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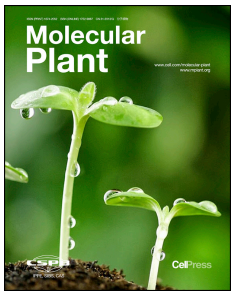

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Carotenoid Metabolism in Plants: The Role of Plastids

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Running title:

Carotenoid metabolism in various types of plastids

Short Summary:

Plastids, the sites for carotenoid biosynthesis and storage, play a central role in governing carotenogenic activity, carotenoid stability, and pigment diversity in plants. This review provides a comprehensive overview of the impact of various plastids on carotenoid biosynthesis and accumulation, as well as the effect of plastid types to the multifaceted regulation of carotenoid metabolism and metabolic engineering of carotenoids.

ABSTRACTS

Carotenoids are indispensable to plants and critical in human diets. Plastids are the organelles for carotenoid biosynthesis and storage in plant cells, and exist in various types, which include proplastids, etioplasts, chloroplasts, amyloplasts, and chromoplasts. These plastids have dramatic differences in their capacity to synthesize and sequester carotenoids. Clearly, plastids play a central role in governing carotenogenic activity, carotenoid stability, and pigment diversity. Understanding of carotenoid metabolism and accumulation in various plastids expands our view on the multifaceted regulation of carotenogenesis and facilitates our efforts toward developing nutrient enriched food crops. In this review, we provide a comprehensive overview of the impact of various types of plastids on carotenoid biosynthesis and accumulation, and discuss recent advances in our understanding of the regulatory control of carotenogenesis and metabolic engineering of carotenoids in light of plastid types in plants.

Key words:
Carotenoid, plastid, regulation, metabolism, metabolic engineering

INTRODUCTION

Carotenoids are a group of natural tetraterpenoid pigments distributed widely in plants, algae, fungi, and bacteria. Many flowers, fruits, and roots owe their vivid orange, yellow and red hues to carotenoids. Carotenoids play essential roles in photosynthesis and photoprotection (Domonkos et al., 2013; Niyogi and Truong, 2013; Hashimoto et al., 2016). They provide precursors for the biosynthesis of phytohormones, abscisic acids (ABA) and strigolactones (SLs) (Nambara and Marion-Poll, 2005; Al-Babili and Bouwmeester, 2015). Carotenoid derivatives also act as signaling molecules to mediate plant development and responses to environmental cues (Havaux, 2014; Tian, 2015; Hou et al., 2016). Apart from their central functions in plants, carotenoids play critical roles in human nutrition and health as essential components of human diets. They provide dietary sources of provitamin A and serve as antioxidants to reduce the onset

of some chronic diseases, such as cardiovascular diseases, cancers, and age-related eye diseases (Fraser and Bramley, 2004; Rao and Rao, 2007; Fiedor and Burda, 2014).

