Assignment of 82 Known Genes and Gene Clusters on the Genome of the Unicellular Cyanobacterium *Synechocystis* sp. Strain PCC6803

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(Received 12 June 1995)

Abstract

We have previously constructed the physical map of a cyanobacterium, *Synechocystis* sp. strain PCC6803 on the basis of restriction and linking clone analysis. Since a total of 82 genes and gene clusters have been isolated from this strain, most of which are involved in oxygenic photosynthesis, portions of their sequences were amplified by the PCR method and assigned on the physical map of the genome by hybridization with restriction fragments, ordered clones, which were obtained from cosmid and A libraries, and long PCR-products. An exception was the gene *psbG2* which was mapped on an extra-chromosomal unit of 45 kb. Since genetic maps of some of genes assigned above, especially those for photosynthesis, have been reported for two other cyanobacterial strains, *Anabaena* sp. PCC7120 and *Synechococcus* sp. PCC7002, gene organizations were compared among the three strains. However, no significant correlation was observed, suggesting that rearrangement of genes occurred in the respective strains during or after establishment of the species.

Key words: *Synechocystis* PCC6803; Genome map; Ordered clones

For understanding of the entire genetic system involved in oxygenic photosynthesis as well as that carried by a single organism which was born a very ancient age, we have started the sequencing project of the entire genome of a unicellular cyanobacterium, *Synechocystis* sp. strain PCC6803. As the first step of the project, we constructed a physical map of the genome by restriction analysis using *Asc*I, *Mlu*I and *Spl*I, and confirmed the map by isolation of linking cosmid clones which covered the restriction sites.1 The estimated genome size was 3.6 Mb. A total of 82 genes and gene clusters, most of which are involved in photosynthesis, have been isolated from this strain and characterized at the sequence level. In this paper, we assigned these genes on the physical map of the genome, and compared their organization with those of two other cyanobacterial strains, *Anabaena* sp. PCC71202 and *Synechococcus* sp. PCC7002,3 in which the genetic maps of some of genes assigned in this study have already been reported.

For precise mapping of genes and gene clusters on the genome, we constructed a more detailed physical map by isolation of overlapping clones from cosmid and A libraries of the genome. Since most cosmid clones carrying *Synechocystis* DNA were stably maintained in *Escherichia coli* cells, approximately 1,100 clones from a cosmid library with an average insert size of 40 kb1 were first sorted by cross-hybridization, and the resulting contigs were located on the map by hybridization with restriction fragments. About 90% of the genome could be covered with 127 cosmid clones (Fig. 1). The gap regions are now being filled using a A library which was constructed by cloning of fractionated 15–20 kb *Sau3AI* fragments of the genome into A-*DASH* vector (Stratagene, USA), and also by the long PCR products. So far, more than 90% of the entire genome have been covered by cosmid and A clones and long PCR products (Figs. 1 and 2).

As the sequence information of 82 genes and gene clusters4–7 which are listed in Table 1 is available, we designed a set of primer pairs about 20 bp in length for each gene or gene cluster, and the resulting probes were hybridized to Southern blots of *Asc*I, *Mlu*I and *Spl*I digests of the genome and to membranes dotted with ordered clones. The results obtained are summarized in Fig. 1, in which the circular genome was shown linearized at the *Asc*I site connecting *Asc*I-F and *Asc*I-E fragments (0/100 position), and under the linear map, restriction maps for *Asc*I, *Mlu*I and *Spl*I, regions covered by respective clones, and name of genes or gene clusters are shown. All the genes examined except one were successfully localized on the map.
Table 1. List of the genes and gene clusters used for mapping.

