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Scientific Research

Isolation, Purification and Characterization of Nucleoids from *Synechococcus elongatus* PCC 7942

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Abstract

The genomic DNA of bacteria is highly compacted in one or a few bodies known as nucleoids. In order to understand the overall configuration and physiological activities of the cyanobacterial nucleoid under various growth conditions and the role(s) of each nucleoid protein in clock function, thylakoid membrane-associated nucleoids from the *Synechococcus elongatus* (se) PCC 7942 strain were isolated and purified in presence of spermidine at low salt concentrations by sucrose density gradient centrifugation. The sedimentation rates, protein/DNA composition and microscopic appearances as well as variation in structural components of clock proteins from the isolated nucleoids were compared under identical conditions. Microscopic appearances of the nucleoids were consistent with the sedimentation profiles. The nucleoid structure in the wild type was more tightly compacted than that in the KaiABC mutant strain. Western immunoblot analyses revealed that the KaiC was associated with the nucleoid fraction whereas maximum KaiA was localized in the cytosolic fraction, supposedly in association with the translation machinery.

Keywords

***Synechococcus elongatus*, Sucrose Gradient, Cyanobacterial Nucleoids, Circadian Clock, KaiC Protein**

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1. Introduction

The chromosomes in the eukaryotic nucleus are compacted into the nucleosomes with histone proteins as the basic unit. However, in bacteria there is no such nucleosome, but the DNA is organized into a nucleoid body with particular proteins in the cell, which has a highly organized architecture based on condensation and coiling of DNA [1]-[6]. This condensation state of nucleoid drived DNA is to be negatively supercoiled *in vivo*, and a number of nucleoid-associated proteins (sometimes referred to as histone-like proteins) have been implicated in the organization of bacterial chromatin, with additional roles in transcription, recombination and replication [7]-[10].

In *Escherichia coli* (*E. coli*) the five most abundant among these are Fis (Factor for Inversion Stimulation), H-NS (Histone-Like Nucleoid Structuring Protein), HU (Heat-Unstable Nucleoid Protein), IHF (Integration Host Factor) and Dps [11]-[24]. However, at present a clear picture of cyanobacterial nucleoid organization is far from being complete. This may be due to insufficient documentation of the molecular structure and composition of cyanobacterium *Synechococcus elongatus* (se) PCC 7942 nucleoid and its circadian rhythm dependent variation [25].

In order to understand overall configuration and physiological activities of the se-genome under various growth conditions and the role(s) of each nucleoid protein in determination of the clock function(s), we have performed a systematic analysis for isolation, purification and characterization of the se-nucleoids for the first time by sucrose density gradient centrifugation. Sedimentation profiles of the se-nucleoids are consistent with the microscopic appearances, concluding that the clock proteins KaiA, kaiB and KaiC are necessary to organize genomic DNA into the se-nucloid. Moreover, quantitative Western immunoblot analyses revealed that the KaiC was associated with the nucleoid fraction whereas maximum KaiA was localized in the cytosolic fraction.

2. Materials and Methods

2.1. Bacterial Strains and Growth Conditions

The bacterial strain for analysis of se-nucleoid proteins was the wild type, KaiA, KaiB, KaiC, KaiBC and KaiABC inactivated *Synechococcus* strain as previously described [26]. Cells were grown in a continuous culture system, in BG-11 liquid medium to maintain an OD730 of 0.25, corresponding to 2.5×10^8 cells/ml, under LL (50 $\mu E/m^2/s$) at 30˚C. After two to three 12 h light (LL) and 12 h dark (LD) cycles, cells were released into free-running conditions of continuous light (LL) and then collected every 4 - 6 h intervals.

2.2. Determination of the Total Number of Cells

Aliquots of the culture were taken at various time intervals as indicated in the text. The number of cells was counted with a Coulter Multisizer II (Coulter Electronics Limited, Northwell Drive, Luton, Beds, LU3 3RH, England). Gain and current settings of 2.5 and 5.5, respectively, were used for all measurement. Coulter Multisizer II readings were maintained in the 3×10^4 - 10×10^4 range by dilution with Coulter Multisizer II specific isotonic buffer.

2.3. Nucleoid Preparation and Isolation by Sucrose Density Gradient Centrifugation

Cyanobacterial nucleoids were prepared from the 100 ml cultures, collected from various strains, each contains about 2.5×10^8 cells/ml. Collected cell were chilled in an ice-cold water bath and harvested by centrifugation (12,000 ×g, 4˚C, 10 min). Preparation of nucleoids and their sucrose density gradient centrifugation run were performed essentially according to the protocol devised by Kornberg *et al*. [27] and modified by Murphy and Zimmerman [28] and this study (for details see **Figure 2**). Linear gradients (12% - 60% sucrose in 10 mM Tris-HCl (pH 7.8 at 0˚C) containing 10 mM KCl, 1 mM EDTA, 0.2 mM DTT (dithiothreitol), 1 mM spermidine HCl) were formed in a cold room in 5 ml Ultraclear plastic centrifuge tubes (13 × 51 mm, Beckman) and were run at 10,000 rpm at 4˚C for 120 min in a RPS50-2-517 rotor (the centrifugal force was reduced to 7000 g). After the run, the tubes were placed against a dark background, illuminated with a high-intensity sharp light from the top and immediately photographed. The relative positions of each of the independent nucleoids isolated from different strains were reproducible at different speeds and for longer runs (data not shown). The speed and duration of the runs used in this study were chosen in order to maintain the fastest moving band (nucleoids from the

culture of wild-type cells) within the bottom of the gradient. Fractions in an average 0.45 ml (except nucleoid fraction 0.60 ml) were collected from the bottom of each gradient and mixed with 20 µl of 0.2 M EDTA and stored at 4˚C until further use.

2.4. Determination of Protein and DNA Content

DNA from each of the 14 fractions was digested at 37˚C for 30 min with 20 µg of RNase A per ml and 20 µg of Proteinase K per ml. After digestion, DNA was purified by extraction with phenol/chloroform and then precipitated with ethanol. Total protein estimation was carried out by BCA method using bovine serum albumin (BSA) as a standard. DNA and protein content were measured from optical density at 280 and 550 nm, respectively.

2.5. Polyacrylamide Gel and Agarose Gel Electrophoresis

The sedimentation patterns of total proteins and DNAs from isolated nucleoids were compared with those of other cell fractions by SDS-containing either 12.5% or 15% Polyacrylamide gels and 1.0% Agarose gel electrophoresis, respectively. Photographs were taken and shown where necessary after Polyacrylamide or Agarose gels were stained with either Coomassie Brilliant Blue (CBB) or Ethidium Bromide, respectively.

