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Analysis of 39 cyanobacterial species reveals rbcX subunit to be present between L and S Subunits

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Abstract

The impact of great oxidation event on RuBisCO has been tremendous. It has led to the competition between carbon dioxide and oxygen at the active site of the enzyme. Cyanobacteria developed strategies to combat this change by concentrating carbon dioxide in organelles called carboxysomes. RbcX helps in proper folding of RuBisCO by interacting with RbcL. However, it is not an absolute requirement for RuBisCO to attain proper folding only with the aid of RbcX. RbcX has a chaperone like activity. The present analysis led to the finding that cyanobacterial species lacking RbcX contain multitude of proteins showing homology to chaperone like proteins. These proteins might be playing the same role as RbcX in these cyanobacterial species to help RuBisCO acquire proper folding. Analyses also indicated that in general the rbcX motif is present between rbcL and rbcS.

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Introduction

Photosynthesis is the major process that helps in the survival of majority of organisms on earth by producing oxygen as the by-product. Photosynthesis is directly or indirectly the primary feeding pathway for all living organisms and is hence the subject of study from several perspectives.

Cyanobacteria, also known as blue green algae have existed on earth since 3.5 billion years. Cyanobacteria were the initial organisms to develop oxygenic photosynthesis, which led to the increase in the oxygen concentration in the environment. Initially the oxygen evolved was used up in oxidizing the large amounts of iron present in the environment which led to the banded iron formation (Rae et al., 2013). Later on, upon the depletion of iron reserves, when all iron got oxidized, oxygen started accumulating in the environment (known as great oxidation event) which was a not a desirable change for RuBisCO, the primary photosynthetic enzyme as this led to competition between oxygen and carbon dioxide at the binding site of RuBisCO.

As oxygen concentration in the atmosphere was increasing, the efficiency of RuBisCO declined further. To cope up with this loss, cyanobacteria developed a carbon concentrating mechanism (CCM), which involves carboxysomes and CO_2:bicarbonate transporters (Rae et al., 2013). The primary function of carboxysome is to concentrate CO_2 around RubisCO and reduce the efflux of CO_2 (Price and Badger, 2003). Two types of carboxysomes came into existence, possibly by convergent evolution with similar designs and function but with different protein makeup. The α-carboxysome is predominantly found in oceanic cyanobacteria (e.g., Prochlorococcus marinus) and β-carboxysome is found in freshwater cyanobacteria and accordingly cyanobacteria are named as α and β cyanobacteria (Rae et al. 2013) respectively.

Rubisco structure and function

RubisCO is the most abundant enzyme present on earth. The catalytic incorporation of CO_2 into RuBP (Ribulose 1, 5 bisphosphate) by Rubisco is the first step in the production of carbohydrates by plants (Whitney et al., 2011). Prior to catalysis, RubisCO needs activation via the reaction with CO_2 molecule with active site Lys residue to form a carbamate, which is then stabilized by Mg^{2+} binding. Following activation, RubisCO can productively bind RuBP, add H_2O and CO_2 molecule to RuBP to give two molecules of 3-PGA. RubisCO is produced in high quantities in plants to overcome its inefficiency in catalyzing carboxylation reactions. Plants use 25% of their nitrogen to synthesize RuBisCO. Inefficiency of RubisCO is due to electrochemical similarity of O_2 and CO_2 and atmospheric level of O_2, which is much higher than CO_2, therefore it undergoes oxygenation reaction too, forming one molecule of 3-PGA and one molecule of 2-phosphoglycolate (it is recycled back to form 3-PGA during photorespiration which is an energy intensive process), thereby decreasing the overall carbon fixation (Whitney et al., 2011).

RuBisCO comprises of at least two large subunits of approximately 50kD called rbcL. RubisCO shows conservation in C terminal domain of L-subunit structure which forms α/β barrel. It has one active site in CTD and NTD each, therefore L2 dimer has two active sites. Different forms of RubisCO exist in various organisms. Form I RuBisCO is arranged as L8S8 (S is small subunit, which binds to L8). Although not strictly required for CO_2 fixation, the S-subunits are essential for maximal activity and provide structural stability of RuBisCO (Andersson and Backlund, 2008). Form II and III do not have small subunit. These forms are present in archaea and proteobacteria. Form IV RuBisCO is also called RLP (RuBisCO like protein). It does not have any catalytic site for carboxylation (Whitney et al., 2011).