The pivotal roles of carotenoids to plants and humans have prompted significant efforts toward understanding of carotenoid metabolism in plants (Hirschberg, 2001; Fraser and Bramley, 2004; Farré et al., 2010; Walter et al., 2010; Zhu et al., 2010; Cazzonelli, 2011; Ruiz-Sola and Rodríguez-Concepción, 2012; Moise et al., 2013; Liu et al., 2015; Nisar et al., 2015). Carotenoid biosynthesis pathway is well established (Figure 1). It starts with the formation of C5 building blocks isopentenyl diphosphate (IDP) and dimethylallyldiphosphate (DMAPP) with isomeration by isopentenyl diphosphate isomerase (IPI) (Pankratov et al., 2016) via the methylerythritol 4-phosphate (MEP) pathway (Eisenreich et al., 2004). Geranylgeranyl diphosphate (GGPP) synthase 11 (GGPS11) is a hub isozyme that interacts with phytoene synthase (PSY) and GGPS recruiting protein to channel GGPP toward carotenoids and chlorophylls, respectively (Ruiz-Sola et al., 2016; Zhou et al., 2017). The condensation of two GGPP by PSY generates the first carotenoid product phytoene. This step is considered as a major rate-limiting step of carotenoid biosynthesis (Cazzonelli and Pogson, 2010). Following a series of desaturation and isomerization reactions catalyzed by phytoene desaturase (PDS), ζ-carotene desaturase (ZDS), ζ-carotene isomerase (Z-ISO), and carotenoid isomerase (CRTISO), a red colored carotenoid lycopene is produced from uncolored phytoene. Cyclization of lycopene by lycopene ε-cyclase (LycE) and/or lycopene β-cyclase (LycB) produces orange α-carotene and β-carotene, representing the α- and β-branch of the pathway, respectively. Subsequent hydroxylation of α-carotene and β-carotene by two non-heme carotene hydroxylases (BCH1 and BCH2) and two heme hydroxylases (CYP97A and CYP97C) generates yellow xanthophylls of lutein in the α-branch and zeaxanthin in the β-branch. Epoxidation and de-epoxidation of zeaxanthin by zeaxanthin epoxidase (ZEP) and violaxanthin de-epoxidase (VDE) form the xanthophyll cycle, a mechanism to protect plants against photodamage (Jahns and Holzwarth, 2012). The conversion of violaxanthin into neoxanthin by neoxanthin synthase (NXS) concludes the core biosynthetic pathway (Neuman et al., 2014). Further modifications of carotenes and xanthophylls produce various species-specific carotenoids (Giuliano, 2014; Giuliano, 2017). Non-enzymatic or enzymatic oxidative cleavages of carotenoids by carotenoid cleavage dioxygenases (CCDs) produce apocarotenoids, which include phytohormones, signaling molecules, and volatiles (Al-

Babili and Bouwmeester, 2015; Beltran and Stange, 2016; Hou et al., 2016). CCDs contribute to the steady-state levels of carotenoids (Gonzalez-Jorge et al., 2013; Lätari et al., 2015) (Figure 1). Carotenoid sequestration is also known to profoundly affect total carotenoid accumulation (Vishnevetsky et al., 1999; Li and Yuan, 2013) (Figure 1). All the genes and enzymes in the core carotenoid biosynthetic pathway have been isolated and well characterized (Ruiz-Sola and Rodríguez-Concepción, 2012; Nisar et al., 2015; Yuan et al., 2015b). However, less is known about the regulatory control of carotenoid metabolism despite recent advances.

In plant cells, plastids are the sites for carotenoid biosynthesis and storage. Plastids are found ubiquitously in plants and exist in various types, such as proplastids, amyloplasts, etioplasts, chloroplasts, and chromoplasts (Lopez-Juez and Pyke, 2004; Jarvis and Lopez-Juez, 2013). All types of plastids except proplastids possess the capacity for carotenoid production (Howitt and Pogson, 2006; Li et al., 2016). Proplastids are small, undifferentiated plastids that enrich in meristem and reproductive tissues. Although not being carotenogenic sites, proplastids are the progenitors for all other types of plastids (Figure 2). Amyloplasts are starch-storing plastids found in seeds, roots and tubers. Amyloplasts in general contain relatively low levels of carotenoids, mainly as xanthophylls (i.e. lutein, zeaxanthin, and violaxanthin) (Howitt and Pogson, 2006; Wurtzel et al., 2012). Etioplasts are regarded as an intermediary stage of chloroplast development in dark-grown plants. Etioplasts contain small amounts of photosynthetic carotenoids primarily lutein and violaxanthin and the chlorophyll precursor protochlorophyllide for rapid adaptation of photomorphogenesis upon illumination (Rodríguez-Villalón et al., 2009). Chloroplasts define plants and are the photosynthetic plastids in green tissues. Abundant carotenoids are localized in chloroplast thylakoid membranes for photosynthesis and photoprotection. Chromoplasts are characterized by massive accumulation of carotenoid pigments in many colored flowers, fruits, and vegetables (Egea et al., 2010; Li and Yuan, 2013; Schweiggert and Carle, 2017). Because of their specific functions and unique morphologies, various types of plastids exhibit different capacities to synthesize and accumulate carotenoids (Figure 2) (Ruiz-Sola and Rodríguez-Concepción, 2012; Li et al., 2016). This review will discuss the impact of plastid types on carotenoid biosynthesis and accumulation, and cover recent progress in understanding the regulatory mechanisms underlying carotenoid metabolism and metabolic engineering of carotenoids in light of plastid types in plants.

