Cell. Mol. Life Sci. 64 (2007) 2607 – 2619
1420-682X/07/192607-13
DOI 10.1007/s00018-007-7190-z

Cellular and Molecular Life Sciences

Review

Carotenoids and carotenogenesis in cyanobacteria: unique ketocarotenoids and carotenoid glycosides

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Received 19 April 2007; received after revision 4 June 2007; accepted 19 June 2007
Online First 23 July 2007

Abstract. Cyanobacteria grow by photosynthesis, and necessarily contain chlorophyll and carotenoids, whose main functions are light harvesting and photoprotection. In this review, we discuss the carotenoids, carotenogenesis pathways, and characteristics of carotenogenesis enzymes and genes in some cyanobacteria, whose carotenogenesis enzymes have been functionally confirmed. In these cyanobacteria, various carotenoids have been identified, including the unique ketocarotenoids, echinenone and 4-ketomyxol; and the carotenoid glycosides, myxol glycosides and oscillol diglycosides. From these findings, certain carotenogenesis pathways can be proposed. The different compositions of carotenoids among these species might be due to the presence or absence of certain gene(s), or to different enzyme characteristics. For instance, two distinct β-carotene ketolases, CrtO and CrtW, are properly used in two pathways depending on the species. One β-carotene hydroxylase, CrtR, has been identified, and its substrate specificities vary across species. At present, functionally confirmed genes have been found in only a few species, and further studies are needed.

Keywords. Carotenogenesis, carotenoid, carotenoid glycoside, cyanobacteria, echinenone, ketocarotenoid, 4-ketomyxol, myxol glycoside.

Introduction

Cyanobacteria are the oldest known oxygenic photosynthetic organisms, and they played a key role in terrestrial history. They are regarded as the origin of plant chloroplasts because of their ability to perform the oxygen-evolving photosynthesis reaction, a characteristic of photosynthetic eukaryotes. They were formerly classified as 'blue-green algae' based on the provisions of the Botanical Code, but they are in fact prokaryotes, lacking internal organelles, and having cell walls that contain peptidoglycans, similar to eubacteria. Rippka et al. revised their generic definitions based on the differential characters in cultured material [1]. Many cyanobacteria contain chlorophyll, carotenoids and several accessory pigments, such as phycoerythrin and phycocyanin, on their membranes. Photosynthesis is performed on the thylakoid membranes, which are developed inside the cells. For a good overview of the physiology, biochemistry, cell structure and taxon, see *Bergey's Manual of Systematic Bacteriology* [2]. Their unique photosynthetic abilities combined with their usefulness in experimental models have made the cyanobacteria popular subjects for genetic and physiological studies. Ge-

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nomes of the most popular cyanobacteria have been analyzed extensively, and complete genome sequencing data for more than 30 strains are now available. Further, the characteristics of cyanobacteria as observed in recent experiments have been well summarized in the book *The Molecular Biology of Cyanobacteria* [3].

Cyanobacteria grow by photosynthesis, and necessarily contain chlorophyll and carotenoids, whose main functions are light harvesting and photoprotection. Three-dimensional structures of both photosystem I and II have been obtained from *Thermosynechococcus elongatus*, and they contain only β-carotene as carotenoids [4, 5]. Myxol glycosides are located in the cytoplasmic and the outer membranes without chlorophyll *a*, and they seem to have important roles in photoprotection [6–8]. Further, the functions of carotenoids in cyanobacteria have been well summarized in the book *The Photochemistry of Carotenoids* [9].

Some studies of carotenoids in cyanobacteria were published in the 1960 s and 1970 s [10, 11]. In 1980, Goodwin compiled the first list of the carotenoids from about 40 species of cyanobacteria [12], with several species added thereafter [13]. In these early studies, several carotenoids were doubtful or were listed with insufficient data. Further, some novel genera and species have been recognized in cyanobacteria, and some species have been reclassified and/or renamed using new classification techniques [2].

The major carotenoids in cyanobacteria are β-carotene, its hydroxyl derivatives, its keto derivatives and the carotenoid glycosides. Ketocarotenoids and carotenoid glycosides are quite unique in nature, especially among the photosynthetic organisms [14]. In contrast, among the anoxygenic photosynthetic bacteria, purple bacteria contain acyclic carotenoids, such as spirilloxanthin and spheroidene; both green-sulfur and green filamentous bacteria contain derivatives of β-carotene and γ-carotene, such as isorenieratene and chlorobactene; and heliobacteria contain C_{30} carotenoids, such as diaponeurosporene [15, 16]. The major carotenoids in land plants are β-carotene, lutein, violaxanthin, and 9'-*cis* neoxanthin, which are derivatives of β-carotene and α-carotene [17].

By comparison, there have been relatively few carotenogenesis enzymes or genes functionally identified in cyanobacteria [13, 18–21]. For example, several carotenogenesis genes are still unknown, since they appear to be randomly distributed in the cyanobacterial genomes. It is possible that the elucidation of the complete genome sequences allow us to identify the genes homologous with the functionally confirmed genes. In the present review, we summarize the carotenoids, carotenogenesis pathways, and carotenogenesis enzymes and genes in some cyanobacteria, whose carotenogenesis genes have been functionally confirmed, and we also discuss related species.

Most carotenoids have trivial names; moreover all carotenoids have been named semisystematically based on IUPAC-IUB nomenclature according to the IUPAC Commission on Nomenclature of Organic Chemistry and the IUPAC-IUB Commission on Biochemical Nomenclature, 1975 [22]. A list of both names and structures of all known naturally occurring carotenoids and references giving data for each compound are presented in *Carotenoids Handbook* [14], and on the Web in the Bioactive Lipid Database (Carotenoids) (http://lipidbank.jp/).

Carotenoids in cyanobacteria

Table 1 summarizes the carotenoid compositions of some cyanobacteria whose genome sequences are known, and also lists their related species. The major carotenoids are β-carotene; its hydroxyl derivatives, zeaxanthin and nostoxanthin; its keto derivatives, echinenone and canthaxanthin; and the carotenoid glycosides, myxol 2'-glycosides and oscillol 2,2'-diglycosides (see Fig. 1). Cyanobacteria can be classified into several groups based on their carotenoid composition.