| Genes and Gene clusters | Function                                                                 | Map position (min) | Accession b) | References |
|-------------------------|--------------------------------------------------------------------------|--------------------|--------------|------------|
| psbA3                   | psbA3 gene for D1 protein of photosystem II (QB binding protein)         | 0-0.5              | X56000       | 5,6        |
| psaD                    | subunit II of photosystem I                                              | 3-4               | J04195       | 7          |
| dfr                     | resistance to the herbicide difunone                                      | 3-4               | X72586       | unpublished |
| ndhB                    | subunit-2 of NADH-dehydrogenase (inorganic carbon transport)              | 4-4.5             | D90288       | 8          |
| rnpB                    | RNase P RNA subunit                                                      | 4-4.5             | X65707       | 9          |
| orf184                  | putative membrane protein                                                | 4-4.5             | Z27404       | unpublished |
| ccmM-N                  | required for construction of carboxysome                                  | 6-7               |              | c)         |
| atpI-H-G-F-D-A-C        | F0F1 ATP synthase I operon                                               | 7                 | X58128, Y07532 | 10,11     |
| ndhD2                   | subunit IV of NADH dehydrogenase                                         | 8                 | U14130       | unpublished |
| ccmA                    | carboxysome formation                                                    | 9                 | D26444       | 12         |
| apeA-B-C                | allophycocyanin and linker proteins                                      | 12.5-13           | M77135       | 13         |
| gltB                    | ferredoxin-glutamate synthase                                            | 13.5-14           | X580485      | unpublished |
| trxA                    | thioredoxin                                                             | 13.5-14           | X580486      | unpublished |
| psbE,F                  | cytochrome 5559 alpha and beta subunits in photosystem II               | 15.5-17.5         | X06988, M33897 | 14,15     |
| psbK                    | small component of photosystem II                                       | 15.5-17.5         | M74841       | 16,65      |
| cozB-A-C                | subunit I, II, III of cytochrome c oxidase (aa3 type)                    | 22-23             | X53746       | 17,69      |
| groEL                   | second groEL like gene                                                  | 24-26             | D12677       | 18         |
| rpoB-C                  | RNA polymerase $\beta$, $\beta'$ subunit                                | 24-26             | D12677       | 18         |
| petJ                    | cytochrome c553                                                         | 24-26             | L25252       | 19,72      |
| rpsS                    | ribosomal protein L19                                                   | 26-27             | X72697       | 20         |
| trnW                    | tryptophan-specific tRNA                                                | 26-27             | X72697       | 66         |
| nusG                    | antiterminator                                                          | 26-27             | X72697       | 20,67      |
| aroC                    | chorismate synthetase                                                   | 26-27             | X73005       | 20,67      |
| rplK-A-J-L              | ribosomal protein (L11,1,10,12)                                          | 26-27             | X73005       | 20,67      |
| psaA-B                  | psaA and psaB genes for P700 apoprotein of photosystem II              | 26.5-28.5         | X58825       | 21         |
| psbN-H                  | low molecular mass component and 9 kDa thylakoid phoshoprotein of photosystem II | 32                | X17687, X58532 | 22        |
| petC-A                  | rieske iron-sulfur protein and apocytocrome f of cytochrome b6-f complex | 32                | X58532       | 22         |
| iclG                    | inorganic carbon and glucose metabolism                                 | 33-34             | X75568       | 23         |
| psbD-C                  | CP-43 chlorophyll a-binding protein                                      | 38-39             | M21538       | 24,74      |
| purT                    | glycaminide ribonucleotide transformylase                               | 38-39             | L36958       | unpublished |
| dnaA                    | DNA synthesis (initiation)                                              | 38-39             | L36958       | unpublished |
| pds                     | phytoene desaturase and herbicide norfluron resistance                   | 39-40             | X62574       | 25         |
| psy                     | phytoene synthetase                                                     | 39-40             | X69172       | 26         |
| rne                    | glutamate-specific transfer RNA                                         | 39-40             | M32099       | 27         |
| pmaI                    | P-type ATPase (homology of eukaryotic Ca$^{++}$ ATPase)                  | 43                | X71022       | 28         |
Table 1. Continued.

