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· Invited Review ·

Carotenoid Metabolism: Biosynthesis, Regulation, and Beyond

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Abstract

Carotenoids are indispensable to plants and play a critical role in human nutrition and health. Significant progress has been made in our understanding of carotenoid metabolism in plants. The biosynthetic pathway has been extensively studied. Nearly all the genes encoding the biosynthetic enzymes have been isolated and characterized from various organisms. In recent years, there is an increasing body of work on the signaling pathways and plastid development, which might provide global control of carotenoid biosynthesis and accumulation. Herein, we will highlight recent progress on the biosynthesis, regulation, and metabolic engineering of carotenoids in plants, as well as the future research towards elucidating the regulatory mechanisms and metabolic network that control carotenoid metabolism.

Key words: carotenoids; isoprenoids; metabolism; metabolic engineering; plastid; regulation.

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Carotenoids represent a diverse group of pigments widely distributed in nature. They contribute to the red, orange and yellow colors found in many flowers, fruits and vegetables. Carotenoids are synthesized de novo not only in all photosynthetic organisms, such as plants (including algae) and cyanobacteria, but also in some non-photosynthetic bacteria and fungi. In plants, carotenoids fulfill a variety of critical functions. They serve as accessory pigments to harvest light for photosynthesis and constitute the basic structural units of photosynthesis apparatus. They act as photoprotectors for plants to adapt high light stress and furnish flowers and fruits with distinct colors to attract insects and animals for pollination and seed dispersal. In addition, oxidative cleavage of carotenoids produces apocarotenoids. Some of the apocarotenoids are signals in plant development, serve as antifungal agents, and contribute to the flavor and aroma of flowers and fruits (Auldridge et al. 2006). The phytohormone abscisic acid, the most well known apocarotenoid derivative, regulates a wide range of biological processes in plants.

Carotenoids are important not only to those organisms where they are synthesized, but also to animals and humans. Carotenoids have long been recognized as essential nutrients and important health beneficial compounds (Fraser and Bramley 2004). As animals and humans are unable to synthesize carotenoids de novo, they have to depend on diet for these essential products. "Pro-vitamin A" carotenoids, such as β-carotene and α-carotene, provide the primary dietary sources of vitamin A. The deficiency of vitamin A is one of the most noticeable nutritional problems in many parts of the world and affects an estimated 250 million children under 5 years of age (World Health Organization, http://www.who.int/nutrition/topics/vad/en/index.html). Food biofortification with enhanced pro-vitamin A carotenoids offers a sustainable way to combat vitamin A deficiency in developing countries, and thus comes to be one of the major

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driving forces stimulating carotenoid research and metabolic engineering of carotenoids in food crops. Apart from the well-established function of carotenoids as pro-vitamin A, some carotenoids, such as lycopene, rich in tomato, are strong antioxidants and have a protective function in reducing the risk of cancer and cardiovascular diseases (Hadley et al. 2002). Xanthophylls, such as lutein and zeaxanthin, are essential components of the macular pigments in eyes and offer protection against macular degeneration, the leading cause of age-related blindness (Krinsky et al. 2003).

The indispensable roles of carotenoids in plants and their important health benefits to animals and humans make the research on carotenoid metabolism exceptionally important. Significant progress has been made in our understanding of carotenoid biosynthesis and catabolism in plants. Nearly all the genes involved in the carotenoid biosynthetic pathway have been isolated and characterized in the past two decades. The availability of a large number of carotenoid biosynthetic genes from bacteria and plants provides the necessary molecular tools to facilitate the genetic engineering of carotenoid content and composition in food plants.

Previous reviews describing different aspects of carotenoid biosynthesis, regulation, manipulation, and their functions in plants, animals and humans are available (Cunningham and Gantt 1998; Niyogi 1999; Hirschberg 2001; Fraser and Bramley 2004; Taylor and Ramsay 2005; Botella-Pavia and Rodriguez-Concepcion 2006; DellaPenna and Pogson 2006; Sandmann et al. 2006; Matthews and Wurtzel 2007; Giuliano et al. 2008). Herein, we will give an overview of carotenoid research, with a major focus on updating the recent progress on carotenoid metabolism in plants.

