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Genome analysis of *Synechococcus* sp. strain PCC6301

M Sugita[1], T Tsudzuki[2], C Sugita[1], M Ishiura[1], K Ogata[3], H Jikuya[3], J Takano[3], M Sugiura[4]

[1]*Center for Gene Research., Nagoya University, Nagoya 464-8602, Japan. sugita@gene.nagoya-u.ac.jp*

[2]*Department of Information and Policy Studies, Aichi-Gakuin University, Nisshin 470-0195, Japan. tsdzki@psis.aichi-gakuin.ac.jp*

[3]*Shimadzu Co.,Genomic Research, Nakagyo-ku, Kyoto 604-8511, Japan.*

[4] *Department of Natural Sciences, Nagoya City University, Nagoya 467-8501, Japan. sugiura@nsc.nagoya-cu.ac.jp*

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Introduction

Cyanobacteria are model organisms for the molecular study of plant-type photosynthesis (Sugiura, 1999). The complete nucleotide sequence of the genome of *Synechocystis* sp. strain PCC6803 revealed 138 genes for photosynthesis and respiration (Kaneko et al. 1996b). Interestingly, several photosynthesis genes, e.g. *ccmK*, *petC*, *ndhD*, *ndhF*, *psbA* or *petF*, are present as multiple copies. The sizes of cyanobacterial genomes vary from 2 to over 10 Mb and their GC contents differ from strain to strain (Herdman M, 1982). The genome of the unicellular cyanobacterium *Synechococcus* sp. strain PCC6301 (*Anacystis nidulans* 6301) is 2.7 Mb in size and the physical and gene maps were constructed (Kaneko et al. 1996a). To further understand the genome structure and function we constructed a set of ordered λclones that covered the entire region of the *Synechococcus* PCC6301 genome. Moreover, we started the project of sequencing the entire genome of *Synechococcus* PCC6301. Here we represent a list of the ordered λ clones and photosynthesis and respiration genes tentatively assigned so far.

Materials and methods

A *Synechococcus* PCC6301 genomic library was constructed using λ Dash II-*Bam*HI digested arms and 10-20 kb DNA fragments partially digested with *Sau*3AI. The λ clones were screened by plaque hybridisation using restriction fragments (P300, P320, PW85, W90 and so on, see Fig. 1) separated by pulsed-field gel electrophoresis (Kaneko et al. 1996a). λ DNAs were digested with *Eco*RI and performed to Southern blot analysis and confirmed to overlap with several clones. Total number of 519 λ clones were ordered and a minimum set of 198 clones was selected for further analysis. The insert DNA of λ clones was amplified by long and accurate polymerase chain reaction and sheared to short DNA fragments. The resultant DNA fragments were subcloned into an *Sma*I site of pUC18, and then shotgun sequenced using Shimadzu multi-capillary DNA sequencers (RISA-384). DNA sequences were assembled and potential open reading frames were subjected to similarity search against the CyanoBase (http://www.kazusa.or.jp/cyano/cyano.html).

Results and discussion

We constructed restriction fragment-specific sublibraries. For instance, the sub-library of P300 and P320 fragment consists of 129 and 150 λ clones, respectively (Fig. 1). We first ordered the λ clones of the respective sub-library and subsequently a set of 198 clones were ordered from L103 to P280-84 (Table 1). The sum of the insert DNA of the ordered clones is approximately 3.5 Mb in size, larger than the genome size of 2.7 Mb. In the present stage, contigs of larger than 9 kb and 1 kb are 106 and 1158, respectively. Our genome sequencing project is in finishing phase. Photosynthesis and respiration genes assigned so far are listed in Table 1.