Three *Anabaena* species, *Nostoc punctiforme* and, *Gloeobacter violaceus* contain β-carotene and little or no zeaxanthin, while *Synechocystis* sp. PCC 6803 contains β-carotene as well as zeaxanthin. Further, *Thermosynechococcus elongatus* contains nostoxanthin in addition to β-carotene and zeaxanthin. All of these species contain ketocarotenoids as well as myxol glycosides or oscillol diglycoside, which are characteristic carotenoids in cyanobacteria.

Three species of *Synechococcus* contain β-carotene, zeaxanthin and nostoxanthin, while, *Prochlorococcus marinus* contains β-carotene and zeaxanthin as well as α-carotene and related carotenoids (Fig. 2) [23]. However, none of the species contain either ketocarotenoids or carotenoid glycosides.

This variation in carotenoid composition might be due to the presence or absence of specific carotenogenesis pathways and genes, as well as to the different characteristics of the specific enzyme(s), which will be described below. However, it is well known that the carotenoid composition depends on growth conditions such as growth stage, light intensity, nitrogen source, and concentration of nitrogen in the cultures, as well as on the strain type within a given species [13, 24].

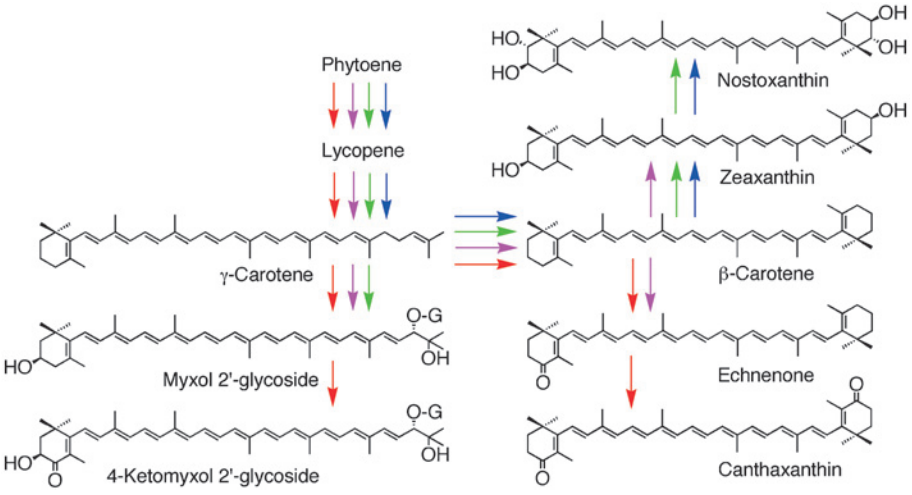

Figure 1. Major carotenoids and main carotenogenesis pathways in some cyanobacteria mentioned in this review. Red, *Anabaena* and *Nostoc*; Purple, *Synechocystis*; Green, *Thermosynechococcus*; and Blue, *Synechococcus*. G, Glycoside. See the text and figures for precise explanations.

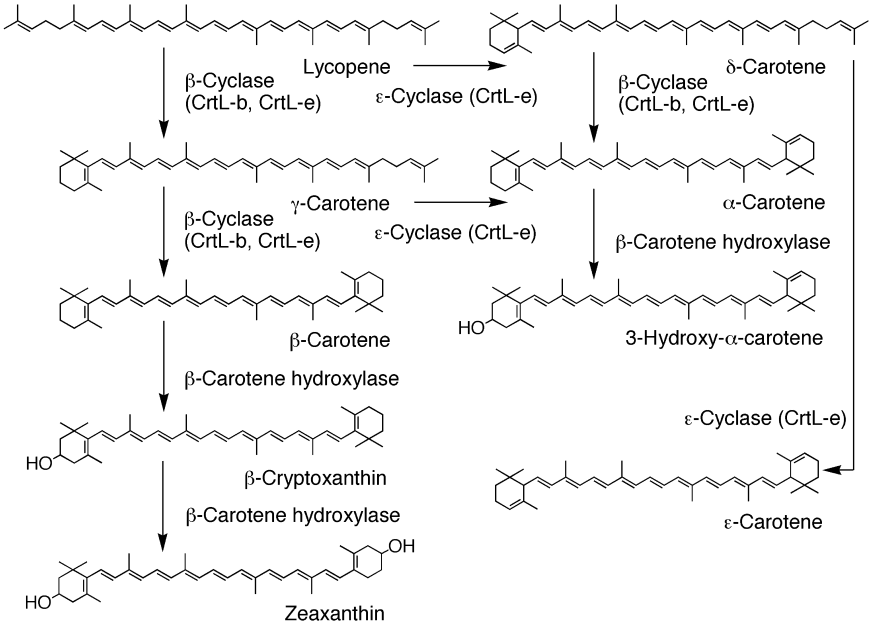

Figure 2. Carotenoids and carotenogenesis pathways in *Prochlorococcus marinus* MED4. See the text for precise explanations.

Myxol glycosides in cyanobacteria

Myxoxanthophyll is a major carotenoid glycoside, widely distributed in cyanobacteria; some species also contain oscillaxanthin (Fig. 3) [12, 13]. These carotenoids have a very unique glycoside linkage: a hydroxyl group at C-2' of the ψ end group of the carotenoids is bonded to glycoside in a (2'*S*)-configuration. This structure is only found in these carotenoids; its presence is limited to cyanobacteria, with no reports of it in any other bacteria, including in the photosynthetic bacteria or eukaryotic algae [12, 14, 25], although some carotenoid glycosides and carotenoid glycosyl esters are found in bacteria, mostly glucoside [25, 26]. Myxoxanthophyll was first isolated in 1936 from *Oscillatoria rubescens*, and its structure was proposed to be a polyhydroxyl carotenoid. The structures of myxoxanthophyll and oscillaxanthin from *Arthrospira* sp. were ultimately determined to be myxol 2'-rhamnoside and oscillol 2,2'-di(L-rhamnoside) [27, 28], respectively, although the glycoside moiety of rhamnoside was reidentified as chinovoside [29].

The determination of the sugar moieties of these carotenoids, including the L- or D-type and the α- or β-linkage, has been made only for a few species of cyanobacteria. To our knowledge, the following structures are the only ones reported to date, even with insufficient determination of the sugar moieties (Fig. 4): myxol 2'-*O*-methyl-methylpentoside, 4-keto-

Table 1. Carotenoid compositions of some cyanobacteria.