Carotenoid Metabolic Pathway in Plants

Carotenoids are mainly C40 isoprenoids. Like all other isoprenoids occurring in nature, carotenoid biosynthesis initiates with the synthesis of the 5-carbon building block, isopentenyl pyrophosphate (IPP) and its allylic isomer dimethylallyl pyrophosphate (DMAPP). IPP and DMAPP used for carotenoid biosynthesis were recently found to be derived from a plastid-localized methylerythritol phosphate (MEP) pathway (Eisenreich et al. 2001; Hunter 2007). The MEP pathway uses glyceraldehy 3-phosphate and pyruvate as initial substrates. Three IPP molecules are added to DMAPP by geranylgeranyl diphosphate (GGDP) synthase (GGPS) to produce C20 GGDP, the common precursor for the biosynthesis of carotenoids and several groups of other plastidic isoprenoids (Figure 1).

The condensation of two molecules of GGDP by phytoene synthase (PSY) to produce C40 phytoene represents the first committed step in the carotenoid biosynthetic pathway. In many plants, PSY is thought to be a rate-limiting step and multiple PSYs share the regulation of carotenoid metabolic flux. Indeed, a new PSY3 in maize and rice was recently found to control carotenoid biosynthesis in roots in response to abiotic stress (Li et al. 2008; Welsch et al. 2008). The PSY catalyzed product, phytoene, is desaturated into red colored lycopene by two related enzymes of phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS) in plants. Two *cis-trans* isomerases of Z-ISO (Li et al. 2007) and CRTISO (Isaacson et al. 2002; Park et al. 2002) are required to convert poly-*cis*-configured phytoene into the all-*trans* form lycopene. Interestingly, in bacteria, a single enzyme, carotene desaturase (crtI), is apparently able to confer the same desaturation and isomerization reactions catalyzed by those four plant enzymes.

Lycopene is the branching point of this pathway. It is cyclized either to yield α-carotene (β,ε-carotene) by lycopene ε-cyclase (LCYE) and lycopene β-cyclase (LYCB) or to produce β-carotene by lycopene β-cyclase (LCYB) alone. LCYE plays a key role in determination of the β-carotene/α-carotene ratio (Harjes et al. 2008). α-Carotene and β-carotene are hydroxylated to produce lutein and zeaxanthin, respectively. These reactions are catalyzed by the β-ring carotene hydroxylase (including the non-heme di-iron type HYDB and the cytochrome P450 type CYP93A) and ε-ring carotene hydroxylase (the cytochrome P450 type CYP93C) (Tian et al. 2004; Galpaz et al. 2006; Kim and DellaPenna 2006). Lutein is the most abundant carotenoid found in leaf tissue of plants. Further epoxidation of zeaxanthin by zeaxanthin epoxidase (ZEP) produces violaxanthin. This reaction is reversed by violaxanthin deepoxidase (VDE) to give rise to the xanthophyll cycle for plants to adapt high light stress. Violaxanthin is converted into neoxanthin by neoxanthin synthase (NSY). A recent study shows that a novel gene product of *ABA4* is needed for neoxanthin synthesis (North et al. 2007). The formation of neoxanthin represents the final step in the classical carotenoid biosynthetic pathway.

The synthesized carotenoid end products can be catabolized to produce apocarotenoids. A family of carotenoid cleavage dioxygenases (CCDs) catalyzes the oxidative cleavage of carotenoids (Auldridge et al. 2006). The first *CCD* gene identified is 9-*cis*-epoxycarotenoid dioxygenase, which cleaves violaxanthin and neoxanthin to produce xanthoxin, the direct substrate for phytohormone abscisic acid (ABA) synthesis (Schwartz et al. 1997). CCDs display complicated cleavage site specificity but many are less specific with their substrate selection. This probably contributes to the diversity of apocarotenoids found in nature (Auldridge et al. 2006).

