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Microalgae as a Source of Lutein: Chemistry, Biosynthesis, and Carotenogenesis

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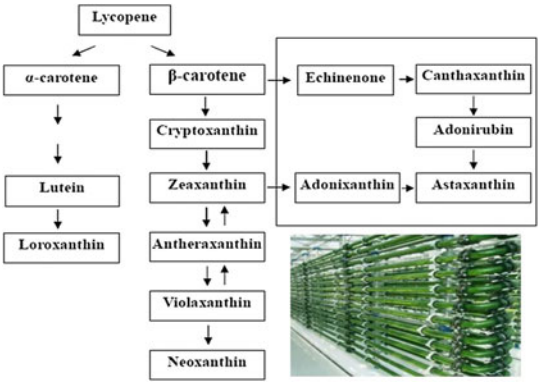
Abstract Microalgae represent a sustainable source of natural products, and over 15,000 novel compounds originated from algal biomass have been identified. This chapter focuses on algae-derived lutein, a group of high-value products. Lutein belongs to carotenoids which have extensive applications in feed, food, nutraceutical, and pharmaceutical industries. The production of carotenoids has been one of the most successful activities in microalgal biotechnology. This chapter gives a mini review of microalgae-based lutein, where emphasis is placed on the biosynthetic pathway and the regulation of carotenogenesis.

Graphical Abstract



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Keywords Biosynthesis · Carotenogenesis · Health effects · Mass cultivation

Contents

1 Introduction

Carotenoids are a group of organic pigments synthesized de novo in higher plants and some other photosynthetic organisms including algae, some types of fungus, and bacteria. They are widely distributed in nature and responsible for a broad variety of colors, such as brilliant yellow, orange, and red of fruits, leaves, and aquatic animals [1]. Thus far, over 700 carotenoids have been identified [2] in which lutein has achieved noticeable attention in both academic and industry research areas. As a natural colorant, lutein has been widely applied in the food industry, especially after the notorious "Sudan red" event [3]. It is also used for coloring fish and poultry, drugs, and cosmetics. In addition, lutein and zeaxanthin are the only carotenoids reported to be present in the eye retina and lens [4]. As these organs are susceptible to oxidant damage, lutein exerts antioxidant action to protect the eyes. These important properties make lutein a highly valuable chemical. According to the report from financial organizations, the global market of lutein is expected to grow to $309 million by 2018 with a compound annual growth rate of 3.6 % (http://www.companiesandmarkets.com/Market/Food-and-Drink/Market-Research/The-Global-Market-for-Carotenoids/RPT988273).

Microalgae are a group of small photosynthetic organisms commonly found in aquatic environments. A number of microalgal species accumulate high contents of carotenoids (including lutein) as a part of their biomass and therefore represent an outstanding natural source of carotenoids. Production of lutein from microalgae is widely studied, for example, *Chlorella protothecoides* [5, 6], *Dunaliella salina* [7], *Scenedesmus almeriensis* [8], and *Galdieria sulphuraria* [9], which has been one of the most successful activities in microalgal biotechnology.

2 Structure

Carotenoids are of isoprenoid origin derived from a 40-carbon polyene chain backbone structure with cyclic groups [10]. There are two types of carotenoids: the hydrocarbon ones are known as carotenes (e.g., α-carotene, β-carotene, and lycopene), and the oxygenated derivatives are named xanthophylls. In the latter, oxygen may be present as OH groups (e.g., lutein), as oxo-groups (e.g., canthaxanthin), or in a combination of both (e.g., astaxanthin). Elements other than carbon, hydrogen, and oxygen are not directly attached to the carbon skeleton in naturally occurring carotenoids [11]. The molecular formula of lutein is $C_{40}H_{52}O_2$, as shown in Fig. 1. The most typical feature of lutein as well as other carotenoids is the long polyene chain. Such a conjugated double-bond system determines the photochemical properties and chemical reactivity that give the basic biological functions of carotenoids, such as antioxidant activities [12].

3 Bioactivities and Impact on Health

The most notable bioactivity of lutein is its antioxidant property. Lutein could be rapidly oxidized by a series of oxidants, which greatly reduces the availability of free radicals to react with other cellular components, such as unsaturated lipids, protein and DNA [13]. Similar to other carotenoid members, the antioxidative power of lutein is attributed to its conjugated carbon double-bond system. This structure allows the quenching of singlet oxygen and scavenging of free radicals. The singlet oxygen-quenching activity includes physical and chemical quenching,

