interpretations of the observed circadian appearance of the cell division, however, should not be ignored, since the functional operations of the multimeric oscillator in controlling rhythmic cell division and rhythmic gene expression have not been worked out at present. For example, gating of circadian cell divisions could be due to deletions and insertions of 'determinate compensation-segment time loops' during progression of the cell divi-

sion cycle as suggested by the cytochron model of *Euglena* [143]. In brief, certain phases of D_1 or D_2 can exist as 'variable segment time loops'. When the time loops are deleted, DNA replication could be initiated at the usual time, but cell division would occur earlier in the cell cycle. Upon insertion of the variable segment time loops in the cell cycle, cell division is slowed to allow DNA replication to catch up. The model is flexible in that any combination of deletion and insertion of time loops could generate the circadian cell division mode. The cytochron model could be modified to be applicable to the circadian cell division of *Synechococcus elongatus*. Deletion and insertion could involve inactivation and activation of regulatory molecules that control certain functions of the cell cycle rather than deletion and insertion of variable segment time loops. A different interpretation is that high light intensity decreases the C period (i.e. it increases the rate of DNA replication) as in *Synechococcus* WH8101 [76], but not the rate of cell septum formation as in *Synechococcus* PCC 6301 [29]. In *Synechococcus* PCC 6301 [29] protein, rRNA and DNA synthesis periods occur sequentially and are completed within a doubling time shortened by high light. Under these conditions, the rate of cell septum formation is actually slower than the rate of DNA replication, and the cell number increases exponentially (see fig. 4C). At the end of one doubling time, DNA replication is uncoupled to cell division septum formation such that cell division does not occur at the end of the cell cycle causing a pause in cell division. These cell events recur to generate a circadian cell division pattern. Of course, these alternate interpretations could be manifestations of oscilloid regulation.

The circadian appearance of cell division presents a puzzling predicament. L/D entrainment could cause circadian cell division in *Synechococcus elongatus* PCC 7942 or induction of synchronized cultures in *Synechococcus* PCC 6301 and *Prochlorococcus*. A possible resolution of this puzzle is that regulatory systems that regulate circadian cell division and synchronized growth can exist and be expressed as independent regulatory mechanisms. Note that in L/D entrained cultures, stepwise growth is observed in the first 12 h in light in experiments displaying synchronized growth [11, 29, 31] or circadian cell divisions [51, 135, 144]. There could be two or more cell division periods within 15 h of light in synchronized cultures of *Synechococcus* PCC 6301. In L/D diel environment, synchronized cell divisions occur at ~24-h intervals in *Prochlorococcus* [56]. Circadian cell divisions are observed only in continuous light conditions. It is possible that coordinate regulation of cell events can occur in or out of circadian cell division time frames. That is, the oscilloid controls the circadian cell divisions under continuous light exposure. The light-responsive coordinate regulatory scheme controls the sequential appearance of the macromolecular synthesis periods in light conditions, but curtails the cell division cycle in the dark.

Cell cycles of unicellular, photoautotrophic, marine cyanobacteria, *Synechococcus* and *Prochlorococcus*

A growing number of studies are being done on the cell cycle of marine photoautotrophs, *Synechococcus* and *Prochlorococcus*. Marine cyanobacterial strains make up a vital microbial community that contributes significantly to biomass and primary production in oligotrophic environments [145–150]. Most but not all of these studies evaluate the cell cycle in order to understand the population dynamics of marine cyanobacteria in the open oceans.