2.6. Western Immunoblot Analysis

For the measurement of each of the three nucleoid proteins in se-nucleoid and cytosolic fractions, a quantitative Western blot analysis was employed using the polyclonal anti-KaiA, anti-KaiB, anti-KaiC and anti-NrtA (internal control) antibodies, as described [26] [29]. Protein concentration was determined by the BCA method using bovine serum albumin (BSA) as a standard. In brief, either nucleoid or cytosolic fractions were treated with a sodium dodecyl sulfate (SDS) sample buffer (50 mMTris-HCl [pH 6.8], 2% SDS, 1% 2-mercaptoethanol, 10% glycerol, 0.025% bromophenol blue) and separated on SDS-15% polyacrylamide gels. Proteins in the gel were directly electroblotted either onto polyvinylidene fluoride (PVDF) membranes (Nippon Genetics) or nitrocellulose membranes. Blots were blocked overnight at 4˚C in 3% bovine serum albumin (BSA) in phosphate-buffered saline (PBS), probed with the specific antibodies against each protein, washed with 0.5% Tween 20 in PBS, and incubated with goat anti-rabbit immunoglobulin G conjugated with hydroxyl peroxidase (Cappel). The blots were developed with ECL Western blotting detection reagents (Amersham).

2.7. Immunofluorescent Microscopy of Se-Nucleoid

Intact cell nucleoids, thylakoid membrane associated crude nucleoids and purified nucleoids were collected and fixed with 0.1% Glutaraldehyde (final concentration) where necessary. The fixed nucleoids (5 µl) were dropped into the well of a 10–well multitest microscope slide (76 × 26 mm with 24 × 60 mm coverslip; Matsunami glass Ind., Ltd., Japan) and air dried at 27˚C. The nucleoids were stained with 5 µl DAPI (4', 6-diamidino-2-phenylindole dihydrochloride) solution (5 µg/ml) which bind specifically to DNA and immediately photographed, using the method developed by Talukder *et al*. [22] and Hiraga *et al*. [30].

3. Results and Discussion

3.1. Cell Growth and Expression Patterns of Se-Total Proteins

In the routine assays herewith described, an early-stationary-phase culture was diluted 200-fold with fresh BG-11 medium, and the culture was continued at 30˚C until 360 hours. At various times, aliquots of the culture were collected and used for determination of the number of cells, measured with a Coulter Multisizer-II. Viable cells were also measured with a viable colony plate counting method and the concentration of total proteins were measured by OD at 550 nm and visualized by CBB staining. In the experiment whose results are shown in **Figure 1(A)**, the analysis was continued for 360 h after transfer of the early stationary phase culture (36 h) into a fresh medium. The growth was measured by turbidity and remaining constant at least 360 h, the condition we employed herewith. However, viable colony forming units was maximum at 120 h, and thereafter goes down slowly (**Figure 1(B)**). These results indicate that at the stationary phase of growth, heterogenous cell populations were not only observed in *E. coli* but also in Cyanobacteria [31] [32].

The composition of total cellular proteins was analyzed by SDS-PAGE. For this purpose, cell lysates were

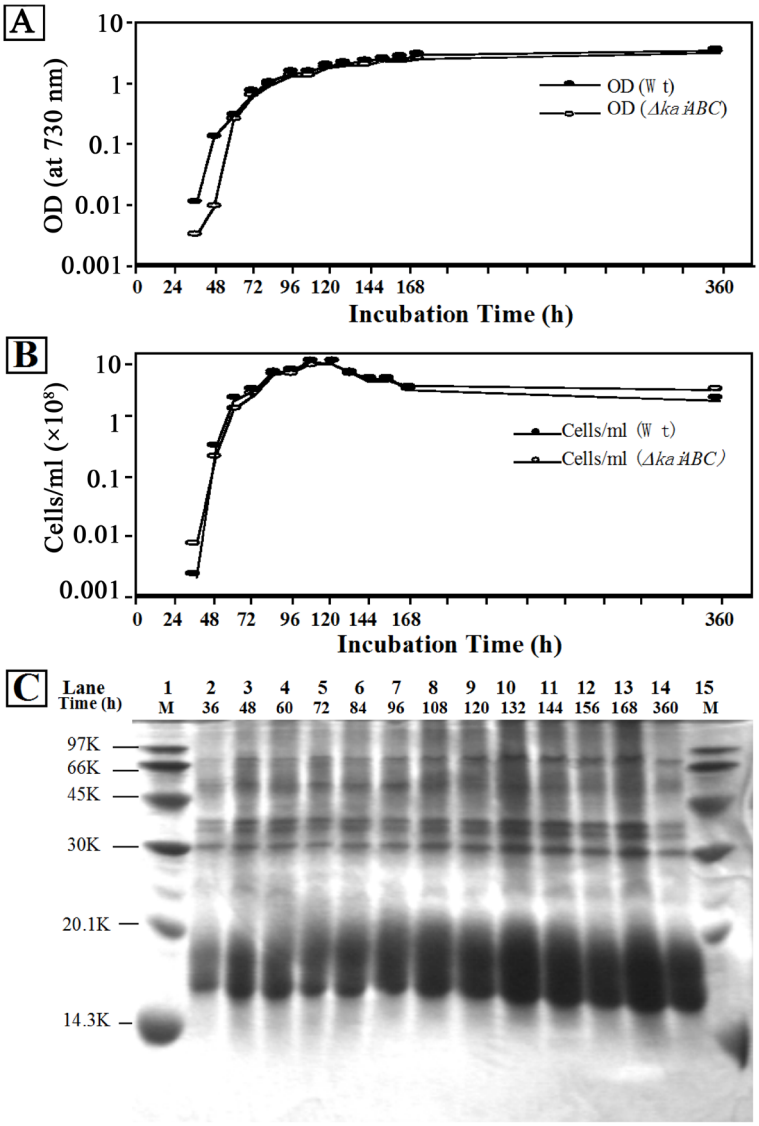

Figure 1. Expression patterns of *Synechococcus elongatus* strain 7942 total proteins at various growth phases. An early-stationary phase (36 h) culture of strain 7942 in BG-11 medium at 30˚C was diluted 200-fold with fresh BG-11 medium, and the culture was continued at 30˚C in a constant light chamber. (A) Optical density and in (B) the total number of cells/ml was measured by OD at 730 nm and the viable plate count method, respectively. (C) At the indicated time points (above the lane markers), aliquots of the culture were collected for the preparation of cell lysates. Portions of the cell lysates containing 15 - 20 μg of total proteins were subjected to SDS-15% PAGE. Gel was stained with CBB (Coomassie Brilliant Blue) and photograph.

prepared at different growth phases, and aliquots containing 15 - 20 μg of total proteins were loaded to SDS-15% PAGE as shown in **Figure 1(C)**. The gel-staining patterns of total se-protein from the various cultures (samples 1 - 14) were essentially identical to the typical pattern of growing cyanobacterial cells. We could not see any changes of total protein level, indicated that the expression of cyanobacterial proteins are growth-phase independent [33]. However, many microorganisms including *E. coli* maintained growth-phase dependent variations in the structures and protein-compositions of nucleoids were reported previously [21] [22].