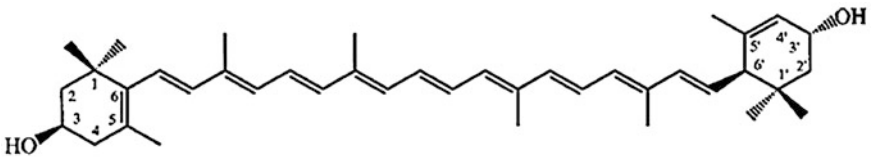

Fig. 1 Structure of lutein

Table 1 Scavenging capacity of carotenoids

Carotenoids	Scavenging capacity[a]			
	$ROO^{\bullet}$	HOCl	$ONOO^{-}$	$HO^{\bullet}$
lutein	**0.6**	**0.97**	**4.81**	**0.78**
zeaxanthin	0.56	1.41	3.87	0.77
β-carotene	0.14	0.71	NA[b]	1.02
lycopene	0.08	0.35	0.4	0.31
fucoxanthin	0.43	1.18	6.26	NA
canthaxanthin	0.04	0.28	0.1	NA
astaxanthin	0.64	1.66	9.4	0.73
α-tocopherol	0.48	1.77	NA	0.37
quercetin	0.84	1.42	5.63	0.97
trolox	1.00	1.00	NA	NA
ascorbic acid	NA	NA	0.41	1.00
cysteine	0.04	NA	1.00	0.02

Peroxyl radical ($ROO^{\bullet}$), hydroxyl radical ($HO^{\bullet}$), hypochlorous acid (HOCl), and peroxynitrite anion ($ONOO^{-}$)

Source Adapted from Ref. [14]

[a]The scavenging capacity was calculated considering as reference: trolox for $ROO^{\bullet}$ and $HO^{\bullet}$, cysteine for HOCl, and ascorbic acid for $ONOO^{-}$

[b]NA: no activity was found within the tested concentrations

both of which require very close interaction between lutein and singlet oxygen. The energy transfer reaction absorbs the excited energy of singlet oxygen onto the carotenoid chain, which results in the formation of the carotenoid triplet state. This triplet state can readily return to the stable ground state by releasing the excess energy of singlet oxygen as heat. Rodrigues et al. [14] conducted a membrane-mimicking system to evaluate the scavenging activity of carotenoids against various reactive species based on the fluorescence loss of a fluorescent lipid ($C11\text{-}BODIPY^{581/591}$). The results are shown in Table 1.

The antioxidative properties make lutein a class of important nutrients in health promotion, and there has been ever-increasing evidence supporting their protective effects in preventing or delaying chronic diseases (Table 2). Previous studies indicated that lutein inhibited the oxidation of low-density lipoprotein (LDL), which suppressed the progression of cardiovascular diseases [15, 16]. Dwyer et al. [17] reported that the dietary intake of lutein effectively prevented the occurrence of early atherosclerosis. Seddon et al. [18] reported that there was a direct relationship between carotenoid intake and a decreased risk of age-related macular degeneration (AMD). Among those selected carotenoids, lutein and zeaxanthin were found to exhibit the strongest protective effects. Lutein is also well known to ameliorate the onset or progression of cataracts [19]. Oxidative damage to lens cell membranes is considered to be mainly responsible for the initiation and aggravation of age-related cataracts [20]. Recently, our studies have revealed a novel physiological function of lutein: the antiglycoxidative effect. Glycoxidation, also known as the Maillard

Table 2 Health benefits of lutein

Therapeutic indications	References	Model
AMD[a]	[24, 25]	EIU[b] in Lewis rats CNV[c] in C57BL/6 J mice induced by laser photocoagulation and b-End3, RAW264.7, and ARPE-19 cell lines
Atherosclerosis	[26, 27]	
Retinal neural damage	[28, 29]	
Ultraviolet radiation	[30, 31]	

[a]AMD, age-related macular degeneration
[b]EIU, endotoxin-induced uveitis
[c]CNV, choroidal neovascularization

reaction, is a process in which reducing sugars react spontaneously with amino acids and amino groups of proteins. This reaction is a key factor contributing to the pathogenesis of diabetic complications [21]. We evaluated over 20 microalgal species and found that the extract of several *Chlorella* exhibited a potent antiglycoxidative effect, where lutein was identified as a major effective ingredient [22]. In cultured human-derived retinal pigment epithelial ARPE-19 cells, lutein inhibited the formation of endogenous advanced glycation end products (AGEs) through the suppression of intracellular oxidative stress, for example, nitric oxide, reactive oxygen species (ROS), and lipid peroxidation [23]. These results strongly suggested the significant potential of lutein in the management of diabetic retinopathy.

4 Distribution

Lutein is widely distributed among fruits, vegetables, and flowers, in which marigold flowers are by far the most abundant natural source of commercial lutein. However, a large amount of lutein present in marigold flowers is esterified with half of the weight corresponding to fatty acids [32], and therefore, chemical saponification is necessary in the manufacture of lutein products. In addition, the production of lutein from marigold is also limited by seasons, planting area, and the high manpower cost [33].