Cell division cycle

In *Synechococcus* sp. strain WH8101 and related strains, distinct phases of G_1, S and G_2 phases – gaps G_1 and G_2 before and after DNA synthesis or S phase – were observed under light growth conditions [21, 48]. G_1, S and G_2 phases were determined from histograms displaying bimodal distribution of DNA florescence. Cells contain one genome in G_1 phase, two genomes after S phase, and end up with one genome following cell division [21, 76]. When light grown cultures were placed in the dark, cell cycles were arrested at G_1 or G_2 phases. This result was explained as follows. There are two cohorts of cells. G_1 phased cells that enter the dark incubation will not be able to initiate DNA replication and will consequently be arrested at G_1. On the other hand, cells in the S phase complete DNA replication in the dark and remain in G_2 phase. Upon reexposure to continuous light, the cell number exhibited an initial lag, then increased exponentially thereafter. The results also indicated that dark incubation does not induce synchronized growth in *Synechococcus* WH8101. The cell cycle characteristics of WH7803, are noticeably different from the other marine strains. *Synechococcus* WH7803 contains 1, 2, 3, 4, 5 or 6 genomes per cell, and the cell cycle is similar to freshwater *Synechococcus* PCC 6301 [48]. Asynchronous initiation of DNA replication is indicated in cells with a high number of genomes per cell. The timing of cell division is not tightly coupled to DNA replication in these cells. There appear to be two or more cohorts of cells; each cohort goes through the cell cycle independent of each other. Dark incubation induces circadian cell divisions in *Synechococcus* WH 7803 [135]. The characteristics of marine *Synechococcus* WH7803 is more akin to freshwater *Synechococcus* PCC 6301 [48] and *Synechococcus* PCC 7942 [51].

Cell cycle of marine cyanobacterium, *Prochlorococcus,* was investigated in the marine environment and in con-

trolled laboratory conditions [22, 56, 145–153]. Maximum estimated doubling time in the equatorial Pacific Ocean at 30 m is 17.52–21.6 h [56]. Data from 24-h samplings showed an orderly appearance of G_1, S and G_2 phases, indicating that cell growth was synchronized in the natural environment [56]. G_1 phase occurs in late night and early morning; S phase occurs during midafternoon (about 14.00 h), and DNA replication is completed in about 3 h (or about 17.00 h). The cells go through G_2 phase by late afternoon to early night; cells divide at night, but the cell cycle is arrested at G_1 phase. A similar pattern of cell cycles was observed in other studies in the field [147, 154] and in the laboratory [151, 152].

There are notable similarities and differences in the cell cycles of marine *Synechococcus* and *Prochlorococcus*. Both strains exhibit distinct G_1, S and G_2 phases. However, in marine *Synechococcus* DNA replication and cell division do not occur in the dark. Cell cycles are, therefore, arrested in G_1 or G_2 phases in the dark. In *Prochlorococcus,* cell division does occur in the dark, and the cell cycle is arrested at G_1 phase. Cell synchrony occurs in *Prochlorococcus* [56] but not in marine *Synechococcus* [21]. The observed differences in cell cycles in the dark cannot be resolved at present unless the nature of the cellular activities of G_1 and G_2 phases, and the regulatory factors that coordinate the G_1, S and G_2 phases, are known.

Cell cycles of marine *Synechococcus* and *Prochlorococcus* are regulated by light

Growth rates in marine *Synechococcus* and *Prochlorococcus* increased in response to increases in light intensities, as found in fresh water Sy*nechococcus*. In marine *Synechococcus* sp. strain WH 8101, WH 7803 and related strains, physical characteristics of cells and cell growth phases varied in relation to increases in growth rates [21, 76, 155]. C (DNA replication) values decreased at low growth rates (0.15–0.5 d^{-1}), increased gradually at moderate growth rates (0.6–1.5 d^{-1}) and decreased sharply at high growth rates (beyond 1.5 d^{-1}). D (post-replication period) values declined rapidly as growth rates increased [76]. The pattern of rRNA content $cell^{-1}$ and cell volume was similar to the behavior of C values at moderate growth rates [155]. The abrupt decreases in rRNA content $cell^{-1}$ and cell volume at high growth rates indicate a physiological response to light-saturated growth conditions. The small but significant variations in cell volume as growth rates are increased could be responsible for the observed pattern of rRNA content $cell^{-1}$. Several sources of information indicate that initiation of chromosome replication is regulated by cell volume-cell mass. The information sources are (i) calculated cell volume and M_i (initiation mass) at the time of initiation of chromosome replication, (ii) estimated initiation volume and (iii) direct measurements of cell volume. However, the underlying basis for the behavior of the values described above in response to growth rates and light intensities remains to be explained.

L/D shift can induce synchronized growth in *Prochlorococcus* strains, as indicated earlier. A series of experiments were performed in the laboratory to determine the factors that could be involved in induction of synchronized growth. Significant results were obtained, and the interpretations of the data made by the researchers are summarized as follows [22]. Cultures of *Prochlorococcus* PCC 9511and CCMD 1375 strains were first grown in alternating 12-h L/D cycles.

