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Roles of xanthophyll carotenoids in protection against photoinhibition and oxidative stress in the cyanobacterium *Synechococcus* sp. strain PCC 7002

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ABSTRACT

Synechococcus sp. strain PCC 7002 is a robust, genetically tractable cyanobacterium that produces six different xanthophyll carotenoids (zeaxanthin, cryptoxanthin, myxoxanthophyll (myxol-2′-fucoside), echinenone, 3′-hydroxyechinenone, and synechoxanthin) and tolerates many environmental stresses, including high light intensities. Targeted mutations were introduced to block the branches of the carotenoid biosynthetic pathway leading to specific xanthophylls, and a mutant lacking all xanthophylls was constructed. Some of the mutants showed severe growth defects at high light intensities, and multi-locus mutants had somewhat lower chlorophyll contents and lower photosystem I levels. The results suggested that xanthophylls, particularly zeaxanthin and echinenone, might play regulatory roles in thylakoid biogenesis. Measurements of reactive oxygen (ROS) and nitrogen (RNS) species in the mutants showed that all xanthophylls participate in preventing ROS/RNS accumulation and that a mutant lacking all xanthophylls accumulated very high levels of ROS/RNS. Results from transcription profiling showed that mRNA levels for most genes encoding the enzymes of carotenogenesis are significantly more abundant after exposure to high light. These studies indicated that all xanthophylls contribute to protection against photo-oxidative stress.

Introduction

Carotenoids are principally C-40 isoprenoid pigments that have ⩾9 conjugated double bonds and possess colors ranging from light yellow to orange-red. Although there are two biosynthetic pathways in nature that can produce the precursors for their synthesis, the methyl-erythritol-phosphate and mevalonic acid pathways, a wide range of carotenoids are produced from the universal precursor molecules isopentenyl pyrophosphate and dimethylallyl diphosphate [1–6]. Carotenoids play crucially important physiological and structural roles in cyanobacteria and other chlorophototrophic organisms, in which they function in both light harvesting and photoprotection [7–9]. In particular, carotenoids help to prevent the production of singlet oxygen, a potent oxidant, by quenching chlorophyll (Chl)[2] triplet states and dissipating the energy as heat [10,11]. Considering the broad distribution of carotenoids in both phototrophic and non-phototrophic organisms, and considering their enormous chemical diversity—more than 700 carotenoids have been described—the importance of carotenoids in biology cannot be overstated. Knowledge of carotenogenesis pathways has increased rapidly in recent years because of rapid advances in genome sequencing and comparative bioinformatics [6,9,12,13].

Xanthophylls, *i.e.*, oxygenated carotenoids, are abundant pigments found in oxygenic chlorophototrophs (*i.e.*, cyanobacteria, algae, and plants), in which their synthesis is often up-regulated in response to high light intensity, ultraviolet radiation, and desiccation [14–17]. In plants and green algae, xanthophyll carotenoids play critical roles in non-photochemical quenching [18–20]. Xanthophylls are also widely, albeit irregularly, distributed across a wide spectrum of non-phototrophic organisms found in the domains *Bacteria* and *Archaea*. With the exception of the acyclic carotenoid, oscillaxanthin, all of the major xanthophylls of cyanobacteria are at least partly formed by oxygenation of one or more cyclic end groups [9,21–23]. In particular, β-rings are the target of numerous enzymes that introduce oxygen to form a variety of xanthophylls with hydroxyl, keto, and epoxide moieties, although the latter appear to be specifically absent from cyanobacterial carotenoids [24]. Carotenoids with a single β-ring are the precursors of myxoxanthophyll and its derivatives; xanthophylls with two β-rings include cryptoxanthin, zeaxanthin, echinenone, canthaxanthin, 3′-hydroxyechinenone, nostoxanthin, and caloxanthin, among others [9]. It should also be

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[2] *Abbreviations used:* CM-H2DCFDA, 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetyl ester; Chl, chlorophyll; OCP, orange carotenoid protein; $OD_{730\ nm}$, optical density at 730 nm; ROS, reactive oxygen species; *Synechococcus* 7002, *Synechococcus* sp. strain PCC 7002; *Synechocystis* 6803, *Synechocystis* sp. strain PCC 6803.

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noted that carotenoids with ε- and ϕ-end groups can also be hydroxylated at the C(3) position [25–27]. Synechoxanthin (18,18′-χ,χ-caroten-dioic acid), a unique aromatic dicarboxylate carotenoid that is synthesized from β-carotene, was recently discovered in some cyanobacteria [9,28,29].

Synechococcus sp. PCC 7002 (hereafter *Synechococcus* 7002) is a unicellular, euryhaline cyanobacterium that naturally synthesizes β-carotene and six xanthophyll carotenoids: zeaxanthin, cryptoxanthin, myxoxanthophyll (myxol-2′-fucoside), echinenone, 3′-hydroxyechinenone, and synechoxanthin [9,28–32]. In addition to possessing a wide range of xanthophylls, this organism tolerates extremely high light intensity and is tolerant to many other environmental stressors, making it an ideal system for stepwise and combinatorial inactivation of carotenoid biosynthetic genes that would otherwise be difficult in a less robust cyanobacterium [33]. The complete carotenogenesis pathway for *Synechococcus* 7002 was recently deduced, and nearly all of the genes encoding enzymes of this multi-branched pathway are now known (see Fig. 1)[9,28–32,34]. Importantly, all key branch-point enzymes in this pathway have been identified.