3.2. Isolation, Purification and Characterization of Se-Nucleoid

We have decided to isolate *Synechococcus elongatus* PCC 7942 nucleoids by sucrose density gradient centrifugation and examine nucleoids for characterization under various conditions. Bacterial nucleoids are generally

released from cells in a Tris-EDTA buffer by treatment with lysozyme, followed by application of one or several detergents [34]-[38]. We have checked several experimental conditions to deduce the most suitable procedure for isolation of cyanobacterial nucleoid, namely, the nucleoid buffer compositions (different concentrations of salt, lysozyme, spermidine), various types of detergents (SDS, Brij-58, Deoxychlolate, NP-40, etc.), reaction conditions for preparation of cell lysates, different centrifugation speeds and times which are very important parameters for nucleoids isolation from the whole cell extracts.

After checking several conditions, we have developed a procedure for the isolation and purification of se-nucleoids in a single visible fraction from the sucrose density gradient centrifugation as shown in **Figure 2**. Our established method was mainly based on nucleoid isolation procedures described previously by Kornberg *et al*. [27] and modified by Murphy and Zimmerman [28]. In this method, cells were treated with lysozyme and then lysed with detergent NP-40 in low salt media containing spermidine. In our cell lysate preparation, we used 1 mg lysozyme/ml and decided to the limit exposure to lysozyme to 15 min at 30˚C. This lysozyme concentration gave efficient detergent lysis under the conditions employed, as judged by recovery of cellular DNA in a nucleoid fraction (see below). Lysates made at the low-salt concentrations were centrifuged under different rotation speeds and times on step sucrose gradients (12% to 60% sucrose), yielded similar patterns. Whole cell lysates from different growth phases were completely sedimented under these conditions.

Two-day-old se-cultures were diluted into 200-fold by adding fresh BG 11 medium and the culture was grown in a 30˚C at a constant light. Cells were cultured until three days and harvested for preparation of cell

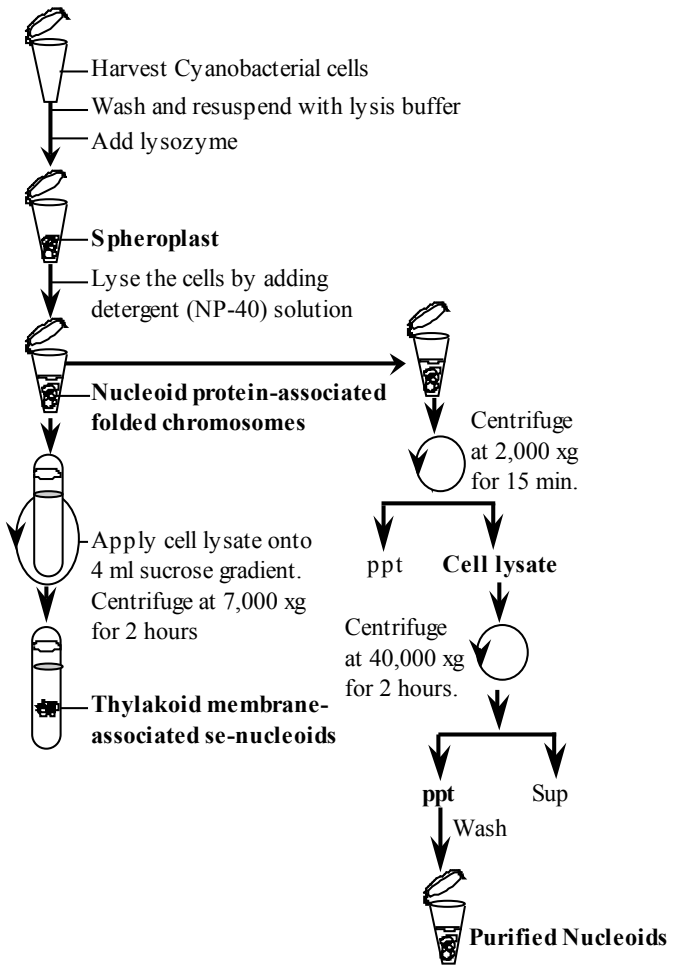

Figure 2. Schematic procedure for isolation and purification of *Synechococcus elongatus* nucleoids from the whole cell extracts. Cell growth, sample collection, sample preparation, sample isolation and preparation of se-nucleoids were described in the Materials and Methods.

lysates. Equal numbers of cells from each of the two strains (wild-type and KaiABC mutants) were gently lysed in the presence of spermidine under low salt conditions and were then layered on the top of a 12% - 60% sucrose density gradient formed in Ultraclear centrifuge tubes (13 × 51 mm, Beckman). Centrifugation was performed at 7000 g at 4˚C for 120 min in a RPS50-2-517 rotor. The positions of the thylakoid membrane-associated nucleoids isolated from the wild-type and KaiABC mutant strains are indicated by arrow in the gradient after the 120 min run described (**Figure 3(A)**). The tubes containing a single visible band in addition to an opalescent sample zone at the top of the gradient is referred to as "isolated se-nucleoids". Interestingly, sedimentation patterns of isolated nucleoids were different from different strain genotypes; from slow to rapid sedimentations were observed in KaiABC mutant to wild type, respectively, which indicate that chromosome compaction or condensation occurred in wild type strain. It was reported previously that the membrane-associated nucleoids exhibit similar rotor speed dependence as membrane-free nucleoids isolated in the presence of high-salt concentrations, suggesting