ETIOPLASTS 'READY TO GO'

Under natural conditions, etioplasts are in a stand-by state for chloroplast development before seedlings merge out from soil for photosynthesis. The most distinctive feature of etioplasts is the formation of prolamellar bodies (PLBs), which consist of large paracrystalline lattices of interconnected tubules with both chlorophyll precursor and carotenoids (Kowalewska et al., 2016). Etioplasts in etiolated seedlings accumulate only limited amounts of carotenoids (von Lintig et al., 1997; Welsch et al., 2000). The low level of carotenoid accumulation in etioplasts is believed to result from both low expression of the major rate-limiting enzyme PSY and its topological localization within the plastids. In etiolated tissues, *PSY* expression was found to be regulated by phytochromes through phytochrome-interacting factors (PIFs), a family of bHLH transcription factors that are required to repress photomorphogenesis in the dark (von Lintig et al., 1997; Leivar et al., 2009; Toledo-Ortiz et al., 2010). PIFs bind directly to the G-box element in the *PSY* promoter to suppress its expression, resulting in down-regulating carotenoid biosynthesis in etioplasts (Toledo-Ortiz et al., 2010). In addition, the topological localization of PSY protein also regulates PSY at activity level in etioplasts. Membrane association is required for PSY activity (Welsch et al., 2000). In etiolated seedlings, most of the PSY proteins are located within PLBs and display low enzymatic activity due to the lack of competent membranes, which reduces its ability for carotenoid biosynthesis (Welsch et al., 2000). Although carotenoid biosynthetic activity is limited, continuous metabolic flux through the carotenogenic pathway occurs in etioplasts. This is evidenced by phytoene accumulation when PDS activity is disrupted by plastid terminal oxidase (PTOX) dysfunction in etiolated seedlings (Kambakam et al., 2016).

Despite being present at low levels, carotenoids are essential for etioplast formation and function. Lutein and violaxanthin are the main carotenoids in etioplasts (Park et al., 2002; Rodríguez-Villalón et al., 2009). Absence of lutein and violaxanthin causes a lack of the representative lattice-like PLBs in etioplasts and delays photomorphogenesis in the dark-grown seedlings of a lutein deficient mutant *ccr2* (Park et al., 2002; Cuttriss et al., 2007). This establishes the crucial role of carotenogenesis for etioplast formation and function. Lutein and

violaxanthin in etioplasts are also believed to be critical in subsequent de-etiolation and photoprotection (Pogson et al., 1998; Barrero et al., 2008). Indeed, higher carotenoid content in etiolated seedlings was found to result in better photosynthetic development (Rodríguez-Villalón et al., 2009). It is clear that biosynthesis and accumulation of carotenoids in etioplasts aid dark-grown seedlings in rapid adaption of photomorphogenesis upon illumination.