Carotenoids (mol%)	*Anabaena* sp. PCC 7120[a]	*Anabaena variabilis* ATCC 29413 (=IAM M-204)	*Anabaena variabilis* IAM M-3 (=PCC 7118, ATCC 27893)	*Nostoc punctiforme* PCC 73102 (=ATCC 29133)	*Synechocystis* sp. PCC 6803	*Thermo-synechococcus elongatus* BP-1	*Gloeobacter violaceus* PCC 7421	*Synechococcus* sp. PCC 6301 (=ATCC 27144, IAM M-6)[b]	*Synechococcus elongatus* PCC 7942 (=IAM M-201)[c]	*Synechococcus* sp. PCC 7002 (=ATCC 27264)	*Prochlorococcus marinus* MED4[d]
β-Carotene	62	51	38	45	26	54	48	28	34	33	11
β-Cryptoxanthin	< 1			< 1		1		2	3	1	3
Zeaxanthin	< 1			< 1	14	8		37	53	49	16
Caloxanthin						6		22	8	13	
Nostoxanthin						14		11	2	4	
Echinenone	25	20	33	17	18	1	3				
Canthaxanthin	1	22	4	13							
3-OH-Echinenone	< 1		1		4						
Deoxymyxol G					1	1					
Myxol G	8	5[e]	11	11	36	4					
Ketomyxol G	4		13	13							
Hydroxymyxol G	< 1	2[e]	< 1	< 1		13					
Oscillol diG							48				
G	fucoside		fucoside	fucoside	dimethyl fucoside	methylpentoside	fucoside				
Reference	[33]	[66]	[33]	[33]	[8]	UP[f]	[34]	[77]	UP [f]	UP [f]	[23]

[a] Also known as *Nostoc* sp. PCC 7120.
[b] Previously *Anacystis nidulans*
[c] Previously *Anacystis nidulans* R2.
[d] Other carotenoids: 1% δ-carotene, 63% α-carotene, 4% 3-hydroxy-α-carotene and 1% ε-carotene.
[e] Not glycosides, but free forms.
[f] UP:, Unpublished observation.

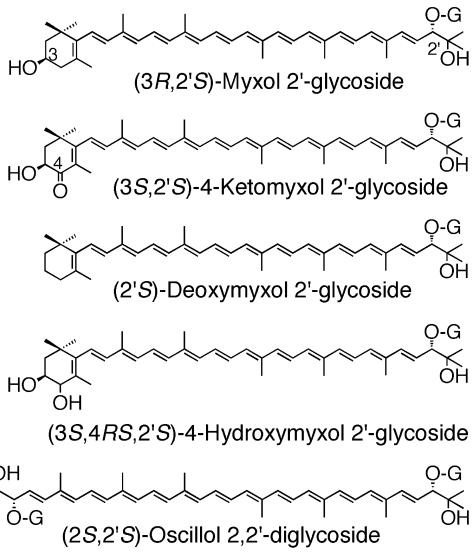

Figure 3. Structure of myxol glycosides and oscillol diglycoside in cyanobacteria. G, Glycoside. Structures of glycosides are shown in Figure 4.

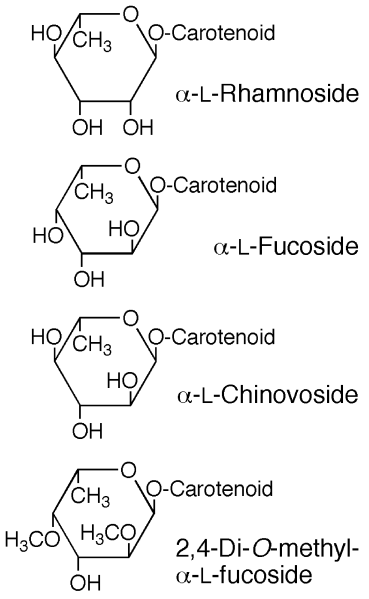

Figure 4. Structures of glycosides in myxol glycosides and oscillol diglycosides. Structures of carotenoid moieties are shown in Figure. 3.

myxol 2'-*O*-methyl-methylpentoside and oscillol 2,2'-di(*O*-methyl-methylpentoside) from *Oscillatoria limosa* [30], myxol 2'-α-L-chinovoside and oscillol 2,2'-di(α-L-chinovoside) from *Oscillatoria agardhii*, and myxol 2'-(3-*O*-methyl-α-L-fucoside) and oscillol 2,2'-di(3-*O*-methyl-α-L-fucoside) from *Oscillatoria bornetii* [29], myxol 2'-α-L-chinovoside and myxol 2'-α-L-fucoside from *Oscillatoria limnotica*, and oscillol 2,2'-di(α-L-chinovoside) and oscillol 2,2'-di(α-L-fucoside) from *Spirulina platemsis* [31]. The stereochemical structure of the hydroxyl groups is determined to be (3*R*,2'*S*)-myxol 2'-rhamnoside from *Phormidium luridum*, and (3*S*,2'*S*)-4-ketomyxol 2'-methylpentoside and (3*R*,2'*S*)-myxol 2'-*O*-methyl-methylpentoside from *Oscillatoria limosa* [32]. Recently, we identified (3*R*,2'*S*)-myxol 2'-(2,4-di-*O*-methyl-α-L-fucoside) from *Synechocystis* sp. PCC 6803 [8], (3*R*,2'*S*)-myxol 2'-α-L-fucoside and (3*S*,2'*S*)-4-ketomyxol 2'-α-L-fucoside from *Anabaena* sp. PCC 7120, *A. variabilis* IAM M3 and, *N. punctiforme* [33], and (2*S*,2'*S*)-oscillol 2,2'-di(α-L-fucoside) from, *G. violaceus* [34] (Table 1). The polar carotenoids from cyanobacteria have been provisionally named myxoxanthophyll and oscillaxanthin, and their sugar moieties are thought to be myxol 2'-rhamnoside and oscillol 2,2'-dirhamnoside [12, 13, 25], even though the various sugar moieties described above have been found.

We have proposed the following nomenclature for the trivial names of myxoxanthophyll, oscillaxanthin, and related compounds [8]: the carotenoid moieties (aglycone) of myxoxanthophyll and oscillaxanthin are myxol and oscillol, respectively; that of aphanizophyll is 4-hydroxymyxol [35], although 4-ketomyxol is also found [30] (Fig. 2). When the sugar moieties have not been determined, they should be named myxol glycoside and oscillol glycoside. If the sugar moieties have been identified, the names should be, for example, myxol 2'-chinovoside, myxol 2'-α-L-rhamnoside, (3*S*,2'*S*)-4-ketomyxol 2'-α-L-fucoside, and (3*R*,2'*S*)-myxol 2'-(2,4-di-*O*-methyl-α-L-fucoside) (Figs. 3, 4). Since names such as myxoxanthophyll and oscillaxanthin cannot specify the sugar moieties, the use of such indefinite terms should be avoided.