Microalgae have long been regarded as an excellent alternative to conventional plant sources as algae-derived lutein is in the free nonesterified form. Green algae *Chlorella* are good examples: *Chlorella pyrenoidosa* can synthesize lutein and other xanthophylls under heterotrophic cultivation using glucose as the carbon source [34]; a high content of lutein was produced by *C. protothecoides* grown in the nitrogen-limited fed-batch culture [5]; and in *Chlorella zofingiensis*, astaxanthin and lutein were biosynthesized in divergent pathways using the common lycopene precursor [35]. Several species of marine microalgae such as *Dunaliella bardawil* and *D. salina*, which have been commonly applied in the production of β-carotene

Table 3 Distribution of lutein in some representative microalgae

Microalgae	Quantity (mg g^{-1})	Reference
Chlorella protothecoides	4.6	[6]
Chlorella protothecoides	5.4	[5]
Dunaliella salina	6.6 ± 0.9	[7]
Scenedesmus almeriensis	5.3	[8]
Galdieria sulphuraria	0.4 ± 0.1	[9]

and phytoene, were also reported to accumulate lutein under specified conditions [36, 37]. Distribution of lutein in some representative algae is summarized in Table 3.

5 Mode of Cultivation

5.1 Photoautotrophic Cultivation

There are two major cultivation modes for microalgae: photoautotrophic and heterotrophic cultures (if a strain is able to grow heterotrophically, a mixotrophic mode may also be developed). Due to the presence of chloroplast, most microalgae grow photoautotrophically through photosynthesis. The idea of photoautotrophic cultivation was first proposed in Germany in the early 1940s, in which CO_2 and light energy are used as the carbon and energy sources, respectively. In the 1990s, Australian researchers proposed the method of "extensive cultivation," which is easy to operate and economical [38]. It consists of large open ponds without CO_2 addition and with minimal control. Facilities are usually located in places where solar irradiance is maximal, cloudiness is minimal, and the climate is warm. The photoautotrophic mode has been commonly employed in the culture of *Dunaliella* for β-carotene production, because this alga can survive under extreme conditions (e.g., high salinity and high temperature) and its cultivation is easily maintained. To reduce the contamination of plankton, hypersaline water is usually used. A two-step approach, namely "intensive cultivation" has also been employed for the large-scale production of *Dunaliella* β-carotene. The aim of stage 1 is to optimize biomass production of cells with a low β-carotene-to-chlorophyll ratio; and in stage 2, the culture is diluted to about one-third to increase the light availability to cells, and carotenogenesis is further enhanced by nitrogen deficiency [39].

Although photoautotrophic cultivation is easily maintained, a high growth rate is barely achieved due to the hostile or unsteady environment. In addition, both light intensity and photosynthesis efficiency are low inside the cultures because of the absorption and scattering of light [40]. As a result, algal growth is largely limited by such inhomogeneous distribution of light. For example, the cell mass production of *Chlamydomonas reinhardtii* only reached 0.57 g cells L^{-1} under photoautotrophic mode with optimized culture conditions [41]. The biomass of *C. zofingiensis*

cultivated photoautotrophically at 68 μmol m^{-2} s^{-1} was confined to 0.72 g L^{-1} [42]. To solve the problems, another culture system, heterotrophic cultivation, has been developed.

5.2 Heterotrophic Cultivation

Heterotrophic cultivation refers to the organism's use of organic carbon substances as carbon and energy sources rather than CO_2 and light to support the cell growth and other physiological events [43, 44]. Due to the elimination of light restriction, cells grow quickly and a high cell mass production can be achieved easily. Thus far, the maximum biomass content achieved in photoautotrophic cultures is 40 g L^{-1} of cell dry weight [45], much lower than that in heterotrophic cultures, where 100 g L^{-1} to greater than 150 g L^{-1} is available [46].

Given the high costs of heterotrophic cultivation, this mode is generally used for the production of value-added compounds from microalgae, such as lutein and astaxanthin (Table 4). When *C. protothecoides* was heterotrophically cultivated, the algal cell dry weight and lutein yield reached 19.6 g L^{-1} and 83.8 mg L^{-1}, respectively, in the fermentor, and even achieved as high as 46.9 g L^{-1} and 225.3 mg L^{-1} using the fed-batch culture strategy [5, 6]. Wu and Shi [51] conducted a heterotrophic cultivation using another *Chlorella* species, *C. pyrenoidosa*; with the artificial neural network (ANN) model employed as the optimization strategy, the highest biomass concentration and the maximum productivity achieved 104.9 g L^{-1} dry cell weight and 0.613 g L^{-1} h^{-1}, respectively. The high cell density can be achieved by the employment of fed-batch, continuous, and perfusion culture strategies that are commonly used in the fermentation of bacteria or yeasts. Fed-batch culture is an effective way to minimize substrate inhibition because a high initial concentration of substrates (e.g., sugars) may lead to growth inhibition. On the other hand, the fed-batch culture cannot overcome the inhibition caused by the toxic metabolites generated during the algal culture. They may accumulate as the cells build up and prevent further enhancement of cell density. Such metabolite-driven inhibition can be eliminated by continuous or semicontinuous cultivation systems, in which the fresh medium is continuously added to a well-mixed culture, while cells or products are simultaneously removed to keep a constant volume. Perfusion culture is a technique combining the advantages of fed-batch and continuous culture systems. Cells are physically retained by a retention device, whereas the spent medium is removed. Meanwhile, fresh medium is fed into the bioreactor to maintain a sufficient nutrient supply. Park et al. [52] established a two-stage mixotrophic culture system for *Haematococcus pluvialis*. By the perfusion process, the biomass density achieved was 3.09 and 1.67 times higher than batch and fed-batch processes, respectively. Zhang et al. [53] established an "attached cultivation" using the immobilized biofilm. Under the optimized condition, the maximum astaxanthin productivity reached 160 mg m^{-2} d^{-1}.