1) In the first set of experiments, cell size and chlorophyll fluorescence (chl fl) increased to peak values at the end of the 12-h light period. Upon transfer of the culture to darkness, both parameters decreased to a low level at the end of the 12-h dark period. This result suggested that pigment synthesis and cell growth were tightly coupled. Similar results were found in a field study [154] performed on subsurface samplings. The sequential appearances of S and G_2 phases in the light period were similar to the patterns described earlier. However, a second minor peak in S phase was observed soon after the culture was subjected to the dark, but no minor peak in G_2 phase occurred. (An unusual sequence of cell events in the dark was found in another study on *Prochlorococcus* which will be described below).

2) In cultures grown in L/D cycles and subjected to prolonged dark periods, the cell cycle was disrupted, and most cells were arrested at G_1 phase. However, a fraction of cells that initiate S phase in the dark cannot complete DNA replication and enter the G_2 phase as observed in phosphorus-starved cells [156]. Cells arrested in S phase apparently die in the dark. The results indicated that light is critical for reinitiation of the cell cycle.

3) In cultures subjected to 12-h L/D entrainment and subsequently exposed to continuous light, S and G_2 phases were reset, that is S and G_2 values peaked at about 12- 18- 24 h intervals. On the other hand, cell size and chl fl peaked at ~12-h intervals. Resetting of these cell cycle events suggested that they could be under the control of circadian clocks [22, 151] or alternatively by exposure to direct light [153].

4) Growth rates were found to be tightly coupled to light intensity. For example, a shift from low light (LL) to high light (HL) induced a rapid increase in cell number and, in a high proportion of cells, S and G_2 phases occurred earlier in the cell cycle; the reverse was found when cultures were shifted from HL to LL. Cell size and growth rates peaked at about the same time interval, suggesting that cells must reach a critical size before they divide.

In another interesting study [151], ultraradian growth (or growth faster than one division per day) was observed in the field and in laboratory conditions (12 h L/12 h D,

26 °C incubation temperature under 40–120 μE m^{-2} s^{-1}). When a light-grown culture of *Prochlorococcus* strain MIT 9302 was placed in the dark, all cells carried out DNA replication and cell division. However, one-third of the cell population was able to go through a 'second wave' of DNA replication and cell division. The occurrence of the second nonoverlapping cell events was not affected by light intensity or duration of light exposure. The second wave of cell cycle was completed within 8 h and was characterized as ultraradian growth. It was concluded that the second cell cycle events were controlled by a 'light-triggered timer' or circadian clock and not by 'assimilation phase'. It would be of interest to learn why two-thirds of the cell population escaped control by circadian clock. Experiments directed toward this question could lead to the basis in the control of cell division in *Prochlorococcus* [22, 151].

Cell cycle events within G_1 and G_2 phases

Distinct G_1 and G_2 phases occur in the cell cycle of marine cyanobacteria; however, the cell events in the gap phases are not clearly described. The crucial activities in G_1 phases most likely would involve initiation of DNA replication and cell division septum formation in G_2 phases. In *Prochlorococcus* sp. strain PCC 9511, mRNA transcripts of *dnaA* and *ftsZ* occurred maximally during the S phase near the end of the light period. However, the peak concentration of FtsZ occurred at night during the cell division period [105]. The results suggest that a regulatory factor in coordinating the initiation of *dnaA* and *ftsZ* transcripts would most likely exist. At present, the phases in which other cellular structures such as ribosomes are assembled or produced in the cell cycle have not been determined.

Are there common regulatory systems that control the cell division cycle in fresh water and marine phototoautotrophs?