Synthesis of phytoene

Isopentenyl pyrophosphate (IPP), a C_5 compound, is the source of isoprenoids, terpenes, quinones, sterols and carotenoids. There are two known independent pathways of IPP synthesis: the classical mevalonate (NVA) pathway and the alternative, non-mevalonate, 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway [36, 37]. The distribution of these two pathways among prokaryotes follows no obvious pattern of taxonomic classification, although only the DOXP pathway has been observed in cyanobacteria. Further, IPP is synthesized by the DOXP pathway in the plastids of higher plants, where carotenoids are synthesized, whereas the MVA pathway is used in the cytoplasm [36–38].

Most carotenoids are tetraterpenoids consisting of eight IPP units. Farnesyl pyrophosphate (C_{15}) is synthesized from three IPPs. Then, one IPP is added to farnesyl pyrophosphate by CrtE (geranylgeranyl

Table 2. Functionally confirmed carotenogenesis genes or enzymes in cyanobacteria.

Abbreviation	Enzyme	Species	Gene	References
CrtE	geranylgeranyl pyrophosphate synthase	*Thermosynechococcus elongatus*	*tll0020*	[39]
CrtB	phytoene synthase	*Synecocystis* sp. PCC 6803	*slr1255*	[40]
		Gloeobacter violaceus	*glr1744*	[41]
		Synechococcus elongatus	*Syn_pcc79421984*	[42]
CrtP	phytoene desaturase (plant type)	*Synecocystis* sp. PCC 6803	*slr1254*	[45, 46]
		Synechococcus elongatus	*Syn_pcc79421983*	[42]
CrtQa	ζ carotene desaturase (CrtI type)	*Anabaena* sp. PCC 7120	*all7255*	[47, 48]
CrtQb	ζ carotene desaturase (plant type)	*Synecocystis* sp. PCC 6803	*slr0940*	[46, 49]
CrtH	*cis* carotene isomerase	*Synecocystis* sp. PCC 6803	*sll0033*	[44, 50]
CrtI	phytoene desaturase (bacterial type)	*Gloeobacter violaceus*	*glr0867*	[34, 41]
CrtL	lycopene cyclase	*Synechococcus elongatus*	*Syn_pcc79422062*	[56]
CrtL-b	lycopene β-cyclase	*Prochlorococcus marinus*	*PMM1064*	[23]
CrtL-e	lycopene ε-cyclase	*Prochlorococcus marinus*	*PMM0633*	[23]
CrtR	β-carotene hydroxylase	*Anabaena* sp. PCC 7120	*alr4009*	UP[a]
		Synecocystis sp. PCC 6803	*sll1468*	[60, 68]
CrtO	β-carotene ketolase	*Anabaena* sp. PCC 7120	*all3744*	[61]
		Synecocystis sp. PCC 6803	*slr0088*	[71]
		Gloeobacter violaceus	*gll0394*	[34, 41]
CrtW	β-carotene ketolase	Anabaena sp. PCC 7120	*alr3189*	[61]
		Nostoc punctiforme	*NpF4798*	[62]
			NpF5919	[62]
		Gloeobacter violaceus	*gll1728*	[41]
WcaG	GDP-fucose synthase	*Anabaena* sp. PCC 7120	*all4826*	UP[a]
		Synecocystis sp. PCC 6803	*sll1213*	[63]
Diox1	apo-carotenoid oxygenase	*Anabaena* sp. PCC 7120	*all4284*	[76]
		Synecocystis sp. PCC 6803	*sll1541*	[75]

[a] UP, Unpublished observation.

pyrophosphate synthase) to yield geranylgeranyl pyrophosphate (C_{20}) (Fig. 5). In a condensation of the two C_{20} compounds, the first carotene of phytoene (C_{40}) is formed by CrtB (phytoene synthase) [18, 19]. This pathway has been confirmed by cloning genes from two species of *Rhodobacter* and two species of *Pantoea* (previously *Erwinia*) [18, 19]. The functions of CrtE of *T. elongatus* [39], and CrtB of *Synecocystis* sp. PCC 6803 [40], *G. violaceus* [41], and, *S. elongatus* [42] have also been confirmed among cyanobacteria (Table 2).

Desaturation of phytoene to lycopene

Four desaturation steps are needed in the conversion from phytoene to lycopene (Fig. 5), and two distinct pathways are known among cyanobacteria: the plant type and the bacterial type.

The plant-type requires three enzymes: CrtP (phytoene desaturase), CrtQ (ζ-carotene desaturase) and CrtH (*cis*-carotene isomerase) (Fig. 5). CrtP catalyzes the first two desaturation steps, from phytoene to ζ-carotene via phytofluene, and CrtQ catalyzes two further desaturation steps, from ζ-carotene to lycopene via neurosporene. During desaturation by CrtQ, neurosporene and lycopene are isomerized to poly-*cis* forms, and then CrtH isomerizes to all-*trans* forms. Light is also effective for their photoisomerization to all-*trans* forms [43, 44]. The functions of these enzymes have been confirmed (Table 2): CrtP from *Synechocystis* sp. PCC 6803 [45, 46] and, *S. elongatus* [42], CrtQa (CrtI-type) from *Anabaena* sp. PCC 7120 [47, 48], CrtQb (plant-type) from *Synechocystis* sp. PCC 6803 [46, 49], and CrtH from *Synechocystis* sp. PCC 6803 [44, 50]. The CrtP of *S. elongatus* is stimulated by NAD(P) and oxygen as a possible final electron acceptor [51].

Chlorobaculum (previously *Chlorobium*) *tepidum* (green-sulfur bacterium) has the same three genes, and their functions have also been confirmed [52]. The carotenoid composition of *C. tepidum* is different from those of cyanobacteria: the major carotenoids are chlorobactene, 1',2'-dihydrochlorobactene and OH-chlorobactene glucoside laurate, which are derivatives of γ-carotene [53].