Table 4 Heterotrophic cultivation of microalgae for the production of lutein and astaxanthin

Carotenoids	Strain	Device	Culture mode	Cell concentration ($g\ L^{-1}$)	Productivity ($mg\ L^{-1}\ d^{-1}$)	Yield ($mg\ L^{-1}$)	Reference
Astaxanthin	*Chlorella zofingiensis*	Flask	Batch	10.2	0.7	10.3	[47]
	Chlorella zofingiensis	Flask	Batch	7.5	1.3	11.8	[48]
	Chlorella zofingiensis	Flask	Batch	7.3	1.4	12.6	[49]
	Haematococcus pluvialis	Flask	Batch	NA	0.8	9.0	[50]
Lutein	*Chlorella protothecoides*	Fermentor	Batch	19.6	11.3	83.8	[6]
	Chlorella protothecoides	Fermentor	Fed-batch	46.9	22.7	225.3	[5]

Source Adapted from Ref. [46]

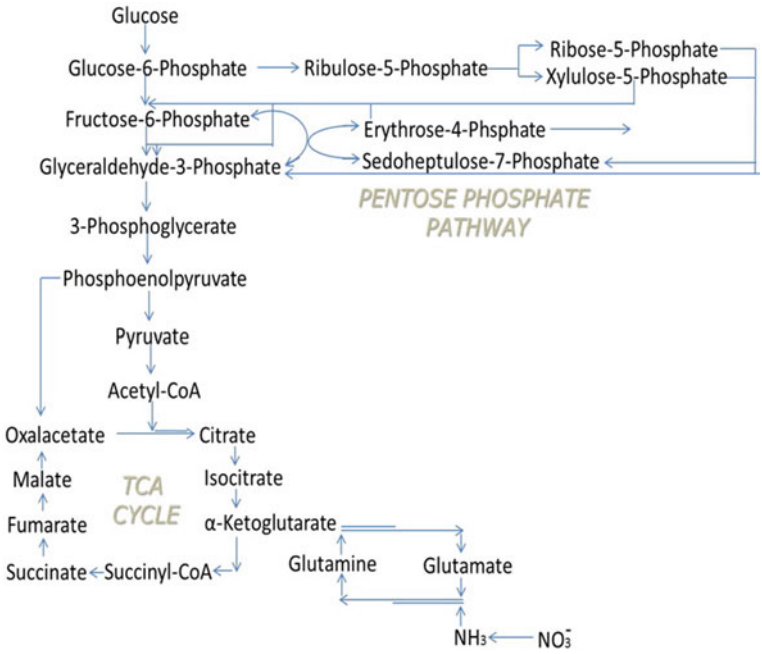

Fig. 2 Heterotrophic metabolism in microalgae (modified from Ref. [55])

The known carbon sources include glucose, glycerol, acetate, fructose, ethanol, and some low-cost media formulations with molasses or carob pulp syrup, as well as wastewater from the sugar or milk processing industries [54]. Among them, glucose is the most superior candidate, stimulating much higher growth rates of microalgae than other carbon sources. Through the comparison between heterotrophic and photoautotrophic modes, it was found that the ATP generated from the former using glucose as the energy supplier exceeded more than 600 % over the latter in which energy was supplied by light [55]. As shown in Fig. 2, in contrast to light conditions where the Embden–Meyerhof–Parnas (EMP) pathway acts as the predominant glycolysis, under darkness, glucose is mainly metabolized via the pentose phosphate (PP) pathway [56]. For example, in alga *Chlorella pyrenoidosa*, the PP pathway accounts for over 90 % of glucose metabolic flux distribution [55].

Heterotrophic cultivation also has some other advantages such as a high degree of process control, low costs for harvesting biomass because of the higher cell densities achieved, and so on [57]. Nevertheless, this is not to say that this cultivation mode is without drawbacks: the number of microalgal species able to grow heterotrophically is limited; the formation of light-induced metabolites will be diminished; contamination and competition with other microorganisms could take place, and so on. Table 5 shows the comparison between photoautotrophic and heterotrophic production of microalgae-derived products.