Etioplasts are 'ready to go' for chloroplast formation and photosynthesis. Light signaling functions as the primary trigger for etioplast differentiation into chloroplasts. De-etiolation involves synchronous formation of chlorophylls, carotenoids, lipids, and proteins for the assembly of photosynthetic apparatus (Cheminant et al., 2011). The rapid production of carotenoids is associated with de-repression of carotenoid biosynthetic genes (Woitsch and Römer, 2003; Rodríguez-Villalón et al., 2009). PIFs and LONG HYPOCOTYL 5 (HY5), which mediates a broad set of photomorphogenic responses, directly regulate *PSY* expression in an antagonistic way. Upon illumination, light triggers degradation of the negative regulators PIFs and stabilizes HY5, resulting in activation of *PSY* expression (Toledo-Ortiz et al., 2010; Toledo-Ortiz et al., 2014; Bou-Torrent et al., 2015). Furthermore, PSY activity greatly enhances following establishment of photosynthetic apparatus (Welsch et al., 2000). A dramatic increase of carotenoids protects plastids from photoxidative damages during de-etiolation (Rodríguez-Villalón et al., 2009; Llorente et al., 2017).

The burst production of carotenoids in coordination with chlorophyll formation during the de-etiolation process is crucial for the etioplast-to-chloroplast transition as well as plant fitness under sunlight. It is believed that carotenoids play different roles in the early and late stages of de-etiolation. During the first several hours, the xanthophyll cycle reaches its highest photoprotection activity before the light-harvesting complex is fully established (Ruban et al., 2007). Relatively high transcript levels of *BCH* and *ZEP* occur in the early stage of this process (Woitsch and Römer, 2003). At the later stage, carotenoid/chlorophyll ratio decreases when photosynthetic machinery is fully developed and PLBs are converted into thylakoids. Sophisticated regulation of chlorophyll formation during de-etiolation has been revealed by the light signaling pathways (Liu et al., 2013; Myśliwa-Kurdziel et al., 2013; Su et al., 2015; Xu et al., 2016) and phytohormones (Humplík et al., 2015; Cortleven et al., 2016). However,

knowledge for the coordinated regulation of carotenoid biosynthesis in this process remains limited.

AMYLOPLASTS 'WITH POTENTIALS'

Amyloplasts are of great economic and agricultural importance because they are enriched in starchy organs such as seeds of wheat, rice, barley, and maize, as well as potato tuber and cassava root. Amyloplasts store starch granules, which are important to plants in energy storage and gravitropism (Jarvis and Lopez-Juez, 2013). Amyloplasts synthesize and accumulate carotenoids mainly as xanthophylls (i.e. lutein, zeaxanthin, and violaxanthin) (Howitt and Pogson, 2006; Wurtzel et al., 2012). With the exception of a possible role in ABA production, the function of carotenoids in amyloplasts remain to be defined. While substantial levels of carotenoids can be found in amyloplasts from some varieties of starchy crops with large genetic variation, such as maize and potato tuber (Vallabhaneni and Wurtzel, 2009; Zhou et al., 2011; Owens et al., 2014), amyloplasts in general accumulate only low levels of carotenoids (Howitt and Pogson, 2006; Zhai et al., 2016). Presumably, there are underlying factors limiting carotenoid biosynthesis and accumulation in amyloplasts, which include biosynthesis capacity, plastid ultrastructures, and metabolic channeling.

Low transcription and activity of key enzymes in the carotenogenic pathway may be the crucial limiting factors for low carotenoid production in amyloplasts (Vallabhaneni and Wurtzel, 2009; Bai et al., 2016). This is supported by considerable data showing that up-regulation of carotenogenic genes leads to significant carotenoid accumulation in the starchy organs such as rice seeds (Paine et al., 2005; Bai et al., 2016), potato tubers (Diretto et al., 2007a; Mortimer et al., 2016), wheat seeds (Wang et al., 2014), and plant calli (Cao et al., 2012; Bai et al., 2014). PSY represents a major rate-limiting step for carotenoid biosynthesis in amyloplasts (Wurtzel et al., 2012). Low PSY gene expression and activity directly affect carotenoid levels in many starchy organs (Schaub et al., 2005; Li et al., 2008; Howitt et al., 2009; Welsch et al., 2010). In addition, degradation of carotenoids in amyloplasts is not a negligible factor for low carotenoid levels in starchy tissues (Schaub et al., 2017). Genome-wide association studies (GWAS) reveal that carotenoid cleavage dioxygenase enzymes, CCD1 and CCD4, are responsible for seed

carotenoid degradation and ZEP is an upstream contributor of carotenoid degradation in Arabidopsis seeds (Gonzalez-Jorge et al., 2013; Gonzalez-Jorge et al., 2016).