Carotenoid biosynthetic enzymes have long been hypothesized to form enzyme complex in participating carotenoid biosynthesis (Cunningham and Gantt 1998). Enzymes from the isoprenoid pathway, isopentenyl pyrophosphate isomerase (IPI) and GGPS, as well as carotenogenic enzyme, PSY, are part of a soluble large protein complex that catalyzes the formation of phytoene in plastid stroma. A hypothetical

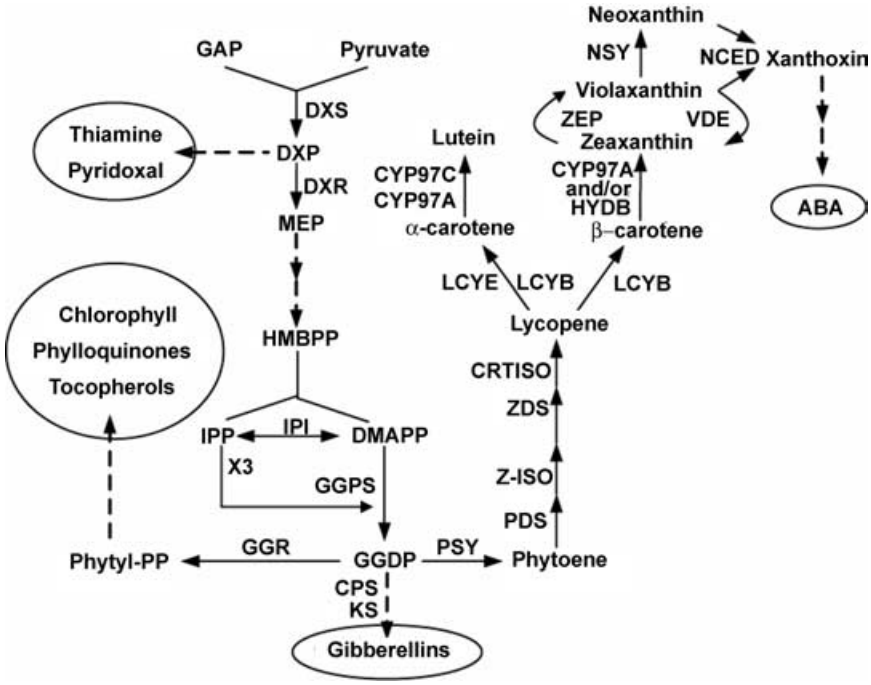

Figure 1. Carotenoid metabolic pathway in plants.

Carotenoid biosynthesis begins with the synthesis of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) via the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway using glyceraldehyde-3-phosphate (GAP) and pyruvate as initial substrates. IPP and DMAPP are used to form the central intermediate geranylgeranyl diphosphate (GGDP) for carotenoid and other isoprenoid biosynthesis via the general isoprenoid biosynthetic pathway. The synthesis of phytoene by condensation of two molecules of GGDP represents the first committed step in the carotenoid biosynthetic pathway. ABA, abscisic acid; CPS, *ent*-copalyl diphosphate synthase; CRTISO, carotene isomerase; CYP93A, carotene β-hydroxylase (cytochrome P450 type); CYP93C, carotene ε-hydroxylase (cytochrome P450 type); DXP, 1-deoxy-D-xylulose 5-phosphate; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; DXS, 1-deoxy-D-xylulose 5-phosphate synthase; GGPS, geranylgeranyl diphosphate synthase; GGR, geranylgeranyl diphosphate reductase; HMBPP, 1-hydroxy-2-methyl-2-butenyl 4-diphosphate; HYDB, carotene β-hydroxylase (non-heme di-iron type); IPI, isopentenyl diphosphate isomerase; KS, *ent*-kaurene synthase; LCYB, lycopene β-cyclase; LCYE, lycopene ε-cyclase; NCED, 9-*cis*-epoxycarotenoid dioxygenase; NSY, neoxanthin synthase; PDS, phytoene desaturase; PSY, phytoene synthase; VDE, violaxanthin de-epoxidase; ZDS, ζ-carotene desaturase; Z-ISO, 15-*cis*-ζ-carotene isomerase; ZEP, zeaxanthin epoxidase.