Table 5 Comparison between photoautotrophic and heterotrophic production of microalgae-derived products

	Photoautotrophic mode	Heterotrophic mode
Available algal species	A lot	Limited
Cell density	Low	High
Cost of production	Low	Highs
Product contents	Low	High
Productivity	Low	High
Scaleup	Easy	Difficult

6 Biosynthesis

Major advances have been made in the elucidation of the carotenoid biosynthetic pathway in bacteria, algae, and higher plants, and it is believed that in these organisms, the biosynthesis of primary carotenoids follows a similar pathway. The biosynthesis process of β-carotene and α-carotene is outlined in Fig. 3, which can be divided into the following steps.

6.1 Formation of Isopentenyl Diphosphate (IPP)

Glyceraldehyde-3-phosphate (GAP) and pyruvate act as the first two precursors. They are utilized to produce 1-deoxy-D-xylulose-5-phosphate (DXP) which is then transformed to IPP through a series of reactions in the 2-C-methyl-d-erythritol 4-phosphate (MEP) pathway present in the plastids of plants, cyanobacteria, algae, and certain bacteria [59].

6.2 Formation of Geranylgeranyl Pyrophosphate (GGPP)

GGPP is the first immediate precursor in the lutein biosynthesis pathway, which is synthesized from IPP. At first, IPP is isomerized to its allylic isomer, dimethylallyl diphosphate (DMAPP). Geranyl pyrophosphate (GPP) is then produced by the condensation reaction of IPP and DMAPP. Elongation of GPP by the addition of IPP results in the formation of farnesyl pyrophosphate (FPP) to which is further added another IPP to produce GGPP [60]. These condensation reactions are catalyzed by a single enzyme called GGPP synthase, which was isolated as a soluble and functional homodimer (74 kDa) from the chromoplasts of *Capsicum* [61].

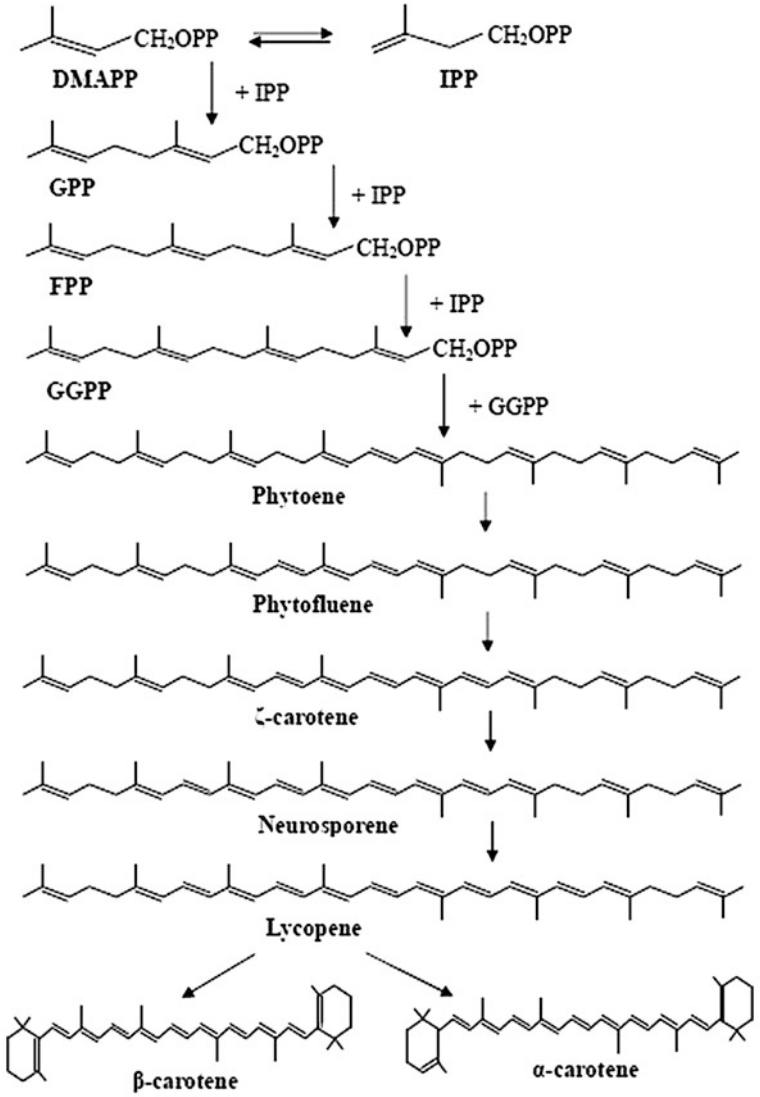

Fig. 3 Carotenoid biosynthetic pathway to form β-carotene and α-carotene (adapted from Ref. [58]). Abbreviations: *DMAPP* dimethylallyl diphosphate; *IPP* isopentenyl diphosphate; *GPP* geranyl diphosphate; *FPP* farnesyl diphosphate; *GGPP* geranylgeranyl diphosphate

6.3 Biosynthesis and Desaturation of Phytoene

Phytoene is synthesized through condensation of two GGPP molecules under the catalysis of phytoene synthase (PSY), a membrane-associated enzyme [62]. PSY has been reported to be a rate-limiting enzyme of carotenoid biosynthesis in

different plant organs, tissues, and locations, such as in canola seeds, ripening tomato fruits, and in marigold flowers [63–65]. In addition, PSY is highly conserved among archaea, bacteria, and eukaryotes [66]. Lycopene is subsequently formed by the conversion of phytoene, which is catalyzed by two desaturases, namely phytoene desaturase (PDS) and zeta-carotene desaturase (ZDS) through a series of reactions [67].