Plastid ultrastructures are important for carotenoid accumulation as they influence both carotenoid biosynthesis and storage capacity (Yuan et al., 2015b; Llorente et al., 2017). Normally, lipid-rich substructures are absent in amyloplasts, and starch granules occupy the inner space. The lack of appropriate lipoprotein sequestering substructures in amyloplasts perhaps restricts their capacities to significantly synthesize and stably store carotenoids (Lopez et al., 2008; Li et al., 2016).

As the key energy storage sites, amyloplasts have huge demand of carbohydrate supply for starch biosynthesis, which may limit the carbon flux into carotenoid pathway. Metabolic channeling maintains the balance between starch and carotenoid biosynthesis in amyloplasts. Because synthesis of starch and carotenoids competes for carbon supplies, it is not surprising to find negative correlations between carotenoid accumulation and starch deposition in some cases, such as in citrus and potato tubers engineered to synthesize high-value carotenoids (Cao et al., 2015; Mortimer et al., 2016).

Nevertheless, amyloplast readjustments regularly occur in special plant organs or tissues (Jarvis and Lopez-Juez, 2013). These readjustments include development of conspicuous crystalline-type carotenoid sequestering substructures (Maass et al., 2009; Cao et al., 2012) and formation of amylochromoplasts (Hempel et al., 2014; Zhang et al., 2014). In amyloplasts, carotenoids are primarily synthesized and stored in amyloplast membranes (Lopez et al., 2008; Pasare et al., 2013; Mortimer et al., 2016). Prominent crystalline-type carotenoid sequestering substructures can be induced in amyloplasts, likely when carotenoid accumulation exceeds the storage capacity of plastid membranes. Amylochromoplasts with starch granules and carotenoid sequestered elements are described in some natural organs such as peach palm fruit, squash, and sweet potato tuber (Jeffery et al., 2012; Hempel et al., 2014; Zhang et al., 2014). This specific kind of plastid was also found in organs engineered to accumulate carotenoids (Maass et al., 2009; Cao et al., 2012) and during the transition from amyloplasts to chromoplasts (Horner et al., 2007).

These readjustments not only impact carotenoid storage capacity, but also possibly affect biosynthetic ability. Prominent carotenoid biosynthesis and accumulation occur in tobacco floral nectaries, citrus fruits, and achiote seed arils following the developmental programmed changes of amyloplasts (Horner et al., 2007; Zeng et al., 2015b; Buah et al., 2016; Louro and Santiago, 2016). These changes are achieved by activation of carotenogenic gene transcription along with other processes (Horner et al., 2007). Apparently, retrograde communication between amyloplasts and nucleus is built to feed back to plastid functions (Enami et al., 2011). Carotenoid metabolites along with carotenoid levels might also act as signals to direct amyloplast modification (Kim et al., 2010; Hou et al., 2016). Furthermore, amyloplasts are compliant with up-regulation of carotenogenic activities and with dynamical partition into different sub-organelle compartments (Bai et al., 2016; Mortimer et al., 2016). These adaptations give starchy organs the potential to synthesize and accumulate substantial levels of carotenoids.