membrane-associated multi-enzyme complex to catalyze the synthesis of α- and β-carotene from phytoene has been postulated to consist of desaturases and lycopene β-cyclase and/or lycopene ε-cyclase in the plastid membrane (Cunningham and Gantt 1998). A large protein complex of approximately 350 kDa containing PDS was recently found in the plastid membrane of plants (Lopez et al. 2008b).

Several important metabolic branches alongside the carotenoid pathway exist. The first one is at 1-deoxy-D-xylulose 5-phosphate (DXP), from where a side branch leads to the biosynthesis of thiamine and pyridoxal (Figure 1). The second one is at GGDP. Geranylgeranyl reductase (GGR) catalyzes the reduction of GGDP into phytol, which becomes the side chain of chlorophylls, phylloquinones and tocopherols. GGDP is also the substrate for *ent*-copalyl diphosphate synthase (CPS), which, together with *ent*-kaurene synthase (KS), leads the metabolic flux into gibberellin biosynthesis (Figure 1).

Regulation of Carotenoid Metabolism in Plants

One of the great challenges of studies of carotenoid metabolism has been the identification of mechanisms and processes by which carotenoid biosynthesis is regulated. Plants have developed complex regulatory mechanisms controlling carotenoid biosynthesis and accumulation. While the composition and relative abundance of various carotenoids are remarkably conserved in green tissues, carotenoid identities and amounts vary broadly in non-green tissues or organs. The essential roles of

carotenoids in photosynthesis, photomorphogenesis and plant development suggest that their biosynthesis is coordinately regulated with other processes such as plastid biogenesis, flowering and fruit development (Fraser and Bramley 2004). The fact that the pathway links with plant hormone gibberellin and abscisic acid biosynthesis implies that changes in the constitution and/or content of carotenoids might bring physiological or biochemical shifting of the plants, which in turn affects carotenoid biosynthesis. Therefore, carotenoid metabolism is likely regulated at multifaceted and multiple levels in plants.

Distinct regulatory mechanisms of carotenoid biosynthesis operate in green tissues and in flowers and fruits. Because carotenoids serve as structural pigments in the photosynthesis system, the regulation of carotenoid biosynthesis in green tissues of plants must occur in a coordinated manner with other cellular processes for the assembly of the photosynthesis apparatus. Although many chloroplast metabolic processes are associated with light-mediated regulation of the relevant genes, this is not necessarily the general rule for carotenoid biosynthetic genes. Despite that the expression of certain carotenoid biosynthetic genes such as *PSY* is regulated by light through a phytochrome-mediated process (von Lintig et al. 1997; Woitsch and Romer 2003), the transcript levels of carotenoid genes collectively are not light dependant (Fraser and Bramley 2004). Additional determinants may play a significant role in regulation of carotenoid biosynthesis in green leaves. A recent work shows that the formation of the prolamellar body is directly related to carotenoid accumulation (Cuttriss et al. 2007).

During flower color development and fruit ripening, transcriptional regulation of carotenoid gene expression appears to be a major mechanism by which the biosynthesis and accumulation of specific carotenoids are regulated. Classic examples are found in tomato and pepper fruits and flowers, where the accumulation of specific carotenoids coincides with increased expression of upstream carotenogenic genes and reduced expression of genes downstream of the accumulating carotenoids (Hirschberg 2001). Post-transcriptional regulation at enzymatic levels also plays a role in controlling carotenoid biosynthesis and accumulation.

Metabolic turnover of carotenoids by CCDs not only produces important signaling and accessory apocarotenoid molecules, but also helps to maintain the steady level of carotenoids in plants. Expression of *CCDs* has been found to inversely regulate carotenoid accumulation (Ohmiya et al. 2006). A body of evidence shows that oxidative cleavage of carotenoids is induced under environmental stresses. Circadian rhythm has been shown to affect carotenoid catabolism (Simkin et al. 2004). Moreover, developmental cues also play an important role in conferring metabolic turnover of carotenoids.