In contrast, the bacterial type uses only one enzyme, CrtI (phytoene desaturase), to convert from phytoene to lycopene (Fig. 5), and among cyanobacteria, only, *G. violaceus* uses this type of CrtI [34, 41]. Thus, *G.*

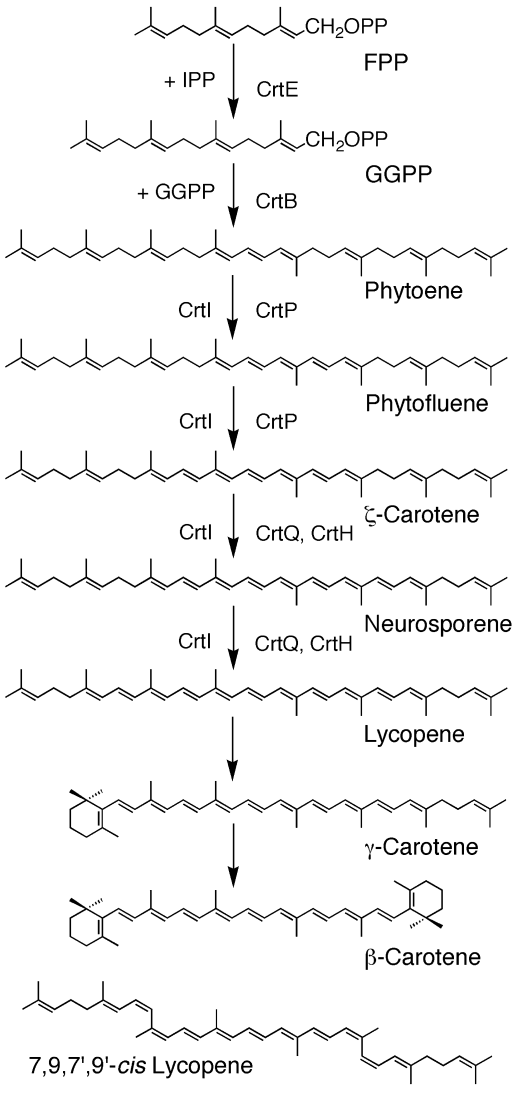

Figure 5. Synthesis of carotenes. Cyanobacteria require three enzymes, CrtP, CrtQ and CrtH, from phytoene to lycopene, while *Gloeobacter violaceus* uses only CrtI. An example of poly-*cis* forms, 7,9,7′,9′-*cis* lycopene, is also shown. IPP, isopentenyl pyrophosphate; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate. See the text for precise explanations.

violaceus is the first oxygenic photosynthetic organism that has been shown to use this type (Table 2). These observations suggest the following evolutionary scheme for this reaction step: the desaturation of phytoene was initially carried out by CrtI in ancestral cyanobacteria, then *crtP* and related desaturase genes were acquired, and, ultimately, there was replacement of *crtI* by *crtP* [34]. Purple photosynthetic bacteria, green filamentous bacteria and heliobacteria contain CrtI [15, 16], as do the other carotenoid-producing bacteria (except for cyanobacteria and green-sulfur bacteria) and fungi.

Lycopene cyclization by lycopene cyclases

Lycopene is cyclized to β-carotene via γ-carotene (Fig. 5), and there are three distinct lycopene cyclases. The first is found in bacteria (CrtY) and plants (CrtL and CrtL-b). These and CrtL-e (lycopene ε-cyclase) in plants exhibit a significant conservation of their amino acid sequences [54], and have an NAD(P)/FAD-binding motif [55]. Some cyanobacteria also contain these enzymes (Table 2). The functions of CrtL from, *S. elongatus* [56] and CrtL-b and CrtL-e from, *P. marinus* [23] have been confirmed. The second type of lycopene cyclase has been found in some bacteria, archaea and fungi [54, 57].

Recently, a new type of functional lycopene cyclase, CruA (CT0456), was found from *Chlorobaculum tepidum*, and the main product is γ-carotene in *Escherichia coli* with a lycopene background [58]. Further, its homologous genes are widely distributed in cyanobacteria, such as in *Synechocystis* sp. PCC 6803 and *Anabaena* sp. PCC 7120 (see Table 4). However, since the functions of lycopene cyclases could not be detected in these *cruA*-like genes [unpublished observation], there may be a fourth type of lycopene cyclase in these cyanobacteria.

There are two possible branching points in the synthesis of β-carotene and its derivatives, and myxol glycosides (see Fig. 1). One is at lycopene, where two lycopene cyclases may be present: lycopene β-cyclase, which produces β-carotene, and lycopene monocyclase, which produces only γ-carotene for myxol. The other possible branching point is at γ-carotene: lycopene cyclase produces β-carotene via γ-carotene, which is also used for myxol synthesis. It is all dependent on the characteristics of the lycopene cyclase and/or myxol synthesis enzymes producing β-carotene and myxol from γ-carotene. In any case, the branching points are still unknown.

P. marinus contains two lycopene cyclases (Table 2), which have sequence homology to CrtL. CrtL-b exhibits lycopene β-cyclase activity, while CrtL-e is a bifunctional enzyme having both lycopene ε-cyclase and lycopene β-cyclase activities (Fig. 2) [23]. The combination of these two cyclases allows the production of β-, α- and ε-carotenes. Both enzymes might have originated from duplication of a single gene. The characteristics of CrtL-e are somewhat different from those in plants [59]. Further, the β-end groups of β-carotene and α-carotene (left half) might be hydroxylated to zeaxanthin via β-cryptoxanthin and 3-hydroxy-α-carotene, respectively.

Synthesis of myxol glycosides

Lycopene is cyclized to γ-carotene (Fig. 5). The left half (β-end group) of the γ-carotene is hydroxylated by CrtR (β-carotene hydroxylase). Its function has been confirmed by the deleted mutants of *Synechocystis* sp. PCC 6803 [60] and *Anabaena* sp. PCC 7120 [unpublished observation], which produces deoxymyxol 2'-glycosides (Fig. 3). Further, a keto group is introduced by the CrtW-type β-carotene ketolase of *Anabaena* sp. PCC 7120 [61] and, *N. punctiforme* [62] (see Table 3) to form 4-ketomyxol 2'-glycoside (Fig. 6). It is not known whether 4-hydroxymyxol 2'-glycoside is produced directly from myxol 2'-glycoside by hydroxylation, or from 4-ketomyxol 2'-glycoside by reduction (Fig. 6).