6.4 Cyclization of Lycopene

Carotenoid cyclization refers to the formation of a six-membered ring at one or both ends of lycopene and an important branching point of the carotenoid biosynthesis pathway in photosynthetic organisms. One branch leads to the formation of β-carotene and its derivative xanthophylls, such as zeaxanthin and astaxanthin, and the other one leads to α-carotene and lutein in plants and microalgae [68]. The formation of β and ε rings at both ends of lycopene results in the yield of α-carotene under the catalysis of β-cyclase (LCY-b) and ε-cyclase (LCY-e), respectively, whereas the formation of two β-rings at the two ends of lycopene gives rise to β-carotene catalyzed by LCY-b alone [67].

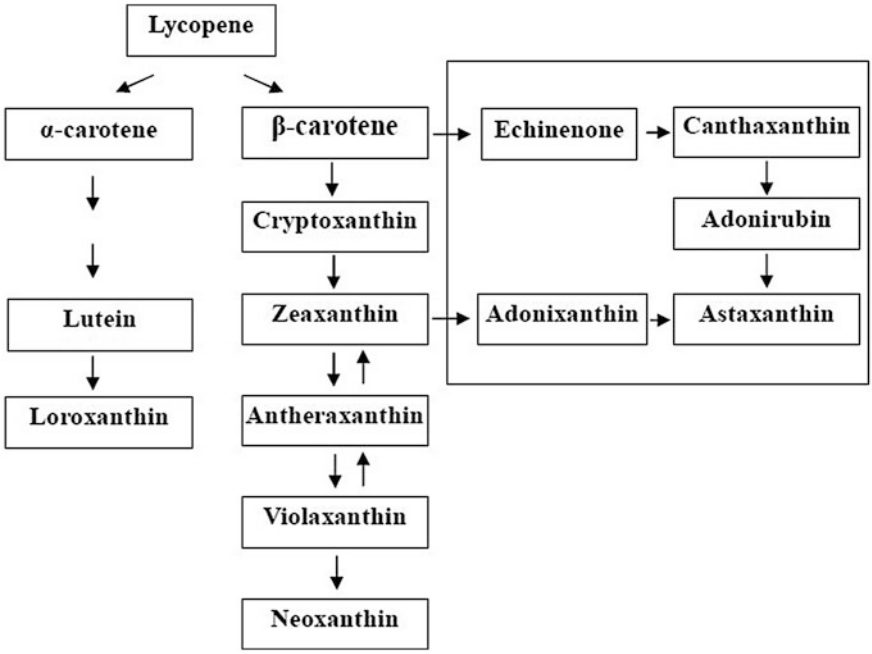

Fig. 4 Schematic diagram of pathway of xanthophyll biosynthesis. In the box is the astaxanthin biosynthetic pathway present in certain algae. Two possible ways are indicated (adapted from Ref. [69])

6.5 Hydroxylation

In higher plants and algae, xanthophylls are enzymically formed through the oxidation of α- and β-carotene. Hydroxylation at the C-3 and C-3′ positions of α-carotene by β- and ε-carotene hydroxylase encoded by genes *CrtR-b* and *CrtR-e* lead to the formation of lutein, whereas hydroxylation of β-carotene by β-carotene hydroxylase alone results in the formation of zeaxanthin [67]. The subsequent epoxidation of zeaxanthin leads to the production of violaxanthin, which is further converted to neoxanthin. Meanwhile, in some algae, additional xanthaphyll biosynthetic pathways could also be present. For example, oxygenation and hydroxylation of β-carotene bring on the synthesis of astaxanthin (Fig. 4).

7 Regulation of Carotenogenesis

7.1 Intercommunication of Cellular Organelles and Retrograde Regulation of Photosynthetic Genes

In higher plants, algae, and other photosynthetic eukaryotic cells, mitochondria, chloroplast, and nucleus are three essential organelles. These cellular components are not isolated or independent, but have interaction and communication with each other. In most cases, chloroplast and mitochondria have their own organelle genome, but these genomes only encode few amounts of proteins. Current proteomics and genomics studies show that these organelles comprise thousands of proteins. Studies in cyanobacterium indicated that most proteins (93–99 %) were encoded by nuclear genes and were transported into the organelles after translation and processing in cytoplasm [70, 71]. Therefore, there must be a mechanism regulating and coordinating the nucleus genome to encode and synthesize the most necessary organelle proteins during cell growth and development.