| Genes and Gene clusters | Function | Map position (min) | Accession | References |
|-------------------------|----------|-------------------|-----------|------------|
| *ndhH*                  | subunit of NADH dehydrogenase | 43.5 | X60650 | 29 |
| *fla*                   | flavodoxin gene | 43.5 | L25881 | 30 |
| *isiA*                  | iron-stress chlorophyll-binding protein | 43.5 | L26530 | unpublished |
| *ntcA*                  | control gene of nitrogen metabolism | 44—45 | X71607 | 31 |
| *atpB-E*                | FOF1 ATP synthase, atpB atpE for ATPase subunits beta and epsilon | 47—48 | X58129 | 10 |
| *ndhL*                  | inorganic carbon transport gene (ictA) | 48—48.5 | M73833 | 32 |
| *psaF-J*                | subunits of photosystem I | 48.5 | L20938, M74801 | 33 |
| *murF*                  | UDP-N-acetylmuramoylalanyl-D-glutamyl-l,2-D-amino-pimelate-D-alanyl-D-alanine ligase | 49—50 | X62437 | unpublished |
| *desA*                  | fatty acid desaturase | ~49—50 | X53508 | 34,71 |
| *rplU*                  | 5OS ribosomal protein L9 | 49—50 | D10716 | 35 |
| *cysM*                  | C553 like cytochrome | 49—50 | D10716 | 35 |
| *psbA2*                 | psbA2 gene for D1 protein of photosystem II (QB binding protein) | 51—52 | X13547 | 5,6 |
| *ndhC-K-J*              | subunits of NADH dehydrogenase | 52.5—53.5 | X17439 | 36 |
| *psbG*                  | subunit subunit of photosystem II | 52.5—53.5 | X17439 | 36 |
| *psaE*                  | 8-kDa subunit III of photosystem I | 55—55.5 | J05079 | 37 |
| *prk*                   | phosphoribulokinase | 57—57.5 | M77134 | 38 |
| *aroA*                  | 5-enolpyruvylshikimate-3-phosphate synthase | 58—58.5 | X75325 | 39,70 |
| *psbO*                  | manganese-stabilizing polypeptide of photosystem II | 58—58.5 | X07986 | 70,73 |
| *trnQ*                  | glutamine-specific transfer RNA | 58—58.5 | X60715 | 70 |
| *glnN*                  | glutamine synthetase | 59—59.5 | X76719 | 40 |
| *petK*                  | low potential cytochrome c | 59—59.5 | U07021 | 41 |
| *des6*                  | delta6 fatty acid desaturase | 59—59.5 | L11421 | 42 |
| *psaC2*                 | iron-sulfur protein of photosystem I | 63.5—64 | X65170 | 43 |
| *ndhD*                  | NAD(P)H-plastoquinone oxidoreductase | 63.5—64 | X65170 | 43 |
| *dnaK*                  | dnaK-like | 64—65 | M57518 | 44 |
| *psbl*                  | low molecular mass I polypeptide of photosystem II | 65—66 | e* | 5 |
| *zfp*                   | putative zinc finger protein | 66—66.5 | S77740 | 45 |
| *tgr*                   | glucose transporter | 66.5—67 | X16472 | 46 |
| *sbaA-cysT*             | sulfate transport operon | 67—67.5 | X67911 | 47 |
| *hemA*                  | glutamyl-tRNA reductase (SYCHEMA, M84218) | 67—67.5 | X65963 | 48,49 |
| *glcP*                  | glucose transport | 67—67.5 | X15988 | unpublished |
| *petD*                  | subunit IV of cytochrome b6/f complex | 68 | X68522 | 50 |
| *rrnA*                  | ribosomal RNA operon | 68.5 | #1 | |
| *ctpA*                  | carboxy-terminal processing protease | 69.5 | L25250 | 51 |
| *rbcL-S*                | large and small subunits of ribulose bis-cocarboxylase | 69.5 | X65960 | 52 |
Table 1. Continued.

| Genes and Gene clusters | Function | Map position (min) | Accession | References |
|-------------------------|----------|-------------------|-----------|------------|
| petE                    | plastocyanin gene | 70-70.5 | X54105      | 53         |
| cpn60                   | chaperonine protein | 70-70.5 | M57517      | 54         |
| psbB                    | CP-47 protein of photosystem II | 77-78 | M17109      | 55         |
| bioF                    | 8-amino-7-oxononanoate synthase | 77.5-79.5 | U10482     | unpublished |
| trnF M (end)            | formylmethionine-specific transfer RNA (putative endonuclease) | 77.5-79.5 | U10482     | unpublished |
| ndhF                    | subunit of NADH dehydrogenase | 79-80 | f^)        |            |
| des9                    | delta9 fatty acid desaturase | 78.5-79 | D16547     | 56         |
| trpB                    | tryptophan synthase beta subunit | 90-90.5 | L14596     | 57         |
| ndhA-I-G-E              | NAD(P)H-plastoquinone oxidoreductase | 91-91.5 | X62517     | 43         |
| rrnB                    | ribosomal RNA operon | 93 |            |            |
| fusB                    | fus-like protein (elongation factor G) | 93-93.5 | X65159     | 58         |
| ilvC                    | acetohydroxyacid isomeroreductase | 93-93.5 | L03713     | 59         |
| frzC                    | chlorophyll biosynthesis; nitrogenase. | 95.5-96 | D10474     | 60         |
| stpA                    | establishment of salt tolerance | 95-95.5 | X75566     | unpublished |
| psaI                    | subunit of photosystem I | 96.5-97 | L24773     | unpublished |
| psaL                    | subunit of photosystem I | 96.5-97 | L11649     | 61         |
| ssp                     | ADP-glucose pyrophosphorylase | 98-98.5 | M83556     | 62         |
| psbA1                   | psbA1 gene for D1 protein of photosystem II (QB binding protein) | 98.5-99 | Y00885     | 63,68      |
| petC2                   | new Rieske Fe-S protein | 99-99.5 | L16885     | unpublished |
| psbG2                   | subunit of NAD(P)H dehydrogenase | pSYSG | X17559     | 64         |