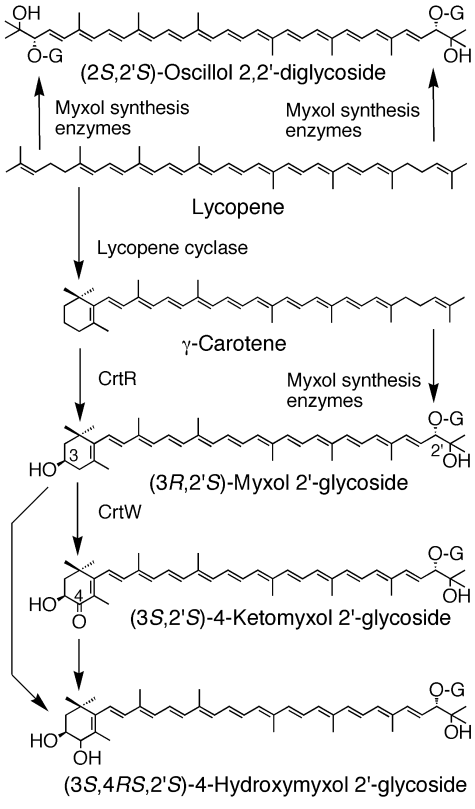

Figure 6. Synthesis of myxol glycosides and oscillol diglycoside from lycopene. G, glycoside. Structures of glycosides are shown in Figure 4. Although certain enzymes should be involved in myxol synthesis, little is known about this process. See the text for precise explanations.

The right half (ψ end group) of myxol has a very unique glycoside linkage, as described above (see Figs. 3, 6). Although certain enzymes should be involved in myxol synthesis, little is known about this process. A deleted mutant of GDP-fucose synthase of *Anabaena* sp. PCC 7120 produces myxol 2'-rhamnoside but not the usual myxol 2'-fucoside, and relatively little free myxol is present [unpublished observation]. GDP-rhamnose could be the substrate of GDP-fucose transferase, which has yet to be identified, instead of the usual GDP-fucose. Concerning the deleted mutant of GDP-fucose synthase of *Synechocystis* sp. PCC 6803 [63], it produces only free myxol, instead of the usual myxol 2'-dimethyl-fucoside [unpublished observation]. This might be due to the absence of the substrate of GDP-fucose transferase. 3,4-Dehydrogenase (CrtD) of *Rhodobacter* seems to play a role in the synthesis of myxol. A *crtD* homolog from a marine bacterium, strain P99–3 (MBIC 03313; previously *Flavobacterium* sp.), which produces free myxol [64], is known to have a function [65], although a *crtD* homolog in *Anabaena* sp. PCC 7120 (*all5123*) has no function [unpublished observation]. In the case of oscillol 2,2'-diglycoside, myxol synthesis enzymes catalyze both end groups of lycopene, and this might be due to the characteristics of myxol synthesis enzymes and/or lycopene cyclase (Fig. 6).

In *A. variabilis* ATCC 29413, myxol glycosides are absent, while the free forms of myxol and 4-hydroxymyxol are present [66]. Another strain of *A. variabilis* IAM M-3 has (3*R*,2'*S*)-myxol 2'-fucoside and (3*S*,2'*S*)-4-ketomyxol 2'-fucoside [33] (Table 1). Thus, *A. variabilis* ATCC 29413 is the first cyanobacterium found to have free myxol and not myxol glycosides, and it seems to lack the gene for, or activity of, glycosyl transferase. Note that the GDP-fucose synthase homologous gene is present (see Table 4). Thus, this strain is considered to be of potential use in investigating the characteristics of myxol glycosides in cyanobacteria. Free myxol with the same stereochemistry to myxol of cyanobacteria has been found in only two marine bacteria, strain P99–3 [64] and strain YM6–073 (MBIC 06409, *Flavobacteriaceae*) [67].

Synthesis of zeaxanthin, nostoxanthin and echinenone

Some cyanobacteria produce zeaxanthin, and some produce both zeaxanthin and nostoxanthin (Table 1, Figs. 1, 7). First, the C-3 and C-3' hydroxyl groups of zeaxanthin are introduced by CrtR (β-carotene hydroxylase); then, the C-2 and C-2' hydroxyl groups of nostoxanthin are introduced by a CrtG-like enzyme. The activities of CrtR from two species have been confirmed (Table 2). CrtR from *Synechocystis* sp. PCC 6803 catalyzes β-carotene to zeaxanthin via β-cryptoxanthin, echinenone to 3'-hydroxyechinenone and deoxymyxol 2'-dimethyl-fucoside to myxol 2'-dimethyl-fucoside [60, 68]. The activity of CrtR from *Anabaena* sp. PCC 7120 for deoxymyxol 2'-fucoside

Table 3. Distribution of functionally confirmed two β-carotene ketolases, CrtO and CrtW, in cyanobacteria.

Species	β-Carotene to echinenone and canthaxanthin[a]	Myxol to 4-ketomyxol[a]	References
Anabaena sp. PCC 7120	CrtO	CrtW	[61]
Synechocystis sp. PCC 6803	CrtO	–	[71]
Nostoc punctiforme	CrtW38[b]	CrtW148[c]	[62]
Gloeobacter violaceus	CrtO and CrtW	–	[34, 41]

[a] See Figure 1.
[b] Gene number: NpF5919.
[c] Gene number: NpF4798.

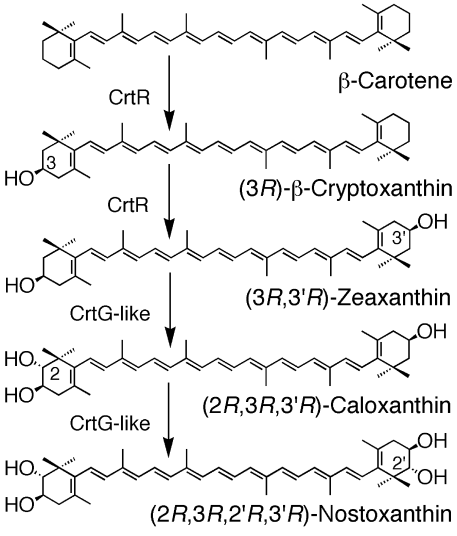

Figure 7. Synthesis of zeaxanthin and nostoxanthin from β-carotene. See the text for precise explanations.