Anterograde regulation refers to the control of the biogenesis and function of organelles by the nuclear genome and has been widely studied in the past decades. Along with anterograde regulation, retrograde regulation refers to the communication between organelles and nucleus. The functional and developmental organelles send signals to the nucleus in order to activate the necessary genes to meet the particular need of the cell. The detailed retrograde regulation mechanisms in chloroplast and mitochondria remain unclear, and thus far, four organelle retrograde regulation mechanisms have been determined: (1) organellar gene expression (OGE), (2) tetrapyrrole biosynthesis, (3) organellar redox state, and (4) ROS [72].

Johanningmeier and Howell [73] studied the regulation of light-harvesting chlorophyll-binding protein (LHCP) mRNA accumulation in *Chlamydomonas reinhardi*. With the block of late chlorophyll biosynthesis, the intermediate of chlorophyll synthesis, namely Mg-protoporphyrin IX monomethyl ester (Mg-ProtoMe), inhibited by a feedback mechanism the light induction of LHCP

mRNA accumulation. Treatments that presumably resulted in an accumulation of Mg-ProtoMe repressed the expression or transcription of chloroplast genes [74, 75]. Walker and Willows [76] reported the roles of Mg-chelatase in the chlorophyll synthesis pathway. Mg-chelatase is a three-component enzyme comprising subunits ChlD, ChlH, and ChlI, which catalyzes the insertion of Mg^{2+} into the porphyrin ring of Proto IX. Mochizuki et al. [77] observed that the plastid-to-nucleus signal transduction pathway was disrupted in two *Arabidopsis* CHlH mutants but not in ChlI mutants. Also, CHlH was found to be able to bind to deuteroporphyrin even in the absence of the other two cheltase subunits [78]. In this regard, Mochizuki et al. [77] proposed that CHlH may not only serve as the chelatase in tetrapyrrole biosynthesis, but also function as the tetrapyrrole sensor in a chloroplast-to-nucleus signaling pathway. Another possibility is that CHlH could directly participate in the plastid-to-nucleus retrograde signaling pathway by forming a CHlH-tetrapyrrole complex with other cofactors without affecting the tetrapyrrole transport [79, 80].

There is another chloroplast-to-nucleus retrograde regulation pathway derived from the redox and ROS signals in plants and green algae. For redox signal retrograde regulation, two possible sources have been proposed, the redox state of the PQ pool in the photorespiration electron transport chain and light-harvesting complex II (LHCII) kinase. Escoubas et al. [81] proposed that in *Dunaliella tertiolecta*, the redox state of PQ may influence the regulation of nuclear photosynthesis genes, and such retrograde regulation was possibly achieved via a chloroplast protein kinase. Similar regulation mechanisms also exist in higher plants [82]. On the other hand, some research groups also suggested that LHC II kinase, rather than the PQ pool, may serve as the retrograde regulation signal source. Pursiheimo et al. [83] demonstrated that the transcription of genes *Lhcb*, *rbcS*, and *psbA* was positively related to LHCII protein phosphorylation. Bonardi et al. [84] also found that STN7, a dual-function chloroplast thylakoid protein kinase, participated in the chloroplast redox signaling pathway in *Arabidoposis thaliana*. These results indicated that protein kinase may also function as a necessary component in the chloroplast redox signaling transduction pathway.

The retrograde regulation also exists in mitochondria. It is believed that the mitochondrion interacts closely with other cellular organelles, such as chloroplast and nucleus, and a wide range of signaling events in plant cells can be strongly influenced by changes in mitochondrial function. Such changes trigger altered nuclear gene expression by a process of mitochondrial retrograde regulation (MRR) [85]. MRR is manifested at mitochondria dysfunctions induced by mutations, chemical agents, or stresses. Previous studies indicated that plant mitochondrial dysfunction led to the induction of nucleus-located genes encoding proteins for the restoration of mitochondria function. Meanwhile, genes encoding antioxidant enzymes were also activated to detoxify the ROS and protect the cell organelle [85]. The most well-studied MRR thus far is on the expression of gene encoding alternative oxidase (AO) in mitochondria. Two possible signal transduction pathways have been proposed: one involves some intermediates of TCA

cycles such as organic acid 2-oxoglutarate, citrate, and malate, and the other is derived from the ROS produced in mitochondria [86]. MRR is still a poorly understood process and very little is known about the exact factors involved in the signal transduction pathway. Components able to react with ROS in mitochondria or cytosol (e.g., lipids, antioxidant enzymes, or small molecular weight compounds) should be considered as the candidates of primary or secondary signal molecules. Matsuo and Obokata [87] studied the MRR in *C. reinhardtii*. Under heterotrophic and mixotrophic conditions, nucleus-located photosynthetic genes were remote-controlled by a mitochondrial respiratory electron transport chain (RET). When algal cells were cultured under autotrophic and mixotrophic conditions, the controller became a photosynthetic electron transport (PET). These results suggested the cooperation between cellular organelles in algae and indicated the regulatory system of photosynthetic genes changes in response to shifts in the dominant energy source between photosynthesis and respiration.