^a^ Each gene and gene cluster indicated is separated by solid lines.  ^b^ Accession numbers of GenEMBL. ^c^ Personal communications from c) Ogawa, T., d) Kawarabayashi, H., e) Ikeuchi, M., f) Steinmüller, K. ^d^ Our preliminary sequence data.

For comparison of the gene organization with those of two other cyanobacterial strains, the gene locations are also illustrated on a circular map (Fig. 2). As the I-Ceu I cleavage site specific for the rrn operon76 was identified at two sites, 68.5' and 93', these sites were designated for rrnA and rrnB, respectively. This has been confirmed by sequence analysis of these regions (to be published). Since the presence of two rrn operons has also been reported for two other cyanobacterial strains, the positions of genes and gene clusters relative to the two rrn operons were compared. However, no significant correlation was observed among the three strains. The result implies that rearrangement of genes occurred in the respective strains during or after establishment of the species. In this connection, it should be noted that we have detected an IS element in the course of sequencing the genome (to be published).

The probe for psbG2,64 the second gene for subunits of NAD(P)H dehydrogenase, and cosmid clones carrying this gene were not hybridized to any restriction fragments generated by Asc I, Mlu I and Spl I, and to cosmid clones assigned to the genome. It is therefore likely that this gene is present on a plasmid, in accordance with the observation of Steinmüller and Bogorad.77 To confirm this, the insert was generated from a psbG2-carrying cosmid clone (CSO208), and PCR amplification was carried out using the sequences from both ends. As a consequence, a product of about 0.5 kb (LA215-216) was obtained, indicating that the gene was located on a 45-kb plasmid, named pSYSG, which was different from the two extra-chromosomal units previously identified, pSYSM (125 kb) and pSYSA (110 kb).

Acknowledgments: We are grateful to S. Sasamoto and T. Hosouchi for their technical assistance. We also thank Drs. C. J. Arntzen, J. Barber, L. Bogorad, M. Ikeuchi, N. Murata, N. Nelson, T. Ogawa, H. Kawarabayashi and K. Steinmüller for providing clones for gene mapping, and Drs. K. Ishikawa and N. Nomura for the preparation of the dotted membranes of cosmid clones.

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Figure 1. A detailed physical map of the *Synechocystis* sp. PCC6803 genome. The physical map was constructed by allocation of overlapping cosmid, λ clones, and long PCR products on the restriction map, and assignment of 82 genes and gene clusters on the map by hybridization with PCR products of the genes. The circular genome was opened at the *AscI* site connecting *AscI*-F and *AscI*-E fragments (0/100 position), and indicated by thick lines on which the map positions from the 0 point are shown by a centesimal scale. The length of each line presents 500 kb. Three thin lines under the bars represent restriction maps for *AscI*, *MluI* and *SplI*. Under the maps, the regions covered by respective cosmid clones (CS), λ clones (λ) and long PCR products (LA). Location of genes and gene clusters are indicated by thick bar. Separately, three extra-chromosomal units one of which contains *psbG2* are indicated.
Figure 2. The gene map of the *Synechocystis* sp. PCC6803 genome. Eighty-two genes and gene clusters assigned are indicated on the circular map of the genome. Restriction maps for *Asc* I, *Mlu* I and *Spl* I which are indicated on the red, blue and yellow circles and the *I-Ceu* I sites indicated by arrows were taken from ref. 1. Inner thick circles with 8 gaps are the regions covered by cosmid and λ clones and by PCR products. Among the assigned genes, those related to oxygenic photosynthesis are shown by green letters. The three small circles below the map represent extra-chromosomal units.

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