Until now, only a few studies report either a transcription factor or other genes which impose global regulatory function on carotenoid metabolism in plants. A member of the APETALA2 (AP2)/ethylene-responsive element-binding protein transcription factor family, AtRAP2.2, binds to one main regulatory region of *PSY* promoter and is shown to modestly regulate the transcript levels of *PSY* and *PDS*, as well as carotenoid content in *Arabidopsis* (Welsch et al. 2007). Two regulatory genes of *UV-DAMAGED DNA-BINDING PROTEIN1* (*DDB1*) and *DE-ETIOLATED1* (*DET1*) control light signaling pathways. Mutations in these genes are responsible for the high pigment mutants (*hp1* and *hp2*) in tomato. These mutants exhibit exaggerated photoresponsiveness and contain elevated levels of carotenoids and flavonoids (Mustilli et al. 1999; Liu et al. 2004). A cystein-rich zinc finger domain-containing OR protein harbors the CxxCxGxG domain that is highly specific to DnaJ-like molecular chaperones for protein folding, assembly and disassembly, and translocation into organelles. The *Or* gene mutation, which is caused by the insertion of a *copia*-type LTR retrotransposon confers high levels of β-carotene accumulation in the cauliflower orange curd mutant and thus represents a novel regulatory gene in mediating carotenoid accumulation (Lu et al. 2006).

Recent studies show that control of plastid biogenesis is an important mechanism by which carotenoid biosynthesis and accumulation are regulated in plants. The *Or* gene mutation in cauliflower exerts its functional role by inducing the differentiation of proplastids or other non-colored plastids into chromoplasts, which act as a metabolic sink for carotenoid biosynthesis and accumulation (Lu et al. 2006; Li and Van Eck 2007). Expression of the cauliflower *Or* gene in a heterologous system provides the first direct evidence demonstrating that induction of chromoplast biogenesis imposes a profound effect on carotenoid accumulation (Lopez et al. 2008a). The *Or* gene product represents the first bona fide molecular switch for chromoplast formation (Giuliano and Diretto 2007). Similar to the cauliflower OR protein, low molecular weight molecular chaperones are also known to be involved in regulation of plastid development. HSP21 from tomato not only protects the photosystem II components from oxidative stress, but also promotes the conversion of chloroplasts into chromoplasts, which in turn leads to carotenoid accumulation (Neta-Sharir et al. 2005). The light signaling proteins responsible for tomato *high pigment 1* (*hp1*) and *high pigment 2* (*hp2*) phenotype apparently impair their regulatory functions in controlling plastid development in these mutants. The carotenoid accumulation in these mutants was found to be linked to an early fruit plastid biogenesis and such accumulation is principally due to an increased plastid number and size to provide a large compartment for carotenoid biosynthesis and deposition (Liu et al. 2004; Kolotilin et al. 2007). Similarly, the *high pigment 3* (*hp3*) mutant in tomato, which is caused by a mutation in the gene of *ZEP*, confers an enhanced level of carotenoid accumulation by increasing the size of plastid compartment in the cells to enable greater biosynthesis and a higher storage capacity (Galpaz et al. 2008).