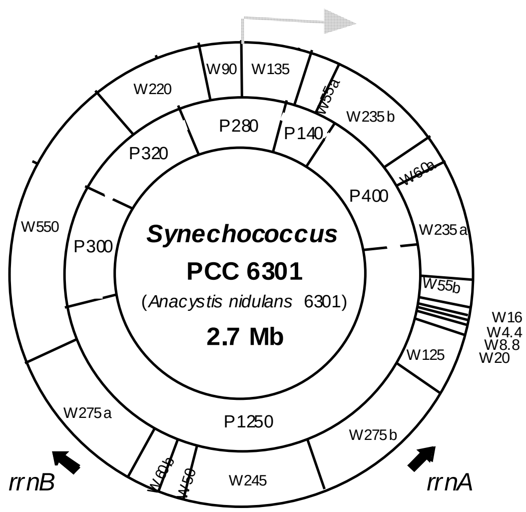

Fig. 1. Physical map of the *Synechococcus* sp. PCC6301 genome (Kaneko et al. 1996). Restriction fragments were named by the enzymes used (P, *Pme*I; W, *Swa*I) followed by their sizes (kb). The λ clones are ordered clockwise from the clone L103 at the position between W90 and W135. Bold arrows indicate the positions and directions of *rrn* operons.

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Table 1. List of a set of the ordered λ clones with the size (kb) of insert DNA and photosynthesis and respiration genes tentatively assigned.

λ clones	Insert	Genes
L103	16.2	
P280-300	18.9	
P280-301	20.0	psbD
P280-42	18.0	psaE
P280-39	18.5	
P280-37	18.2	ndhD2, ndhD3, psbN
P280-4	17.0	pntB
P280-21	17.1	
L17A	18.4	
P140-22	18.0	ndhD3, icfA, cfxA
W55-32	16.2	ccmK, ccmM
W55-49	17.1	ndhB
W55-61	18.0	
W55-26	20.5	
P140-29	18.3	
P140-20	17.9	
PW55-1	20.0	
L32A	16.8	
P400-404	17.0	psaE
P400-407	16.6	psaE
P400-409	10.8	cmpR
P400-321	17.6	ccmK
P400-318	18.0	
P400-317	18.0	tpi
P400-213	19.9	psaF, tpi
P400-292	19.9	petA, petC
P400-208	18.5	
P400-207	17.7	ndhD, ndhJ
P400-206	19.6	psbL, psbE, ndhC, ndhJ, ndhK
P400-204	15.1	
P400-202	19.1	
P400-201	20.5	
P400-849	19.0	
P400-301	22.5	pgk
P400-826	17.9	
P400-822	17.2	petE
P400-823	16.0	
P400-828	12.9	
P400-834	17.5	
P400-840	21.4	cpcA, cpcB, cpcC, cpcD, cpcE, cpcF, apcA, apcB, petH, cfxE
P400-852	19.4	
P400-857	16.8	atpA, atpC, atpD, atpH, atpI, apcA, apcC, apcD, apcE, psbZ
P400-808	16.4	
P400-806	16.7	psaD, cmpR
P400-803	17.7	
P400-801	20.5	ptk, petH
PW70-15	20.0	

λ clones	Insert	Genes
PW70-18	21.2	
PW70-20	21.6	
PW70-8	15.9	psaK
PW70-34	19.4	
W55b-gap1	9.5	psbA
W55b-67	15-20	
W55b-65	15.2	
W55b-8	16.5	
W16-21	16.5	
W16-4	19.0	
W20-C	17.0	
W20-D	16.8	ferredoxin
W125-40	16.2	
W125-11	19.1	
W125-39	18.8	
W125-64	19.9	
W125-20	21.0	
W125-7	19.2	
W125-3	17.0	
W125-52	17.6	
W125-8	18.9	psaK
W275b-501	16.8	
W275b-303	21.6	psbC, psbD, isiA
W275b-305	12.9	psbC, psbD
W275b-P17	16.6	tpi
W275b-P14	15.8	
W275b-P13	15.7	ndhD, apcA, cpcA, cpcC, cpcE, cpcF, cfxE
W275b-P6	15.8	
W275b-610	16.8	
W275b-629	15.2	ndhB, ndhD2, rpiA
W275b-631	18.7	
W275b-633	15.3	
W275b-637	18.5	ndhB, gap2, ferredoxin
W275b-714	17.2	psbC
W275b-707	16.2	petH
W275b-716	16.7	
W275b-718	15.2	
W275b-719	16.3	psbK
W275b-113	16.7	
W275b-112	16.0	psbA, psbD
W245-t3	14.5	psaK
W245-t5	16.2	isiA, psbC, psbD
W245-t7	19.1	
W245-t9	20.0	
W245-t11	15.0	
W245-t15	10.4	
W245-400	14.7	
W245-401	14.0	ccmK