In the studies reported here, the physiological functions of mutants unable to synthesize specific xanthophyll carotenoids of *Synechococcus* 7002 were examined by creating a collection of strains in which genes encoding enzymes for the synthesis of specific xanthophylls were specifically mutated. Additionally, these mutations were combined to produce strains that could only synthesize a single type of xanthophyll or no xanthophylls at all. Results from transcription profiling experiments that describe the mRNA abundances for genes encoding the enzymes of carotenogenesis in cells grown under a wide variety of conditions are also reported. The results demonstrate that xanthophyll carotenoids play important and additive roles in protecting cells from oxidative stress. A xanthophyll-free mutant experienced very severe oxidative stress from elevated levels of ROS/RNS produced endogenously when the cells were exposed to high light intensity.

Materials and methods

Growth conditions of cyanobacteria

The wild-type and mutant strains of *Synechococcus* 7002 were grown in medium A supplemented with 1 mg $NaNO_3$ ml^{-1} (medium A^+) [35]. Cells were grown in liquid and on agar plates (1.5% (w/v)) as described [36]. Standard growth conditions are defined as follows: medium A^+ (supplemented with antibiotics if required), 38 °C, saturating cool-white, fluorescent light intensity ≈250 μmol photons m^{-2} s^{-1}, sparging with 1% (v/v) CO_2 in air for liquid cultures (air for plates). Adjustment of the growth light intensity was achieved by addition of extra light sources (for high light intensity conditions) or by shielding the lights with paper to produce reduced light intensity conditions as required. Because of their sensitivity to high light, some mutants were maintained on stock plates under reduced light conditions. Antibiotic(s) were employed for selection and maintenance of mutant cultures at the following concentrations: kanamycin (100 μg ml^{-1}); spectinomycin (100 μg ml^{-1});

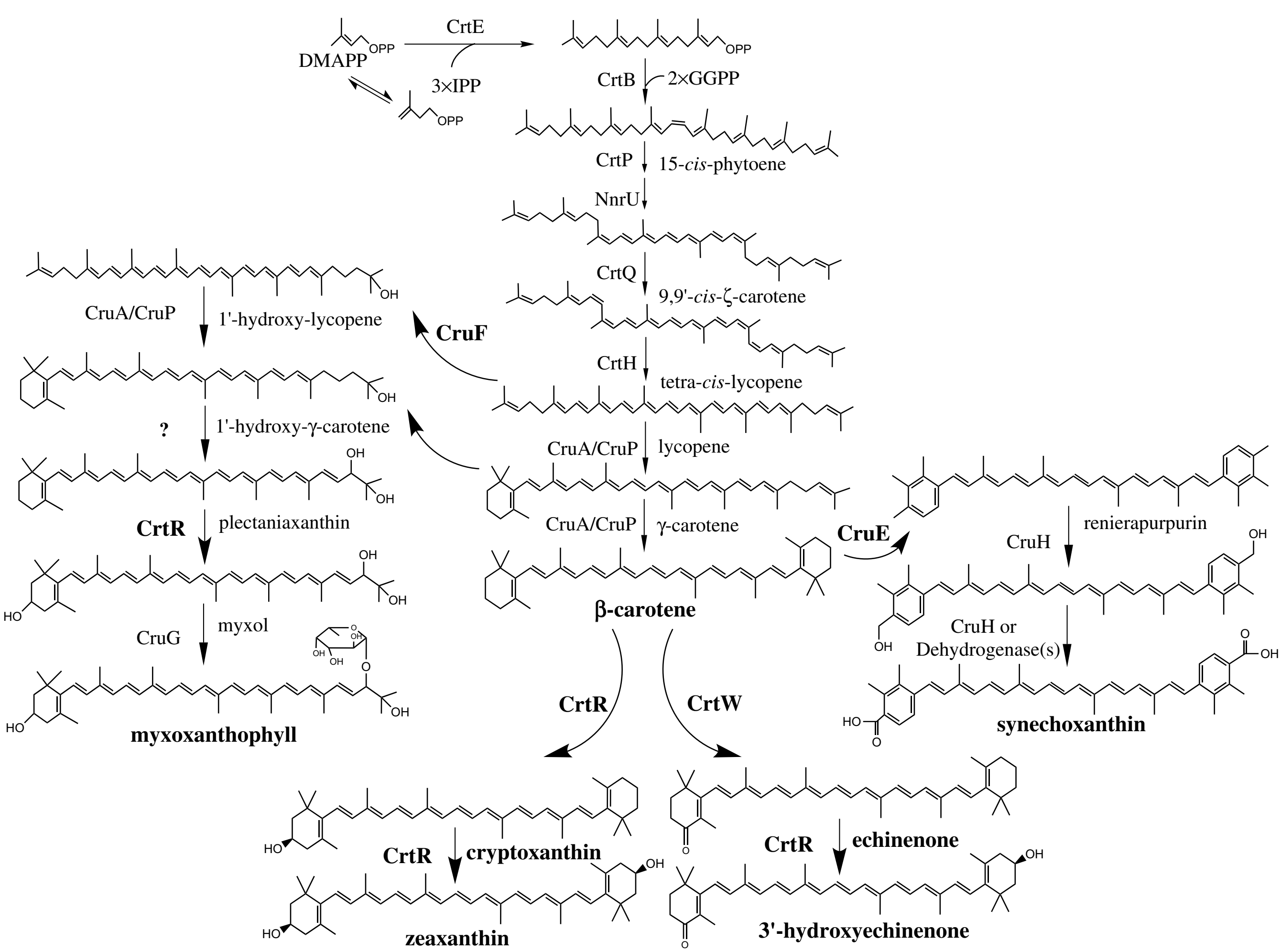

Fig. 1. Scheme showing the biosynthesis of carotenoids in *Synechococcus* 7002.