Carotenoids in plants are synthesized de novo in nearly all types of plastids but accumulate in large quantities in chloroplasts and chromoplasts (Howitt and Pogson 2006). In chloroplasts, carotenoids are located in photosynthetic membranes and integrated with chlorophyll-binding proteins to form pigment-protein complexes, whereas, in chromoplasts, carotenoids are associated with polar lipids and carotenoid associated proteins to form carotenoid-lipoprotein sequestering substructures to effectively sequester and retain a large quantity of carotenoids (Vishnevetsky et al. 1999). Large amounts of carotenoid sequestering structures associated with high levels of carotenoid accumulation were found in the carrot and cauliflower *Or* mutant (Li et al. 2001; Lopez et al. 2008a). Several carotenoid-associated proteins, such as fibrillin, CHRD and CHRC, have been characterized (Vishnevetsky et al. 1999). Fibrillin comprises up to 80% of the total chromoplast proteins from pepper fruits. Expression of the genes encoding these carotenoid-associated proteins is well associated with chromoplast development and carotenoid accumulation (Leitner-Dagan et al. 2006; Simkin et al. 2007). Thus, formation of carotenoid-sequestering structures directly affects carotenoid biosynthesis and accumulation. Additionally, carotenoid sequestering structures may also prevent the carotenoid end products from overloading plastid membranes, the site of carotenoid biosynthesis, and avoid a negative feedback to the biosynthetic pathway by the end products.

A recent study suggests that starch breakdown might cause redox change, providing the signal to trigger β-carotene accumulation (Horner et al. 2007). Tobacco floral nectarines synthesize starch in amyloplasts. During flower development, the starch starts to break down into nectar sugars, which is accompanied by the plastid changes from amyloplasts to chromoplasts and the associated accumulation of β-carotene.

Engineering of Carotenoid Metabolism in Food Plants

Carotenoids are highly beneficial for human nutrition and health. Significant progress has been made recently in quantitative and qualitative manipulation of the carotenoid metabolic pathway in plants (Fraser and Bramley 2004; Taylor and Ramsay 2005; Botella-Pavia and Rodriguez-Concepcion 2006; Sandmann et al. 2006; Giuliano et al. 2008). The strategy commonly used is to alter the expression of a rate-limiting enzyme or several enzymes in the pathway in a tissue-specific manner in seed, fruit or other storage organ. Golden Rice represents one of the best-known successful examples for metabolic engineering of carotenoids for improving crop nutritional value. In Golden Rice 2, ectopic expression of genes in a mini-carotenoid biosynthetic pathway containing a maize *PSY* and *Erwinia uredovora* carotene desaturase (*crtI*) results in the accumulation of β-carotene in rice endosperm up to 31 μg/g dry weight, a level that can substantially fulfill the daily vitamin A requirement (Paine et al. 2005). In another study, tuber-specific overexpression of a bacterial mini-carotenoid biosynthetic pathway leads to the production of "golden" potato with a profound increase of β-carotene to a level of 47 μg/g dry weight (Diretto et al. 2007).

Phytoene synthase controls the committed step of the carotenoid biosynthetic pathway and thus it is a major target for metabolic engineering of carotenoid content in food plants. Overexpression of a bacterial *PSY* gene under a seed-specific promoter results in the production of golden canola seeds with a dramatic increase in total carotenoid and β-carotene content (Shewmaker et al. 1999). A significant increase in total carotenoid levels by expression of plant *PSY* in a tissue-specific manner is achieved in tomato fruit (Fraser et al. 2002) and potato tuber (Ducreux et al. 2005). Overexpression of *PSY1* in tomato also affects other isoprenoid metabolism and causes changes in plastid type to form chromoplasts-like structures prematurely during fruit development (Fraser et al. 2007).

Quantitative modification of carotenoid composition in food crops to increase β-carotene and xanthophyll content has been achieved by tissue-specific upregulation of other carotenoid biosynthetic genes or downregulation of endogenous genes in the pathway. Conversion of lycopene into β-carotene to form orange tomato is obtained by transformation with tomato lycopene β-cyclase (D'Ambrosio et al. 2004), or through plastid transformation to express a bacterial lycopene β-cyclase gene (Wurbs et al. 2007). Metabolic engineering of carotenoids by tissue-specific silencing of lycopene ε-cyclase and β-carotene hydroxylase enhances β-carotene accumulation in potato tuber (Diretto et al. 2006; Van Eck et al. 2007), whereas silencing of *ZEP* results in zeaxanthin enhancement in potato (Romer et al. 2002).