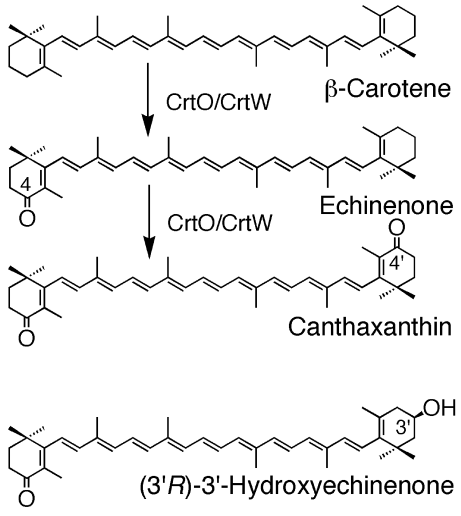

Figure 8. Synthesis of echinenone and canthaxanthin from β-carotene. 3′-Hydroxyechinenone is synthesized by CrtO/CrtW and CrtR via echinenone or β-cryptoxanthin. See the text for precise explanations.

is high, while that for β-carotene is low, judging from the small amount of zeaxanthin present (Table 1). Consequently, deoxymyxol is a good substrate for CrtR in *Anabaena* species, *N. punctiforme*, *Synechocystis* sp. PCC 6803 and *T. elongatus*, while β-carotene may or may not be a substrate, depending on the characteristics of CrtR in these species. On the other hand, β-carotene is a good substrate for CrtR in *Synechococcus* species due to presence of zeaxanthin. Since CrtR in plants and CrtZ in bacteria are known to catalyze β-carotene to zeaxanthin, further functional comparisons are still needed for these genes.

T. elongatus and three *Synechococcus* species contain caloxanthin and nostoxanthin (Table 1), which have additional hydroxyl group(s) at C-2 of the β end group (Fig. 7). CrtG (2,2'-β-hydroxylase) has been found to catalyze this reaction from three species of *Brevundimonas* [69, 70], and homologous genes have been found in these cyanobacteria (see Table 4). A functional analysis has yet to be performed for these genes. Echinenone is one of the major carotenoids in some cyanobacteria, while canthaxanthin is usually among the minor ones (Table 1). For introduction of the keto group, CrtO or CrtW is catalyzed as described below. These cyanobacteria in Table 1 seem to have similar carotenogenesis pathways (Fig. 1). The differences in the composition of the carotenoids among these species might be due to the presence or absence of the gene(s) and/or due to the different characteristics of the enzyme(s).

Synthesis of ketocarotenoids: echinenone and 4-ketomyxol

At present, two distinct β-carotene ketolases, CrtO and CrtW, have been found, and only seven β-carotene ketolases have been functionally confirmed in four species of cyanobacteria (Tables 2, 3). In *Synechocystis* sp. PCC 6803, CrtO catalyzes β-carotene to echinenone [71], and 4-ketomyxol 2'-glycoside is absent [8] (Table 1). *Anabaena* sp. PCC 7120 has two functional enzymes, that is, CrtO catalyzes β-carotene to echinenone and CrtW catalyzes myxol 2'-fucoside to 4-ketomyxol 2'-fucoside [61]. *N. punctiforme* has two CrtW-type β-carotene ketolases, CrtW38 and CrtW148 [62], and both echinenone and 4-ketomyxol 2'-fucoside are present [33]. Although their functions in the cells were not reported [62], based on their substrate specificity CrtW38 might catalyze β-carotene to echinenone and CrtW148 might catalyze

Table 4. Proposed carotenogenesis genes in some cyanobacteria.

Gene or enzyme	*Anabaena* sp. PCC 7120[a]	*Anabaena variabilis* ATCC 29413[b]	*Nostoc punctiforme* PCC 73102[c]	*Synechocystis* sp. PCC 6803[a]	*Thermosynechococcus elongatus* BP-1[a]	*Gloeobacter violaceus* PCC 7421[a]	*Synechococcus* sp. PCC 6301[d]	*Synechococcus elongatus* PCC 7942[e]	*Prochlorococcus marinus* MED4[a]
crtE[f]	*alr0213*	*Ava2704*	*NpF3770*	*slr0739*	*tll0020*	*gll0416*	*YP_171470*	*Syn_pcc79420776*	*PMM1070*
crtB[g]	*alr1833*	*Ava4794*	*NpR2771*	*slr1255*	*tll1560*	*glr1744*	*YP_172822*	*Syn_pcc79421984*	*PMM0143*
crtP[h]	*alr1832*	*Ava4795*	*NpR2772*	*slr1254*	*tll1561*	–	*YP_172823*	*Syn_pcc79421983*	*PMM0144*
crtQa[i] *crtQb*[j]	*all7255* (*alr2382*)	– *Ava0200*	– *Npr0498*	– *slr0940*	– *tll0337*	– –	– *YP_073207*	– *Syn_pcc79421512*	– *PMM0115*
crtH[k]	*alr2064*	*Ava3112*	*NpR4225*	*sll0033*	–	(*gll2133*)	*YP_171014*	*Syn_pcc79421246*	*PMM1155*
crtI[l]	–	–	–	–	–	*glr0867*	–	–	–
crtL-b[m] *crtL-e*[n] *cruA*[o]	– – (*alr3524*)	– – *Ava3214*	– – *NpR4002*	– – (*sll0147*)	– – *tlr1139*	– – (*gll3598*)	*YP_172741* (*YP_172741*) –	*Syn_pcc79422062* (*Syn_pcc79422062*) –	*PMM1064* *PMM0633* –
crtR[p]	*alr4009*	*Ava1693*	*NpR4276*	*sll1468*	*tlr1900*	–	*YP_172377*	*Syn_pcc79422439*	–
crtG[q]	–	–	–	(*slr0224*)	*tlr1917*	–	*YP_171559*	*Syn_pcc79420680*	–
crtO[r]	*all3744*	*Ava1581*	(*NpF3745*)	*slr0088*	–	*gll0394*	–	–	–
crtW[s]	*alr3189*	*Ava3888*	*NpF4798*, *NpF5919*	–	–	*gll1728*	–	–	–
GDP-fucose synthase[t]	*all4826*	*Ava2096*	*NpF3486*	*sll1213*	*tll0633*	*glr3792*	*YP_173100*	*Syn_pcc79421700*	*PMM1207*
Apo-carotenoid oxygenase[u]	*all4284*	*Ava1236*	*NpF0298*	*sll1541*	*tll0015*	*gll3689*	*YP_172025*	*Syn_pcc79420196*	*PMM0280*