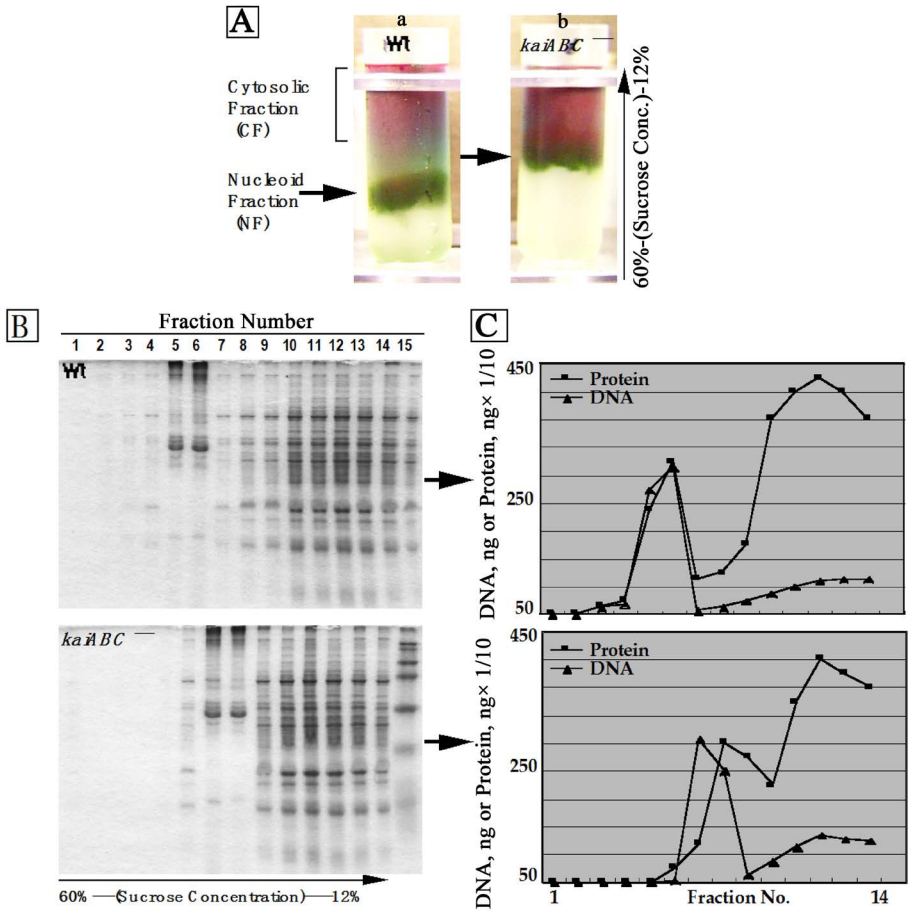

Figure 3. Sucrose density gradient centrifugation of isolated se-nucleoids. (A) Wild type and KaiABC cells were grown in BG-11 medium in continuous light (LL) for 3 days. (A)a and (A)b represent nucleoids fractions (marked by arrow) which were isolated from wild type and KaiABC, respectively. Nucleoid isolation procedure was described detail in Materials and Methods. (B) Electrophoresis patterns of total proteins from isolated nucleoids were compared with those of other cell fractions on a SDS-15% polyacrylamide gel. Five µl of each of the 14 fractions in 2 different strains were applied to lanes 1 - 15 [except 1 µl in lane 5 and 6 in wt (NF) and 1 µl in lane 7 and 8 in KaiABC-(NF), respectively]. Lane 15 on top and bottom gel represents 2.0 µl wt fraction (lane 14) and standard molecular mass markers (molecular mass values in KDa shown from top to bottom: phosphorylase b, 97.4 kDa; bovine serum albumin, 66.2 kDa; ovalbumin, 45.0 kDa; carbonic anhydrase, 31.0 kDa; soybean trypsin inhibitor, 21.5 kDa; and lysozyme, 14.4 kDa, respectively). Gels were stained with Coomassie brilliant blue (CBB). (C) DNA and protein contents in the sucrose density gradient fractions collected from bottom upward (represented from left to right). DNA and protein were measured from optical density at 280 nm and absorbance of Bradford reagent [Bio-Rad Proten Assay Kit (Bio-Rad)], respectively.

that associated membranes do not affect the compactness of the nucleoids [35] [39]. As described above, the differences in sedimentation rates under different strains reflected their different states of folding, condensation states and/or superhelicity. This result indicates that somehow clock proteins involved in the formation of genome DNA into the se-nucleoids. Therefore, we decided to check sedimentation patterns individually in each of 3 clock genes mutants KaiA, KaiB and KaiC, and also double mutants of KaiBC. All mutations caused unfolding of the nucleoid (data not shown). The sedimentation pattern of the isolated nucleoids was generally consistent with large, compact particles, as visualized below. An approximate value of 1.7×10^3 S was estimated as the sedimentation coefficient of the peak fraction of wild type strain. These values are consistent with the value described previously [28] [39].

In order to determine whether these bands corresponded to the nucleic acid-protein complexes that putative nucleoids are presumed to be, the gradients were collected from the bottom in 14 equal 0.45 ml volume fractions except 0.60 ml of nucleoid fraction in both strains. The protein and DNA contents of each fraction were estimated from the absorbance of Bradford reagent at 550 nm and optical density at 280 nm, respectively, as shown in **Figure 3(B)** and **Figure 3(C)**. The sedimentation patterns of total proteins from isolated nucleoids were compared with those of other cell fractions by SDS-polyacrylamide gel. Aliquots of each of the 14 fractions in different strains were applied to lanes 1 - 14 of the SDS-containing 15% polyacrylamide gel. We applied 5 µl of each of the 14 fractions [except 1 µl in lanes 5 and 6 for wild type and in lanes 7 and 8 for KaiABC mutant crude and washed nucleoid fraction, respectively]. An equal volume (1 µl) of both wild type crude and washed nucleoids fractions were applied in lanes 5 and 6; each lane contains 12.5 and 10 µg total proteins which were associated with 5.5 and 4 ng DNA, respectively. On the other hand, lanes 10 to 14 corresponded to the cytosolic fractions and each contains about 17 µg proteins associated with 1 ng of DNA, respectively. All most similar amount of Protein/DNA ratio was determined in KaiABC mutant fractions. The total volume of wild type nucleoid fractions was 600 µl. From an average of 2 independent cultures, each contains about 1.9×10^8 cells/ml. The total proteins of isolated nucleoid and cytosolic fractions were found to be 652 and 4350 µg, respectively (data not shown).