7.2 Stimulation of Carotenogenesis by Oxidative Stress

7.2.1 Enhancement of Carotenoid Synthesis Induced by ROS

Jahnke [88] reported that the accumulation of carotenoids was one of the responses of microalgae to the oxidative stress induced by UV radiation. When *D. bardawil* was exposed to the UV-A radiation of 70 μmol photons m^{-2} s^{-1} for 84 h, the contents of lutein and zeaxanthin within algal cells increased about threefold and fivefold, respectively [89].

Carotenogenesis in algae could also be stimulated in response to chemically generated oxidative stresses. Kobayashi et al. [90] indicated that Fe^{2+} may serve as an $HO^{\bullet}$ generator via an iron-catalyzed Fenton reaction, and the generated ROS played an essential role in enhancing the carotenoid formation in *H. pluvialis*. This viewpoint was verified by Ip and Chen [49]. When the *C. zofingiensis* culture was supplemented with ROS generators Fe^{2+} (18 μM) and H_2O_2 (0.1 mM), the yield of astaxanthin increased from 9.9 to 12.58 mg L^{-1}. The production of lutein was also enhanced in *C. protothecoides* under heterotrophic cultivation. The lutein content increased from 1.75 to 1.98 mg g^{-1} when 0.01 mmol L^{-1} H_2O_2 and 0.5 mmol L^{-1} NaClO were added to generate $^1O_2$ [91]. Inactivation of antioxidant enzymes is another way to stimulate the formation of carotenoids as they act as nonenzymatic antioxidants. For example, sodium azide is known as an inhibitor of catalase (CAT) and superoxide dismutase (SOD), two important ROS scavengers. Shaish et al. [92] reported that when *D. bardawil* cells were treated with sodium azide, the content of β-carotene increased from 11.7 to 27.5 pg $cell^{-1}$.

7.2.2 Expression Variation of Genes Encoding Enzymes Involved in Carotenoid Biosynthesis After Oxidative Stress Treatment

When the microalgae are under stress treatments such as high light and high salt stress, the expression of genes encoding enzymes involved in carotenoid biosynthesis is upregulated, which subsequently increases the accumulation of carotenoids. According to Steinbrenner and Linden [93], in *H. pluvialis*, all four genes encoding PSY, lycopene cyclase, PDS, and carotenoid hydroxylase showed higher transcript levels in response to increased illumination. Similar research was conducted by Ramos et al. [94] using *D. salina*. The highest steady-state mRNA level of *Lcy-β* (encoding lycopene β-cyclase) was observed in algal cells exposed to high light (500 μmol m^{-2} s^{-1}) or low light (45 μmol m^{-2} s^{-1}) combined with nutrient depletion for 24 and 48 h, respectively. As a result, the cellular β-carotene content increased faster in the case of exposure to high light conditions at 24 h.

7.2.3 ROS Sensing Signaling Cascade Involved in Simulating Carotenogenesis

Due to the high cytotoxic and reactive feature of ROS, their concentration must be tightly controlled. Higher plants and algae can sense the ROS signals and make appropriate cellular responses. The redox-sensitive proteins that reversibly become oxidized or reduced depending on the cellular redox state are essential in the response process [19]. Pfannschmidt et al. [82] indicated that heterotrimeric G-proteins participated in the signaling pathway mediated by ROS. Protein phosphorylation controlled by protein Tyr phosphatases and specific mitogen-activated protein (MAP) kinases are two main well-studied molecular mechanisms of redox-sensitive regulation of protein function in cells [19, 95]. In microalgae, a gene encoding a MAP kinase was isolated from *D. salina* and its expression was found to be affected by changes in temperature and salinity conditions that also influenced the accumulation of β-carotene [96]. Eom et al. [97] detected 16 (11 %) differently expressed genes from *H. pluvialis* that were involved or potentially involved in defense or stress responses. Nedelcu [98] indicated that tumor suppressor protein p53, which plays a major role in cellular response to stress, was also present and mediated cellular responses to stress in two algae, *Volvox carteri* and *C. reinhardtii*.

8 Conclusion and Future Perspectives

Lutein production has been one of the most successful activities in the microalgal industry. As the world's market demand is growing, there are still substantial challenges to be addressed to enhance the production capacity and economics of microalgae-based lutein, which urgently requires the development of microalgal

biotechnology. For example, genetic engineering is a feasible approach to improve the biosynthetic pathway of lutein in microalgae and heighten its cellular accumulation. Another important issue to consider is the cost. As mentioned earlier, although the heterotrophic cultivation allows a higher cell density and lutein productivity, the high costs of glucose hinder its commercial application. It is logical to develop low-cost and effective carbon sources from industrial or agricultural wastes. Finally, the production of lutein may be combined with other algal-based metabolisms (such as oil) and coupled with wastewater treatment and biofixation of carbon dioxide to develop an integrated industrial chain that may increase the process economy and bring environmental benefits as well.

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