In plastids, L-subunits interact with general chaperones like Hsp70 chaperone system (DnaK/DnaJ/GrpE), GroEL, GroES and the RuBisCO specific chaperone BSDII (Nishimura et al., 2008), which prevent these subunits from misfolding. Several reports show that in cyanobacteria L-subunits interact with rbcX which exists as an arc shaped homodimer ($RbcX_2$). RbcX binds to the flexible carboxy terminal sequence of RbcL, via domain EIKFEFD (Liu et al., 2010). It facilitates the assembly of L-subunits into (L2)4 complexes which is then displaced by stable binding of S-subunits that produce L8S8 enzyme. But some reports indicate that this RbcX is not the ultimate requirement for folding of RbcL subunits (Emlyn-Jones et al., 2006).

Analysis of the presence or the absence of RbcX among several cyanobacterial species may help us to understand its dispensability. It can lead us to the identification of some other chaperones which play important role in assembly of RuBisCO L-subunits. Studies on chaperone-assisted assembly of cyanobacterial L_8S_8 Rubisco have been almost exclusively conducted in Escherichia coli. Studies indicate that RbcX from Synechococcus PCC7942 plays an apparent chaperonin-like role in RubisCO assembly in E. coli, but it is dispensable in the cyanobacterium. In cyanobacterium, RbcX may work indirectly by activating some other chaperone for assembly of functional RuBisCO enzyme. Indeed, the necessity for and function of RbcX in other cyanobacteria therefore require further attention (Emlyn-Jones et al., 2006). The structurally unrelated products of the cbbQ and cbbX genes that are clustered with the RubisCO genes in proteobacteria have also been shown to promote similar improvements in RuBisCO assembly and activity (Gibson and Tabita 1997; Hayashi et al., 1999; Hayashi and Igarashi 2002). Some cyanobacterial species viz. Prochlorococcus marinus MIT9313, Prochlorococcus marinus MIT9303, Synechococcus WH7803 possess cbbX (genome.microbedb.jp/cyanobase/).

Material and Method

Analysis of RuBisCO subunits: In order to ascertain the role of RbcX, we analysed the genomes of various cyanobacteria retrieved from KAZUSA genome resource for the analysis of the location of RbcS, RbcX, and RbcL and whether these

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subunits are located adjacent to each other or are dispersed in the genome and also to determine whether the distance of RbcX with respect to RbcL and RbcS is making any significant contribution towards its function.

Scanning different Cyanobacterial species for rbcX: Several of the cyanobacterial species were found to lack RbcX encoding genes. The absence of rbcX gene was confirmed by browsing the individual genes as well as by genome blast. RuBisCO being a multimeric protein is expected to require a chaperone for folding of an active protein. The absence of RbcX in several cyanobacteria directs the thought process towards the idea that there exist certain chaperones which are yet to be identified. To investigate this, organisms devoid of RbcX were analysed for the chaperones present in them. The chaperone encoding gene sequences or protein sequences were retrieved from Kazusa genome resource and aligned in MEGA 5.1 software with rbcX to identify any similarity in sequences.

RESULTS

Positional analysis of rbcX: During the analysis of 39 cyanobacterial species we observed that X subunit was found in between L and S subunits. For example, Synechococcus elongatus PCC7002 (Fig. 1). In some cyanobacterial sp. there are multiple S-subunits viz. Anabeana virabilis ATCC 29413, Nostoc punctiforme ATCC29133, Trichodesmium erythraeum IMS101, Cyanothece sp. PCC 7425. Some cyanobacterial sp. also have more than one large subunit, for instance, Cyanothece sp. PCC8801, Cyanothece sp. PCC7424. In other cases like Synechococcus elongatus PCC7942, X subunit is located very far from its L and S subunit. The location of rbcL, rbcS, rbcX of some cyanobacterial species is given in table 1.