The relative composition and cellular distribution ratio of protein and DNA in whole cells, isolated nucleoids, and cytosolic fractions were estimated as shown in **Figure 3(C)**. About 28% - 40% and 52% - 76% of the cellular DNA was recovered in the isolated cytosolic and se-nucleoid fractions, respectively, in both strains. The fractions comprising both the nucleoid and cytosolic peaks have a protein/DNA ratio of roughly 50/1 (**Figure 3(A)**). Although the ratio was about 10 fold higher compared to the protein/DNA ratio reported previously in *Escherichia coli* by Murphy and Zimmerman [28]. The possible reason of such variations might also be due to the different conditions which were employed for isolation of nucleoids from different bacterial species [40]. In wild type culture, about 85% of the protein that is released by cell lysis remains in the cytosolic fractions of the gradients.

Next, we decided to examine the se-nucleoid structures by fluorescence microscopy. **Figure 4(A)** shows the microscopic appearances of intact cell nucleoids, thylakoid membrane associated nucloids and purified nucleoids. Three types of nucleoids were collected, fixed with 0.1% Glutaraldehyde, stained with 5 µg/ml DAPI and examined by fluorescence microcopy. Left and right panels of **Figure 4(A)** show the se-nucleoids of wild type and KaiABC mutant strains, respectively. Clear differences of se-nucleoids in shapes, sizes and structures were observed in between of wide type and mutant strains, indicated that the wild type se-nucleoids are much more compacted than that of KaiABC mutant. Because, fluorescence was more extended for the KaiABC mutant nucleoids whereas that from the wild type nucleoids was relatively compact under various treatments and conditions we examined (compare **Figure 4(A)** 3rd panels, thylakoid membrane associated crude se-nucleoids). Microscopic studies of DAPI stained nucleoids have been previously reported [36] [38] [41]; our pictures of the individual nucleoids isolated from bands in a sucrose density gradient were remarkably similar to the intact cell nucleoid as shown in **Figure 4(A)** (2nd panel). We have found 3 - 5 copy of genomes in each cell nucleoid, which compacted tightly each other. However, these copy numbers and compaction state were not seen in nucleouds isolated from KaiABC mutant strain, probably due to the lack of clock protein (**Figure 4(A)** bottom panels, purified nucleoids). Our data, however, suggest that se-nucleoid structure is dynamic and coupled with clock functions, thus may reflect changes in DNA topology associated with replication or segregation [42].

It is well documented that the KaiC proteins plays a significant role in the repression of transcription of several genes in *Synechococcus elongatus* PCC 7942 [43], suggesting again that KaiC deposition would be primarily concentrated in the nucleoid region of cell where transcription generally exist. In addition to fluorescence mi-

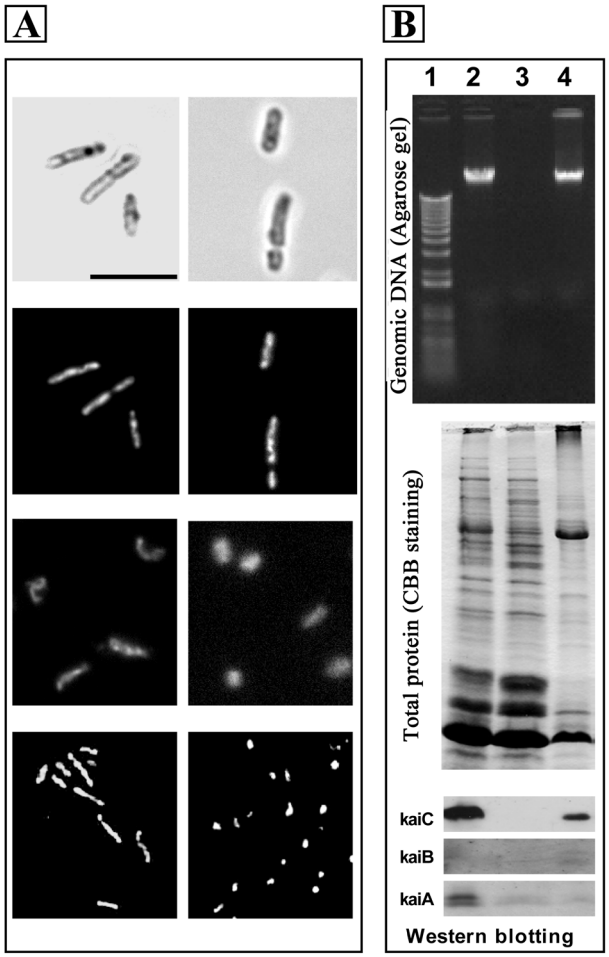

Figure 4. Characterization of se-nucleoids. (A) Fluorescence microscopic view of cyanobacterial nucleoids. Various types of nucleoids with 0.1% Glutaraldehyde where necessary, stained with 5.0 µg/ml DAPI and examined by fluorescence microscope. Left and right panel represent cyanobacterial wild type and mutant strains. Top images represent the phase-contrast views of both wild type and mutant strains of *Synechococcus elongatus* cells. Remaining 3 DAPI staining fluorescent images from top to bottom represent the intact cell nucleoids, isolated crude nucleoids and purified nucleoids of both wild type and mutant strains. Scale bar on the tor left represents 5µM. (B) Top, middle and bottom panels represent the genomic DNA, total protein and 3 cyanobacterial clock proteins expression patterns, which were performed by Agarose gel electrophoresis, Polyacrylamide gel electrophoresis and Western immunoblot analysis, respectively. Lane 1 in the top left panel represents standard DNA markers and lanes 2, 3 and 4 represent the crude cell lysates, cytosolic fraction and nucleoid fraction, respectively. For the measurement of genomic DNA, total cellular protein and each of 3-clock proteins, aliquots of each of 3 lanes (except lane 2, crude cell lysates) were collected from the sucrose gradients. In each lane containing 5 µl of total fractions subjected to 1% Agarose gel electrophoresis for the measurement of genomic DNA after stained with Ethidium bromide (Top panel) and 12.5% SDS-PAGE for the visualization and estimation of total proteins after stained with CBB (Middle panel) and Western immunoblot analysis was carried out in order to estimate the clock proteins KaiA, kaiB and kaiC level on above fractions. The blots were probed with anti-KaiC, anti-KaiB, and anti-KaiA antibodies as mentioned (bottom panel).