All photoautotrophic cyanobacteria exhibit the basic physiological characteristics of photoautotrophy. All photoautotrophic cyanobacteria display the same G_1, S and G_2 (or D_1, C, D_2) phases within the cell division cycle. Except for *Prochlorococcus*, cell division in photoautotrophs is arrested in the dark. DNA replications that are initiated in the dark are not completed in *Synechococcus* PCC 6301 and *Prochlorococcus* MED 4. Cells are arrested in G_1 or G_2 phases in the dark in marine *Synechococcus* WH 8101or G_1 in *Prochlorococcus* strains. On the other hand, the arrest point can occur at any phase in *Synechococcus* PCC 6301. A common requirement in all photoautotrophs is that light initiate the cell growth cycle when dark-incubated cells are reexposed to light. The scheme in the coordinate regulation of light-activated initiation in macromolecular synthesis in *Synechococcus* PCC 6301could occur in marine cyanobacteria as well. Of course there could be some variation in the regulation of cell events. For example, the ability to divide in the dark by *Prochlorococcus* strains can be explained as follows. A putative regulatory factor that initiates cell division septum in *Prochlorococcus* could result by a missense mutation such that the regulatory factor is active in the light or darkness. The control of circadian gene expressions and cell divisions by oscilloid could be an independent regulatory system that is expressed in *Synechococcus* PCC 7942, *Synechococcus* WH 7803 and perhaps in other cyanobacteria under continuous light. These considerations suggest that common genetic regulatory schemes that underly the expression of these common characteristics of the cell cycle most likely exist in all photoautotrophs.

Strategy of obligate photoautotrophic mode of growth

Obligate photoautotrophs must be able to reproduce effectively in the natural environment in order to perpetuate their species. It is therefore ironic that obligate photoautotrophs are able to produce stored energy molecules through photosynthesis but not use them to support sustained growth in the dark. *Prochlorococcus* is able to divide in the dark in order to complete the cell cycle initiated in daylight, but continuous cell division cycle does not occur. Photoautotrophic cyanobacteria evolved two distinct physiologies – physiology of growth in light, and dark physiology. This bipartite physiology is apparently a successful strategy of growth; cyanobacteria have evolved about 2.7×10^9 years bp which attests to their effective scheme of cell reproduction. Both physiologies could be controlled by the common, intrinsic, genetic regulatory systems described earlier. An advantage of this bipartite physiology is that *Synechococcus* is able to initiate cell division cycle immediately when dark-incubated cultures are reexposed to light. It is well known that the photosynthetic apparatus and Rubisco (ribulose bisphosphate carboxylase/oxygenase) are functional immediately upon illumination. This strategy is beneficial since available radiant energy during the daylight hours is ~10–15 h, and the cells must be able to multiply optimally during that time period. Light-to-dark and dark-to-light shifts in growth conditions could alter physiological processes and cause delays in the cell growth process. Photoautotrophs avoid lag-phase transition delays upon transfer from dark to light growth conditions and are able to initiate the cell division cycle immediately upon reexposure to light [49]. If survival of the population depends on effective reproduction, this strategy can permit two

cell division periods and thereby increase the population fourfold well within the daylight hours (see fig. 1 A, B), assuming that sufficient radiant energy and inorganic nutrients are available. Note that in 'ideal' laboratory conditions, the potential of higher growth rates can be increased severalfold when both temperature and light intensity are increased. In the natural environment, both parameters of growth can occur together. The large increase in population during cyanobacterial blooms can be attributed in part to this unique ability to enhance multiplication in favorable environmental conditions [29].

It is interesting to note that *Synechococcus* never evolved elaborate enzymatic processes of importing organic substrates to support photoheterotrophic or heterotrophic growth. In fact, uptake of a number of organic compounds could inhibit the growth of *Synechococcus* PCC 6301. For example, intermediate metabolites of citric acid cycle, cysteine and indole were found to inhibit the growth of *Synechococcus* PCC 6301 [156a]. It is possible that accumulation of key intermediate metabolites in a biosynthetic pathway could inhibit enzymes upstream of the pathway by the mechanism of autogenous or feedback regulation. Fructose is toxic to *Synechococcus* PCC 7942 R2-1 [157] and the photoheterotrophic strains *Synechocystis* PCC 6803 and *Synechocystis* PCC 6714, which utilize glucose as carbon and energy sources [158, 159]. Experimental results [157] suggest that intracellular accumulation of a glucose derivative could disrupt the metabolic activities by feedback inhibition in the *Synechococcus* R2-1 strain. Incidentally, the limited range of nutritional mutations in *Synechococcus* PCC 6301 [160] can alternatively be explained by the accumulation of potentially toxic intermediate metabolites. That is, a mutation that causes an accumulation of potentially toxic metabolites could be lethal.