Astaxanthin, a ketocarotenoid with high-economic value as neutraceutical and aquacultural supplement, is normally found in marine bacteria and microalgae and synthesized via 4-ketolase from zeaxanthin. Astaxanthin occurs rarely in plants, only in a few species such as in the ornamental flowers of *Adonis aestivalis*, and appears to be produced from β-carotene via an indirect pathway (Cunningham and Gantt 2005). Several efforts have been made to engineer plants for astaxanthin production by expression of genes from algae and bacteria in model plants (Mann et al. 2000; Stalberg et al. 2003; Ralley et al. 2004) and in potato tubers (Gerjets and Sandmann 2006; Morris et al. 2006). Recently, successful overproduction of astaxanthin in carrot roots has been obtained by introducing an algal β-carotene ketolase (Jayaraj et al. 2007). Over 70% of total carotenoids in carrot roots are converted into novel ketocarotenoids, providing up to 2.4 mg/g dry weight accumulation.

Genes involved in the light signal transduction pathway have been shown to regulate carotenoid biosynthesis. Fruit-specific suppression of an endogenous *DET1* gene results in enhanced carotenoid accumulation as well as increased flavonoid levels in tomato fruits (Davuluri et al. 2005). Overexpression of a blue

light photoreceptor gene, *CRY2*, also produces increased levels of carotenoids and flavonoids in tomato fruits (Giliberto et al. 2005). These studies provide two examples showing that genes involved in light signal pathways can also be used to engineer carotenoids in plants.

While the common strategy for metabolic engineering of carotenoids in plants is to increase the metabolic flux towards carotenoid biosynthesis, the recent study of the *Or* gene from an orange cauliflower mutant has brought a new endeavor for carotenoid enhancement by alteration of sink strength. Rather than directly controlling carotenoid biosynthesis by altering the transcript levels of carotenoid and upstream isoprenoid biosynthetic genes (Li et al. 2001) or enhancing the metabolic flux into carotenoid pathway (Li et al. 2006), the *Or* gene causes high levels of β-carotene accumulation by enhancing sink strength (Lu et al. 2006). Remarkably, expression of the *Or* transgene in potato produces deep orange yellow-flesh tubers with an over sixfold increase of total carotenoid content. The increased carotenoid accumulation in the *Or* transgenic tubers was found to be associated with the formation of carotenoid sequestration structures in chromoplasts, which serve as an effective metabolic sink to facilitate the sequestration and storage of carotenoids (Lopez et al. 2008a). The success in using the *Or* gene to enhance carotenoid levels in crops opens a door for a complementary strategy to genetically engineer carotenoid metabolism (Li and Van Eck 2007).

It is well known that carotenoid accumulation is a net result of biosynthesis, turnover and finally stable storage of the end products. Studies of the *Or* mutant as well as the tomato *hp* mutants provide a strong notion that increase of the storage capacity can exert a profound influence on carotenoid accumulation. Concomitant increase of sink capacity and the catalytic activity of carotenoid biosynthetic pathway may represent a promising strategy for carotenoid enhancement in food crops.

Remarks

Although significant progress has been made in our understanding of carotenoid metabolism in plants, several key issues are yet to be addressed in detail. For example, very little is known about the global regulatory mechanisms underlying carotenoid metabolism and the genetic elements that regulate the expression of carotenoid biosynthetic genes. Not much is known about the signaling molecules that initiate plastid development for carotenoid biosynthesis and accumulation. Carotenoid biosynthesis is controlled by light in green tissues, but how light ultimately regulates this process remains to be elucidated. Also, limited information is available on how the metabolic flux is distributed among different branches of the general isoprenoid biosynthetic pathway. Similarly, the interactions between the carotenoid biosynthetic pathway and other pathways, and how such interactions influence plant growth and development are unclear. Further, how plants regulate carotenoid turnover in maintaining homeostasis remains unknown. An in-depth investigation on the different aspects of carotenoid regulation will provide insights into the regulatory mechanisms, which will facilitate the transgenic strategy to modify carotenoid content and composition with a predictable outcome across different plant species.

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