croscopy, we applied Western immunoblot technique to estimate the three-clock proteins from the se-nucleoids. Three different samples (see **Figure 4(B)**, lanes 2 - 4 represent crude cell lysates, isolated cytosolic and nucleoid fractions) were collected and loaded onto the 1% Agarose gel for visualization of genome DNA; SDS-12.5% PAGE for visualization of total protein by CBB staining and SDS 15% PAGE for estimation of 3-clock proteins by Western immunoblot analysis are shown in **Figure 4(B)** top, middle and bottom panel, respectively. As expected, genomic DNA is almost absent and present in the cytosolic and nucleoid fraction, respectively (compare lane 3 with lane 4 in 1% Agarose gel). On the other hand, total protein levels were varied among the 3-different

samples, indicating that several proteins are involved in the organization of se-genomic DNA into se-nucleoids, which have yet to be identified (**Figure 4(B)** middle panel),

Finally, we have checked the involvement of 3-clock proteins in the organization of genomic DNA into the se-nucleoids by Western blotting. Among them, the intracellular accumulation patterns were different. Lane 2 corresponded to the protein band, which was cross-reacted with the total cell lysates (before fractionated by sucrose density gradient), in parallel, while lanes 3 and 4 represent the protein of cytosolic and nucleoid fractions, respectively. Significant amount of KaiC was detected in both the crude cell lysates and the nucleoid fraction, but KaiC was not found in cytosolic fraction, indicate that clock protein KaiC plays a key role in the organization of genomic DNA into the se-nucleoids. Under the condition we employed here, we could not detect KaiB protein. In addition to thylakoid-membrane specific marker protein NrtA, significant amount of KaiA was found not only in the cytosolic fractions but also in the nucleoid fraction. This result indicates that certain amount of KaiA protein exists in the cytosol, supposedly in association with the translation machinery [44].

4. Concluding Remarks

Many scientists around the world were tried to purify cyanobacterial nucleoid from thylakoid membranes, but none of them succeeded [42] [45]-[47]. In this paper, for the first time, we have established a procedure for isolation and purification of se-nucleoids. During purification, it was very difficult to separate thylakoid membranes from the se-nucleoid. Among the different detergents applied, only NP-40 detergent was effective for purification of se-nucleoid from the whole cell extract. However, during purification, a huge amount of genomic DNA was released together with the thylakoid membranes (data not shown). Therefore, we conclude that the thylakoid membrane is a part of se-nucleoid, because se-genomic DNA is tightly associated with thylakoid membranes as suggested previously [48]-[50].

We have clearly demonstrated that the clock protein complexes associate directly with the se-nucleoids (**Figures 2-4**). This association leads the compaction of se-nucleoid, which might be controlled cyanobacterial gene expressional mechanism (s) that yet to be determined. Alternatively, clock proteins, especially KaiA, might be involved in facilitating the co-transla- tional membrane insertion and assembly of thylakoid proteins such as the DpsA, D1 and Cytochrome f by contributing to short distances between transcription, translation and protein insertion [51]-[53].

Mori *et al*. [46] report that KaiC is a DNA binding protein and they propose that the ATP dependent hexameric complex is the active form of KaiC, and mediates rhythmic changes in chromosomal torsion. Recently, Smith and Willams [47] found the circadian changes in the compaction state of se-nuceloids. Therefore, it is important to check whether se-nucleoids that we have isolated in this study will show the rhythmic changes in chromosomal torsion. Altogether our results indicate that the major clock component KaiC primarily organizes genomic DNA into the se-nucleoid which may be affected circadian time dependent regulation of gene expression in *Synechococcus elongates* PCC 7942 [54].

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References

[1] Hecht, R.M., Taggart, R.T. and Pettijohn, D.E. (1975) Size and DNA Content of Purified *E. coli* Nucleoids Observed by Fluorescence Microscopy. *Nature*, **253**, 60-62. http://dx.doi.org/10.1038/253060a0

[2] Worcel, A. and Burgi, E. (1972) On the Structure of the Folded Chromosome of *Escherichia coli. Journal of Molecular Biology*, **71**, 127-147. http://dx.doi.org/10.1016/0022-2836(72)90342-7

[3] Pettijohn, D.E. (1982) Structure and Properties of the Bacterial Nucleoid. *Cell*, **30**, 667-669. http://dx.doi.org/10.1016/0092-8674(82)90269-0

[4] Woldringh, C.L. and Nanninga, N. (1985) Structure of the Nucleoid and Cytoplasm in the Intact Cell. In: Nenninga, N., Ed., *Molecular Cytology of Escherichia coli*, Academic Press, London, 161-197.

[5] Schmid, M.B. (1990) More than Just "Histone-Like" Proteins. *Cell*, **63**, 451-453. http://dx.doi.org/10.1016/0092-8674(90)90438-K

[6] Pettijohn, D.E. (1966) The Nucleoid. In: Neidhardt, F.C., Ed., *Escherichia coli and Salmonella*, American Society for Microbiology, Washington DC, 158-166.

[7] Flashner,Y. and Gralla, J.D. (1988) DNA Dynamic Flexibility and Protein Recognition-Differential Stimulation by Bacterial Histone-Like Protein HU. *Cell*, **54**, 713-721. http://dx.doi.org/10.1016/S0092-8674(88)80016-3

[8] Finkel, S.E. and Johnson, R.C. (1992) The Fis Protein: It's Not Just for DNA Inversion Anymore. *Molecular Microbiology*, **6**, 3257-3265. http://dx.doi.org/10.1111/j.1365-2958.1992.tb02193.x

[9] Ishihama, A. (1999) Modulation of the Nucleoid, the Transcription Apparatus and the Translation Machinery for Stationary Phase Survival. *Genes to Cells*, **4**, 135-143. http://dx.doi.org/10.1046/j.1365-2443.1999.00247.x

[10] Ptacin, J.L. and Shapiro, L. (2013) Chromosome Architecture Is a Key Element of Bacterial Cellular Organization. *Cell Microbiol*ogy, **15**, 45-52. http://dx.doi.org/10.1111/cmi.12049

[11] Rouviere-Yaniv, J., Yaniv, M. and Germond, J.E. (1979) *Escherichia coli* DNA-Binding Protein HU Forms Nucleosome-Like Structure with Circular Double-Stranded DNA. *Cell*, **17**, 265-274. http://dx.doi.org/10.1016/0092-8674(79)90152-1

[12] Drlica, K. and Rouviere-Yaniv, J. (1987) Histone-Like Proteins of Bacteria. *Microbial Review*, **51**, 301-319.