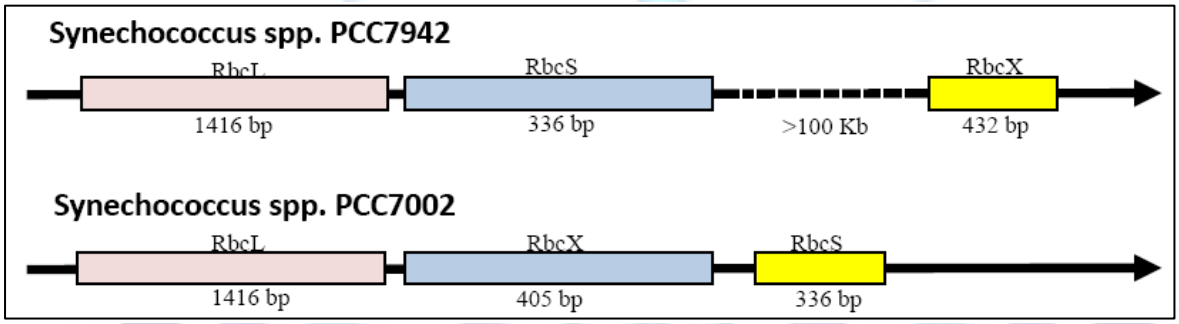

Fig.1. RbcX location in *Synechococcus elongatus* PCC 7942 and 7002

Table 1: Locus of L and S subunit genes of RuBisCO in the genome of the organisms possessing RbcX

Organism	rbcL (locus in bp)	rbcS (locus in bp)	rbcX (locus in bp)
Gloeobacter violaceus PCC 7421	2307046-2308470	2308900-2309200	2308500-2308877
Synechocystis sp. PC C 6803	2748414-2479826	2480477-2480818	2480034-2480444
Anabaena sp. PCC 7120	1785970-1787400	1787950-1788279	1787495-1787893
Thermosynechococcus elongates BP-1	1574633-1576060	1573812-1574168	1574199-1574579
Microcystis aeruginosa NIES-843	4390428-4391843	4389548-4389883	4389899-4390297
Synechococcus elongatus PCC 6301	139920-141338	139494-139829	2692684-2693169
Synechococcus sp. PCC7002	1882749-1884164	1881910-1882245	1882276-1882680
Acaryochloris marina MBIC 11017	1775408-1776838	1774474-1774821	1774874-1775275
Anabaena variabilis ATCC 29413	4857469-4858899	4853884-4855128, 4856590-4856919	4856976-4857374
Synechococcus elongatus PCC 7942	1479461-1480879	1480970-1481305	1595486-1595944
Synechococcus sp. JA-2-3B'a(2-13)	2682338-2683762	2681523-2681855	2681864-2682271
Synechococcus sp. JA-3-3Ab	1207204-1208628	1209115-1209447	1208699-1209106
Cyanothece sp. ATCC 51142	3281510-3282925	3280671-3281006	3281030-3281428
Nostoc punctiforme ATCC 29133	5263600-5265030	5265663-5265992, 5267505-5268779, 5380153-5382162	5265208-5265615
Trichodesmium erythraeum IMS101	6791736-6793166	2407801-2409072, 6790587-6790922	6791096-6791482
Cyanothece sp. PCC 7424	1061844-1063007, 1503225-1504643	1502367-1502702	1502734-1503141
Cyanothece sp. PCC 7425	3372918-3374348	3372003-3372347, 4356729-4358000	3372423-3372818
Cyanothece sp. PCC 8801	1677472-1678890, 2144196-2145281	1679403-1679738	1678968-1679372
Arthrospira platensis NIES-39	4152769-4154199	4151888-4152223	4152272-4152637

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Table 2: Locus of L and S subunit genes of RuBisCO in the genome of the organisms lacking RbcX