Genes which show the highest and more than 30 % homology are listed. The functionally confirmed genes (Table 2) are underlined. Among them, the genes in parentheses have high homology, but experimentally they show no effects on carotenoid composition [unpublished observations] or they should have no functions in carotenogenesis due to absence of corresponding carotenoids. A BLAST search was performed in the following databases: [a]Cyanobase (http://bacteria.kazusa.or.jp/cyanobase/), [b]ver. 26jun05 of ORNL (http://genome.ornl.gov/microbial/avar/), [c]ver. 22dec03 of ORNL (http://genome.ornl.gov/microbial/npun/), [d]genomic database of NCBI (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi) and [e]ver. 21jun05 of ORNL (http://genome.ornl.gov/microbial/syn_PCC7942/). Genes whose functions have already been confirmed are chosen for the query sequences as follows: [f]*tll0020*, [g]*slr1255*, [h]*slr1254*, [i]*all7255*, [j]*slr0940*, [k]*sll0033*, [l]*glr0867*, [m]*Syn_pcc79422062*, [n]*PMM0633*, [o]*cruA* in *Chlorobaculum tepidum* (annotated *CT0456* in Cyanobase), [p]*sll1468*, [q]*crtG* in *Brevundimonas* sp. SD212 (accession no. AB181388 in GenBank), [r]*all3744*, [s]*alr3189*, [t]*all4826* and [u]*sll1541*.

myxol 2'-fucoside to 4-ketomyxol 2'-fucoside [33]. In, *G. violaceus*, both CrtO and CrtW function to catalyze β-carotene to echinenone, and (2*S*,2'*S*)-oscillol 2,2'-di(α-L-fucoside) is present but myxol 2'-glycoside is absent [34, 41]. It is not known whether both or either gene have functions in the cells. In total in cyanobacteria, the reaction from myxol 2'-glycoside to 4-ketomyxol 2'-glycoside is catalyzed by CrtW in two species, while that from β-carotene to echinenone is catalyzed by CrtO in three species and by CrtW in two species. The composition of the products echinenone and canthaxanthin might depend on the characteristics of the enzyme. It would be interesting to determine just how the cyanobacteria obtain both or either β-carotene ketolase, and how they make proper use of them.

Two distinct β-carotene ketolases are widely distributed in bacteria and green algae, as well as in cyanobacteria. CrtO and CrtW, whose functions have been confirmed, have been found in two bacterial species, and in five bacterial and two algal species, respectively [69, 72, 73]. Even though the reactions of both CrtO and CrtW involve the same β-carotene ketolation, the characteristics of the enzymes are different. The CrtO enzymes are almost twice the size of the CrtW enzymes and do not share significant amino acid sequence homology with CrtW. The substrate specificities of CrtO are only the β-end group (β-carotene and γ-carotene), while those of CrtW are the β-end group (β-carotene) and 3-hydroxy-β-end group (zeaxanthin and myxol). CrtO has six conserved regions, including the FAD-binding motif [73], while CrtW has iron-binding motifs [62]. Two β-carotene ketolases might have evolved convergently from different ancestors to acquire the same functions, although further studies are needed to confirm this [61].

Carotenogenesis genes

Table 2 summarizes the functionally confirmed carotenogenesis genes in cyanobacteria; their homologous genes, whose query sequences are chosen only from these functional genes, are summarized in Table 4. The *crtE* and *crtB* genes have high sequence similarity from bacteria to plants, respectively.

Only three CrtQs among prokaryotes have been functionally confirmed: *Anabaena* sp. PCC 7120 (CrtQa, *crtI*-like sequence) [48], *Synechocystis* sp. PCC 6803 (CrtQb, plant *crtQ*-like) [49] and, *C. tepidum* (CrtQb) [52] (Table 2). CrtQa has sequence homology with CrtI and CrtH, and CrtQb with CrtP. Therefore, functional CrtQa is only found from *Anabaena* sp. PCC 7120 among the carotenogenesis organisms. Further, *Anabaena* sp. PCC 7120 possesses a homologous gene (*alr2382*) to *crtQb* of *Synechocystis* sp. PCC 6803 (Table 4), but it seems not to have a function [unpublished observation]. Therefore, it would be interesting to determine the functions of the plant *crtQb*-like gene from *Anabaena* sp. PCC 7120, and the distribution of the two *crtQ* genes among cyanobacteria.

Concerning lycopene cyclase, *Synechococcus* and *Prochlorococcus* contain functional CrtL [23, 56], while the other cyanobacteria in Table 1 possess no *crtL*-like genes (Table 4). Instead, they possess *cruA*-like genes, which seem to have no lycopene cyclase activity in cyanobacteria, as described above. We still do not know which is the actual lycopene cyclase gene(s). Moreover, these *Synechococcus* and *Prochlorococcus* contain no ketocarotenoids, and the *crtO*-like and *crtW*-like genes are absent, although they do contain nostoxanthin, and *crtG*-like genes are present. Further, they contain no carotenoid glycosides.

A sensory rhodopsin has been found in *Anabaena* sp. PCC 7120 [74]. Retinal is known to be formed from apo-carotenoids in *Synechocystis* sp. PCC 6803 [75] and *Anabaena* sp. PCC 7120 [76] by an apo-carotenoid oxygenase. Since the substrates of apo-carotenoids have yet to be found in these cyanobacteria [8, 33], they might be produced from carotenoids by carotenoid oxygenase, which likewise has yet to be found.

In conclusion, we have discussed the carotenoids, carotenogenesis pathways, and characteristics of carotenogenesis enzymes and genes in some cyanobacteria, whose carotenogenesis enzymes have been functionally confirmed. In these cyanobacteria, various carotenoids have been identified, including the unique ketocarotenoids, echinenone and 4-ketomyxol, and the carotenoid glycosides, myxol glycosides and oscillol diglycosides. We propose certain carotenogenesis pathways. The different compositions of carotenoids among these species might be due to the presence or absence of certain gene(s), or to different enzyme characteristics, which are found, for instance, in β-carotene ketolases, CrtO and CrtW, and in β-carotene hydroxylase, CrtR. At present, some genes have not been identified, the functionally confirmed genes have been found in only a few species, and further studies are needed.

Acknowledgments. We thank Dr H. Masukawa for his help in producing *Anabaena* sp. PCC 7120 mutants, Dr T. Maoka for the analysis of myxol 2'-glycosides by NMR, and Dr W. F., J. Vermaas for the gift of the GDP-fucose synthase mutant of *Synechocystis* sp. PCC 6803. This work was supported in part by Grants-in-Aid for Science Research from JSPS to S.T. (no. 16570038).

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