[13] Dürrenberger, M., Bjornsti, M.A., Uetz, T., Hobot, J.A. and Kellenberger, E. (1988) Intracellular Localization of the Histone Like Protein HU in *Escherichia coli*. *Journal of Bacteriology*, **170**, 4757-4768.

[14] Pettijohn, D.E. (1988) Histone-Like Proteins and Bacterial Chromosome Structure. *Journal of Biological Chemistry*, **263**, 12793-12796.

[15] Ball, C.A., Osuna, R., Ferguson, K.C. and Johnson, R.C. (1992) Dramatic Changes in Fis Levels upon Nutrient Upshift in *Escherichia coli*. *Journal of Bacteriology*, **174**, 8043-8056.

[16] Almirón, M., Link, A.J., Furlong, D. and Kolter, R. (1992) A Novel DNA Binding Protein with Regulatory and Protective Roles in Starved *Escherichia coli*. *Genes and Development*, **6**, 2646-2654. http://dx.doi.org/10.1101/gad.6.12b.2646

[17] Ditto, M.D., Roberts, D. and Weisberg, R.A. (1994) Growth Phase Variation of Integration Host Factor level in *Escherichia coli*. *Journal of Bacteriology*, **176**, 3738-3748.

[18] Broyles, S. and Pettijohn, D. (1986) Interaction of the *Escherichia coli* HU Protein with DNA. Evidence for Formation of Nucleosome-Like Structures with Altered DNA Helical Pitch. *Journal of Molecular Biology*, **187**, 47-60. http://dx.doi.org/10.1016/0022-2836(86)90405-5

[19] Atlung, T. and Ingmer, H. (1997) H-NS: A Modulator of Environmentally Regulated Gene Expression. *Molecular Microbiology*, **24**, 7-17. http://dx.doi.org/10.1046/j.1365-2958.1997.3151679.x

[20] Talukder, A.A. and Ishihama, A. (1999) Twelve Species of the Nucleoid-Protein from *Escherichia coli*: Sequence Recognition Specificity and DNA-Binding Affinity. *Journal of Biological Chemistry*, **274**, 33105-33113. http://dx.doi.org/10.1074/jbc.274.46.33105

[21] Talukder, A.A., Iwata, A., Nishimura, A., Ueda, S. and Ishihama, A. (1999) Growth Phase-Dependent Variation in Protein Composition of the *Escherichia coli* Nucleoid. *Journal of Bacteriology*, **181**, 6361-6370.

[22] Talukder, A.A., Hiraga, S. and Ishihama, A. (2000) Two Types of Localization of the DNA-Binding Proteins within the *Escherichia coli* Nucleoid. *Genes Cells*, **5**, 613-626. http://dx.doi.org/10.1046/j.1365-2443.2000.00350.x

[23] Talukder, A.A. (2005) Survival and Death in Bacteria. In: Yamada, M., Ed., *Structure and Composition of Escherichia coli Nucleoid*, Research Signpost, Kerala, 77-101.

[24] Talukder, A.A., Hossain, M.A., Yamada, M. and Ishihama, A. (2006) Nucleoids Dynamics in *Escherichia coli*: A Growth Phase Dependent Process. *Bangladesh Journal of Microbiology*, **23**, 81-88.

[25] Dunlap, J.C. (1999) Molecular Basis for Circadian Clocks. *Cell*, **96**, 271-290. http://dx.doi.org/10.1016/S0092-8674(00)80566-8

[26] Iwasaki, H., Taniguchi, Y., Ishiura, M. and Kondo, T. (1999) Physical Interactions among Circadian Clock Proteins KaiA, KaiB and KaiC in Cyanobacteria. *EMBO Journal*, **18**, 1137-1145. http://dx.doi.org/10.1093/emboj/18.5.1137

[27] Kornberg, T., Lockwood, A. and Worcel, A. (1974) Replication of the *Escherichia coli* Chromosome with a Soluble Enzyme System. *Proceedings of the National Academy of Sciences of the United States of America*, **71**, 3189-3193. http://dx.doi.org/10.1073/pnas.71.8.3189

[28] Murphy, L.D. and Zimmerman, S.B. (1997) Isolation and Characterization of Spermidine Nucleoids from *Escherichia coli*. *Journal of Structural Biology*, **199**, 336-346. http://dx.doi.org/10.1006/jsbi.1997.3884

[29] Jishage, M. and Ishihama, A. (1995) Regulation of RNA Polymerase Sigma Subunit Synthesis in *Escherichia coli*: Intracellular Levels of Sigma 70 and Sigma 38. *Journal of Bacteriology*, **177**, 6832-6835.

[30] Hiraga, S., Ichinose, C., Niki, H. and Yamazoe, M. (1998) Cell Cycle-Dependent Duplication and Bidirectional Migra-

tion of SeqA-Associated DNA-Protein Complexes in *E. coli*. *Molecular Cell*, **1**, 381-387. http://dx.doi.org/10.1016/S1097-2765(00)80038-6

[31] Makinoshima, H., Nishimura, A. and Ishihama, A. (2002) Fractionation of *Escherichia coli* Cell Populations at Different Stages during Growth Transition to Stationary Phase. *Molecular Microbiology*, **43**, 269-279. http://dx.doi.org/10.1046/j.1365-2958.2002.02746.x

[32] Makinoshima, H., Aizawa, S.I., Hayashi, H., Miki, T., Nishimura, A. and Ishihama, A. (2003) Growth Phase-Coupled Alternations in Cell Structure and Function of *Escherichia coli*. *Journal of Bacteriology*, **185**, 1338-1345. http://dx.doi.org/10.1128/JB.185.4.1338-1345.2003

[33] Mori, T., Binder, B. and Johnson, C.H. (1996) Circadian Gating of Cell Division in Cyanobacteria Growing with Average Doubling Times of Less than 24 Hours. *Proceedings of the National Academy of Sciences of the United States of America*, **93**, 10183-10188. http://dx.doi.org/10.1073/pnas.93.19.10183

[34] Stonington, O.G. and Pettijohn, D.E. (1771) The Folded Genome of *Escherichia coli* Isolated in a Protein-DNA-RNA Complex. *Proceedings of the National Academy of Sciences of the United States of America*, **68**, 6-9. http://dx.doi.org/10.1073/pnas.68.1.6

[35] Hecht, R.M., Stimpson, D. and Pettijohn, D.E. (1977) Sedimentation Properties of the Bacterial Chromosomes as an Isolated Nucleoid and as an Unfolded DNA Fiber: Chromosomal DNA Folding Measured by Rotor Speed Effects. *Journal of Molecular Biology*, **111**, 257-277. http://dx.doi.org/10.1016/S0022-2836(77)80051-X

[36] Hinnebusch, B. and Bendich, A.J. (1997) The Bacterial Nucleoid Visualized by Fluorescence Microscopy of Cells Lysed within Agarose: Comparison of *Escherichia coli* and Spirochetes of the Genus *Borrelia*. *Journal of Bacteriology*, **179**, 2228-2237.