Organisms	rbcL (locus in bp)	rbcS (locus in bp)
Prochlorococcusmarinus SS120	524454-525866	525970-526311
Prochlorococcus marinus MED4	519087-520502	520592-520933
Prochlorococcus marinus MIT9313	1293386-1294798	1292937-1293278
Synechococcus sp. WH8102	1651727-1653142	1651326-1651667
Synechococcus sp. CC9902	1563311-1564723	1562850-1563191
Synechococcus sp. CC9605	716826-718241	718301-718642
Prochlorococcus marinus str. MIT 9312	513733-515148	515243-515584
Prochlorococcus marinus str. AS9601	529175-530590	530681-531022
Prochlorococcus marinus str. MIT 9515	549602-551017	551111-551452
Prochlorococcus marinus str. MIT 9303	737613-739025	739134-739475
Prochlorococcus marinus str. NATL1A	549278-550690	550791-551132
Prochlorococcus marinus str. MIT 9301	503604-505019	505109-505450
Synechococcus sp. RCC307	744350-745765	745825-746166
Synechococcus sp. WH 7803	674560-675975	676035-676376
Prochlorococcus marinus str. MIT 9215	555111-556526	556620-556961
Prochlorococcus marinus str. MIT 9211	517060-518472	518572-518913
Chlorobium tepidum TLS	1677980-1679287	
Rhodopseudomonas palustris CGA009	1731700-1733157	1733171-1733593
Synechococcus sp. PCC 9311	1750450-1751862	1750005-1750346
Prochlorococcs marinus NATL2A	536918-538330	538431-538772

Folding of RuBisCO L subunit: We also found certain cyanobacterial sp. in which RbcX was absent or not yet recognized, e.g., Synechococcus sp., Prochlorococcus marinus sp. For confirming the absence of RbcX, genome BLAST of all the organisms from table 2 was done with RbcX of Gloeobacter violaceous (since it is reported to be the most primitive organism according to 16S rRNA phylogenetic analysis) to find similar or near similar proteins. However, the blast result did not show any significant hits. For finding out how these species fold their L subunit without rbcX, we started looking for other chaperonins which might aid in the process. We found out that Prochlorococcus marinus sp. 120 has 17 genes for different chaperones like DNA J, DNA K etc., which showed homology with RbcX. Organisms not having documented RbcX are given in the Table 3.

Table 3: Diverse chaperones present in cyanobacteria**.** The signs '+' and '-' indicate the presence or the absence of the chaperone in the organism respectively.

Organism	HSP40	HSP70	HSP70-2	ATPases	HSP60	GroEL	GroES	Other chaperones
P. marinus SS120	-	DNAK	-	+	+	+	+	-------
P. marinus MED4	-	DNAK	DNAK2	-	-	+	+	-------
P. marinus MIT9313	-	DNAK	DNAK2	-	-	+	+	-------
Synechococcus WH102	-	DNAK	DNAK2	-	-	+	+	HSP33
Synechococcus CC9902	-	DNAK	-	-	-	-	+	HSP33, Cpn60, Tcp-1
Synechococcus CC9605	-	DNAK	-	-	-	-	+	HSP33, Cpn60,Tcp-1
P. marinus SS120	-	-	-	-	+	+	+	-------

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P. marinus MED4	-	DNAK	DNAK2	-	-	+	+	-------
P. marinus MIT9515	-	DNAK	DNAK2	-	-	+	+	-------
P. marinus MIT9303	-	DNAK	DNAK2	-	-	+	-	-------
P. marinus NATL1A	-	DNAK	DNAK2	-	-	+	+	-------
P. marinus MIT9301	-	DNAK	DNAK2	-	-	+	+	-------
Synechococcus RCC307	DNA J	DNAK	-	+	-	+	+	Clpb, Cpn10
Synechococcus WH 7803	DNAJ	DNAK	-	-	-	+	+	Grpe, clpb
Prochlorococcus marinus 9215	-	DNAK	DNAK2	-	-	+	+	Cpn60
Prochlorococcus marinus str. MIT9211	DNAJ	DNAK	DNAK2	-	-	+	+	-------
Rhodopsuedomonas palustris CGA009	-	-	-	-	-	-	-	-------
Synechococcus 9311	DNAJ	DNAK	DNAK2	+	-	+	-	Grpe, HSP 33
Prochlorococcus marinus NATL2A	-	DNAK	-	-	-	+	+	Grpe