Table 1. Continued

λ clones	Insert	Genes	λ clones	Insert	Genes
W245-405	16.1	gap2	P300-202	20.0	fdp
W245-117	15.0	gap2, psbK	P300-207	20.3	atpB, atpE
W245-105	16.8	psbO	P300-216a	17.8	atpB, atpE
W245-104	17.3		P300-220	18.7	
W245-101	18.5	ndhF	P300-302	17.9	psaC
W245-100	19.1	atpH, atpI, apcA, apcB, apcC,	P300-304	17.8	
		apcD	P300-305	21.0	
W245-202	20.9	atpA, atpC, atpH, atpI, apcB	P300-307	13.4	
		apcC, apcD, apcE, psbZ, ndh	P300-308	20.8	
W245-204	18.8	psbZ	P300-310	19.1	
W245-301	19.7	apcA, apcD, gap2	P300-425	19.0	apcF
W245-210	15.0	psbH, psbN	P300-423	19.1	apcA, apcF, cpcG
W245-501	18.3		P300-420	20.8	
W245-206	20.9		P300-418	18.3	
W245-702	18.8	ndh	P300-416	17.0	psaA, psaB
W245-701	16.2		P300-415	19.7	ndhD2, ndhD, ndhF
L712	21.1		P300-412	20.1	
W50-501	15.6		P300-406	17.0	
W50-503	21.6		P300-403	19.5	psaA, psaB
W50-504	17.4		L1	18.4	cpcG
W50-18	20.0		P320-500	16.8	cpcG
W50-507	18.7	ndh	P320-512	24.2	
W60-605	17.0	ndh, ndhF, cmpR	P320-513	24.6	ndhD2, ndhD, ndhF
W60-607	19.7	ndh	P320-516	12.4	ndhD2, ndhD, ndhF
W275a-201	16.9		P320-517	17.0	
W275a-204	18.0		P320-519	17.5	
W275a-206	15.9		P320-521	17.8	
W275a-43	19.3	ccmK	P320-523	18.1	
W275a-50	18.0		P320-525	19.0	
W275a-55	17.0		P320-309	19.9	
W275a-57	16.5	ctaE	P320-531	16.2	
W275a-a7	15.7	ctaB, ctaD, coxB	P320-304	22.8	
W275a-a4	20.1	petF, ctaB, ctaD, ctaE, coxB	P320-302	22.5	
W275a-a2	16.7		P320-301	15.6	
W275a-110	13.9		P320-208	20.8	
W275a-115	16.7		P320-205	20.5	
W275a-125	19.2		P320-200	16.9	
W275a-128	20.0		P320-48	17.6	
W275a-138	16.4	atpA, atpC, apcD, atpH, apcA	P320-402	22.5	cyd-1
		apcD, apcE, psbZ, ndh	P320-26	17.4	ndhH, gap2
W275a-143	15.3	atpA, atpC, apcA, apcB, ndh	P320-411	27.6	ftrC
W275a-150			L23	18.3	
W275a-m5	16.8		PW85-53	16.9	
W275a-32	13.8		PW85-36	17.5	
W275a-12	18.2		PW85-30	21.5	
W275a-13	18.0		PW85-16	19.5	
PW80-19	17.3		PW85-31	18.2	psbD, ndhF, cytM
PW80-16	14.8		P280-840	19.0	ndhD, ndhF, psbD
PW80-11	17.7		P280-67	17.9	ndhD, ndhF, petG, cytM
PW80-1	19.0		P280-13	22.4	
PW80-5	18.0		P280-87	16.2	
PW80-25	17.3		P280-64	16.9	cyd-2
PW80-23	15.0	fdp	P280-84	14.5	
				(3523.6)	