What mechanisms are involved in controlling the obligate phototrophic mode of growth? At least three basic levels of operating systems could exist. The control of macromolecular synthesis by light-activated regulatory factors must be the major control mechanism since initiation of the cell growth cycle is prevented in the dark. Control of macromolecular synthesis by light-activated factors can be considered as the core regulatory system since macromolecules make up the basic cellular structures. Cell division by circadian elements, however, could be an independent control system.

The second level of control appears to be the control of photosynthesis and biosynthetic reactions. The control of photobiochemical and biosynthetic reactions can be considered as the peripheral regulatory system. The biosynthesis of precursor molecules should in turn be coordinated with the synthesis of macromolecules that occur sequentially at specific times during the cell growth cycle.

The third level of control concerns the activities of dark physiology which involve cell maintenance reaction, repair and preparation for growth in light. The nature of cell maintenance is at present ill defined. Perhaps the synthesis of novel RNA molecules [45] and proteins [46] could be involved in some form of maintenance activities. The authors suggested that the RNA molecules are involved in L/D/L transition phase reactions. In photosynthetic organisms, toxic oxygen such as superoxide anion, singlet dioxygen and hydroxyl radicals are produced through interactions with O_2 that are generated during light reaction in photosynthesis [161]. Highly toxic hydroxyl radicals, for example, can cause lipid peroxidation, protein denaturation and DNA damage. In a superoxide dismutase mutant (*sodB*) of *Synechococcus* sp. strain PCC 7942, damage to photosystems I and II was detected [162]. In *Anacystis nidulans* photooxidative death and survival from photooxidative death was correlated with superoxide dismutase activity [163, 164]. The superoxide dismutase is induced by O_2 and requires photosynthesis and protein synthesis [164]. Apparently there are sufficient amounts of residual superoxide dismutase activity in the dark that can detoxify the toxic oxygen molecules. However, in a CO_2-free environment the activity of superoxide dismutase could be compromised. Oxidative damage to DNA could cause mutation and impair DNA replication unless the DNA lesions are repaired in the dark. Excision repair of DNA [165] as well as a host of DNA repair mechanisms could be other forms of survival activities in the dark. However, the question remains regarding the source of required nucleotides that must be available for repair and replication of DNA molecules in the dark. If nucleotide synthesis does not occur in the dark, an alternative method for building adequate pools of precursor molecules is by degradation of macromolecules. As described earlier partially, replicated genomes could be degraded to produce significant amounts of nucleotides. Other precursor molecules must also be available in sufficient quantity to support the initiation of the cell cycle immediately when a light source becomes available.

Concluding remarks

The highly ordered sequential appearances of macromolecular synthesis periods in *Synechococcus* PCC 6301 are most likely coordinated by light-activated regulatory factors that orchestrate the timely and orderly formation of the cellular structures. In the dark, the regulatory factors become inactive preventing further reinitiation of macromolecules. It is likely that a common regulatory program for the cell cycle exists in all unicellular, photoautotrophic cyanobacteria. The characteristics of the cell division cycle in *Synechococcus* sp. strain PCC 6301 could serve as a model for further elucidation in the regulation

of cell events in photoautotrophs that display unique photoautotrophic nutritional physiology. Future investigations should be done to determine:

1) key regulatory factors that control the coordinated appearances of all major cellular events that occur in the cell cycle. The tools of genetics and molecular biology developed for cyanobacteria in conjunction with the analysis of the genome sequences of cyanobacteria could assist in identifying specific genes that serve as genetic elements that coordinate macromolecular synthesis periods. It would be of interest to determine whether genes coding for regulatory factors of the cell division cycle such as cyclins and kinases found in other microorganisms could be identified.

2) The functional operation of the oscilloid model in controlling rhythmic cell divisions and rhythmic gene expressions should be elucidated.

3) Experiments should be done to determine whether the common sequence of cell cycle events is controlled by a common regulatory scheme in all photoautotrophic cyanobacteria.

Certain growth characteristics found in *Synechococcus* could occur in other cyanobacteria and eukaryotic phototrophs. For example, *rrn* operons in plant chloroplasts could be regulated by a light-activated transcription factor [M. Sugiura, personal communication]. The loss of chloroplast in *Euglena* and, perhaps, the etiolation of leaves in higher plants during prolonged incubation in the dark could indeed involve a light-activated factor similar to Rbp1.

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