Discussion

Gene arrangement of rbcL, rbcS and rbcX is different in different cyanobacterial species which suggests that there is no set rule for rbcL to appear at the extreme 5'end and it may be located downstream rbcS and rbcX. This indicates that these subunits were not transferred as a cluster during the course of evolution. Also lack of RbcX indicates that these organisms got these subunits independently and at different points of time. Eventually, RbcX subunit lost its relevance, at least in some species, which further indicates parallel evolution of these species, or development of alternative strategies for protein folding, while other species who continued to have RbcX still utilize it. But at the same time, there are reports that Synechococcus sp. RbcX is not an ultimate requirement for RuBisCO large subunit folding. So it can be deciphered that even if it is present in organism like Synechococcus, it is not the most essential chaperone in RubisCO large subunits folding. Presence of more than one small or large subunit might indicate gene duplication. As we observed that the repeating subunit is found away from the core rubisco operon, it may suggest that these subunits were transferred individually during the course of evolution. Some organisms (e.g., Prochlorococcus marinus) do not have any reported RbcX. In order to know about the overall impact of the presence or the absence of RbcX, we started analyzing their small and large subunits. We found that there is no significant difference in the other two subunits. The only difference that we came across is in having number of chaperones like GroEl and GroES. Species which do not have RbcX, have large number of other folding genes (e.g., Prochlorococcus marinus), which might still provide them with properly folded RbcL subunits.

As mentioned earlier, some organisms are able to perform photosynthesis without RbcX e.g., Synechococcus species. Possible reasons for it are (1) These organisms possess some other proteins that overtake the functioning of RbcX. (2) Structure of some of the RbcL and RbcS have evolved such that they don't require RbcX anymore.

In majority of the species, RbcX interacts with EIKFEFD motif in RbcL. However, some marine species which lack RbcX still have this motif in their large subunits e.g., Prochlorococcus marinus SS120 (table 4). This fact may have the following reasons behind it: (1) Several cyanobacterial sp. have evolved independently of RbcX and adapted such that they do not need RbcX at all. (2) RbcX acts downstream of GroEL and GroES infolding of RbcL partially and RbcX improvises upon the folding; and hence in organisms which do not possess RbcX, their GroEL and GroES might be more efficient than other species due to unknown reasons. (3) Inspite of having EIKFEFD domain, absence of RbcX suggests that some other protein/s (not yet discovered) might interact with this domain and bring about the same effects as RbcX. Surprisingly, upon further investigating the occurrence of the RbcX binding domain in RbcL in organisms both possessing and lacking RbcX, we came across another interesting observation, i.e., the organisms which do not have any reported RbcX have a conserved RbcX interaction domain while the organisms that possess RbcX have a little alteration in the same domain (table 4).

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Table 4: Five organisms lacking RbcX* and five possessing RbcX[$] were analyzed for the presence or the absence of the RbcX interacting domain

Prochlorococcus marinus SS120 0551*	W	K	E	I	K	F	E	F	D
Synechococcus sp W1718*	.	.	.	.	.	.	.	.	.
Prochlorococcus marinus str MIT 9312*	.	.	.	.	.	.	.	.	.
Synechococcus sp. CC9605 0752*	.	.	.	.	.	.	.	.	.
Prochlorococcus marinus NATL1 06041*	.	.	.	.	.	.	.	.	.
Gloeobacter Violaceus PCC 7421[$]	.	.	.	.	.	.	.	Y	E
Synechocystis sp. PCC 6803[$]	.	.	.	.	.	.	.	.	E
Anabanea Sp. PCC 7120[$]	.	.	.	.	.	.	.	.	E
Thermosynechococcus elongatus BP-1[$]	.	.	.	.	.	.	.	.	E
Microcystis aeroginosa NIES-843[$]	.	.	.	.	.	.	.	.	E

Conclusions

The study was performed with the aim to identify the occurrences of RbcS, RbcL and RbcX in the cyanobacterial genome and the relative organization of the genes encoding them. It was revealed in this study that many cyanobacterial species lack RbcX, the protein involved in chaperoning RubisCO assembly. The cyanobacterial species lacking RbcX possess many proteins which show homology to chaperone proteins. Interaction between RbcX and RbcL domain is important for proper folding of RubisCO. Some cyanobacterial species lacking RbcX have evolved different mechanisms for the folding of RbcL subunits. Proteins which are most important for the folding of RbcL subunits in such cyanobacterial sp. can be deciphered only by experimental approaches like yeast two hybrid systems.

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