[37] Murphy, L.D. and Zimmerman, S.B. (1997) Stabilization of Compact Spermidine Nucleoids from *Escherichia coli* under Crowded Conditions: Implication for *in Vivo* Nucleoid Structure. *Journal of Structural Biology*, **199**, 321-335. http://dx.doi.org/10.1006/jsbi.1997.3883

[38] Zimmerman, S.B. and Murphy, L.D. (2001) Release of Compact Nucleoids with Characteristic Shapes from *Escherichia coli*. *Journal of Bacteriology*, **183**, 5041-5049. http://dx.doi.org/10.1128/JB.183.17.5041-5049.2001

[39] Weitao, T., Nordström, K. and Dasgupta, S. (1999) Mutational Suppression of *mukB* and *seqA* Phenotypes Might Arise from Their Opposing Influences on *the Escherichia coli* Nucleoid Structure. *Molecular Microbiology*, **34**, 157-168. http://dx.doi.org/10.1046/j.1365-2958.1999.01589.x

[40] Åkerlurd, T., Nordström, K. and Bernander, R. (1995) Analysis of Cell Size and DNA Content in Exponentially Growing and Stationary-Phase Batch Cultures of *Escherichia coli*. *Journal of Bacteriology*, **177**, 6791-6797.

[41] Nitta, K., Nagayama, K., Danev, R. and Kaneko, Y. (2009) Visualization of BrdU-Labelled DNA in Cyanobacterial Cells by Hilbert Differential Contrast Transmission Electron Microscopy. *Journal of Microscopy*, **234**, 118-123. http://dx.doi.org/10.1111/j.1365-2818.2009.03162.x

[42] Sato, N. (2001) Was the Evolution of Plastid Genetic Machinery Discontinuous? *Trends in Plant Science*, **6**, 151-155. http://dx.doi.org/10.1016/S1360-1385(01)01888-X

[43] Nakahira, Y., Katayama, M., Miyashita, H., Kutsuna, S., Iwasaki, H., Oyama, T. and Kondo, T. (2004) Global Gene Repression by KaiC as a Master Process of Prokaryotic Circadian System. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 881-885. http://dx.doi.org/10.1073/pnas.0307411100

[44] Kitayama, Y., Iwasaki, H., Nishiwaki, T. and Kondo, T. (2003) KaiB Functions as an Attenuator of KaiC Phosphorylation in the Cyanobacterial Circadian Clock System. *EMBO Journal*, **22**, 2127-2134. http://dx.doi.org/10.1093/emboj/cdg212

[45] Sato, N., Nakayama, M. and Hase, T. (2001) The 70-kDa Major DNA-Compacting Protein of the Chloroplast Nucleoid Is Sulfite Reductase. *FEBS Letters*, **318**, 347-350. http://dx.doi.org/10.1016/S0014-5793(00)02342-5

[46] Mori, T., Saveliev, S.V., Xu, Y., Stafford, W.F., Cox, M.M., Inman, R.B. and Johnson, C.H. (2002) Circadian Clock Protein KaiC forms ATP-Dependent Hexameric Rings and Binds DNA. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 17203-17208. http://dx.doi.org/10.1073/pnas.262578499

[47] Smith, R.M. and Williams, S.B. **(**2006**)** Circadian Rhythms in Gene Transcription Impared by Chromosome Compaction in the Cyanobacterium *Synechococcus elongatus*. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 8564-8569. http://dx.doi.org/10.1073/pnas.0508696103

[48] Kobayashi, T., Takahara, M., Miyagishima, S., Kuroiwa, H., Sasaki, N., Ohta, N., Matsuzaki, M. and Kuroiwa, T. (2002) Detection and Localization of a Chloroplast-Encoded HU-Like Protein That Organizes Chloroplast Nucleoids. *The Plant Cell*, **14**, 1579-1589. http://dx.doi.org/10.1105/tpc.002717

[49] Jeong, S.Y., Rose, A. and Meier, I. (2003) MFP1 Is a Thylakoid-Associated, Nucleoid-Binding Protein with a Coiled-Coil Structure. *Nucleic Acids Research*, **31**, 5175-5185. http://dx.doi.org/10.1093/nar/gkg693

[50] Kabeya, Y., Nakanishi, H., Suzuki, K., Ichikawa, T., Kondou, Y., Matsui, M. and Miyagishima, S.Y. (2010) The

YlmG Protein Has a Conserved Function Related to the Distribution of Nucleoids in Chloroplasts and Cyanobacteria. *BMC Plant Biolology*, **10**, 57. http://dx.doi.org/10.1186/1471-2229-10-57

[51] Durham, K.A. and Bullerjahn, G.S. (2002) Immunocytochemical Localization of the Stress-Induced DpsA Protein in the Cyanobacterium *Synechococcus* sp. Strain PCC 7942. *Journal of Basic Microbiology*, **42**, 362-372. http://dx.doi.org/10.1002/1521-4028(200212)42:6<367::AID-JOBM367>3.0.CO;2-T

[52] Zhang, L., Paakkarinen, V., van Wijk, K.L. and Aro, E.M. (1999) Co-Translational Assembly of the D1 Protein into Photosystem II. *Journal of Biological Chemistry*, **274**, 16062-16067. http://dx.doi.org/10.1074/jbc.274.23.16062

[53] Rohl, T. and van Wijk, K.L. (2001) In Vitro Reconstitution of Insertion and Processing of Cytochrome *f* in a Homologous Chloroplast Translation System. *Journal of Biological Chemistry*, **276**, 35465-35472. http://dx.doi.org/10.1074/jbc.M103005200

[54] Johnson, C.H., Eglim, M. and Stewart, P.L. (2008) Structural Insights into a Circadian Oscillator. *Science*, **322**, 697-701. http://dx.doi.org/10.1126/science.1150451

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