CHLOROPLASTS 'FOR CHLOROPLASTS'

Chloroplasts accumulate high levels of carotenoids although carotenoid colors are masked by the green chlorophylls. Lutein, ß-carotene, violaxanthin and neoxanthin are the most abundant carotenoids with astonishingly conserved ratios in chloroplasts (Ruiz-Sola and Rodríguez-Concepción, 2012). Majority of chloroplast carotenoids are localized in the thylakoid membranes for light harvesting and photoprotection (Jahns and Holzwarth, 2012; Niyogi and Truong, 2013; Ruban, 2016). Violaxanthin and other carotenoids can also be found in chloroplast envelope membranes (Schwarz et al., 2014) and plastoglobules, the small lipoprotein droplets (van Wijk and Kessler, 2017). Plastoglobules are identified as a site of carotenoid breakdown for apocarotenoid production (Ytterberg et al., 2006; Lundquist et al., 2012; Rottet et al., 2016) and for nonendogenous carotenoid accumulation (Mortimer et al., 2017).

Active carotenoid biosynthesis in chloroplasts occurs mainly in envelope membranes (Joyard et al., 2009; Ruiz-Sola and Rodríguez-Concepción, 2012). Sub-plastidial proteomic studies localize most carotenogenic enzymes in chloroplast envelopes, with ZEP in both envelopes and thylakoids, where VDE is only in thylakoids (Ytterberg et al., 2006; Joyard et al., 2009; Bruley et al., 2012) (Figure 3). Noticeably, the intra-organelle localizations of carotenogenic enzymes do

not fully overlap with those of carotenoid metabolites. It is possible that an unknown trafficking pathway exists. Plastoglobules containing some carotenoid metabolites and enzymes (Figure 3) are attached to thylakoids, and plastoglobule number in chloroplasts is correlated with thylakoid biogenesis (Brehelin et al., 2007; van Wijk and Kessler, 2017), which make plastoglobules the possible structures for metabolite trafficking to thylakoids.

Carotenoids in chloroplasts function mainly as photosynthetic pigments and photoprotectors. Without carotenoids, chloroplasts could not function properly and thus plants would not survive. While knocking out *PSY* and *PDS* in the photosynthetic tissues result in total abolishment of photosynthesis, mutations in xanthophyll biosynthetic genes do not cause lethal phenotype but negatively impact photoprotection (Pogson et al., 1996; Tian and DellaPenna, 2001; Kim and DellaPenna, 2006). Photoprotection is one mechanism that plants have employed to better survive under high light stress. Carotenoids play irreplaceable roles in this process by quenching chlorophyll-excited states, scavenging reactive oxygen species, and dissipating excess energy into heat. The photoprotective mechanism referred to as non-photochemical quenching (NPQ) in vascular plants includes two xanthophyll cycles, the violaxanthin cycle and lutein epoxide cycle, to convert excess excitation energy into harmless heat (Leonelli et al., 2017; Leuenberger et al., 2017). Other than high light stress, recent studies show that the ethylene signaling and pathogens also affect NPQ (Chen and Gallie, 2015; Zhou et al., 2015a), suggesting that xanthophyll cycles provide plasticity for chloroplasts to better adapt to environmental stresses. Additionally, some carotenoids that accumulate outside of the thylakoids are believed to protect cells and be important stress response (Solovchenko and Neverov, 2017).

Carotenoids in chloroplasts are also converted into apocarotenoids that serve as signal molecules. Beyond the well-known phytohormones ABA and SLs (Finkelstein, 2013; Al-Babili and Bouwmeester, 2015), evidence is emerging that supports the regulatory roles of additional apocarotenoids throughout plant development (McQuinn et al., 2015; Tian, 2015; Hou et al., 2016). An uncharacterized apocarotenoid signal derived from cleavage of phytofluene and/or ζ-carotene in a ζ-carotene desaturase mutant alters leaf development with needle-like, translucent leaves and greatly affects chloroplast biogenesis through regulating the expression of nuclear and plastidial encoded genes (Avendano-Vazquez et al., 2014). Non-enzymatically generated

apocarotenoids, β-cyclocitral and dihydroactinidiolide, regulate the expression of stress related genes to enhance high light acclimation, possibly via salicylic acid signaling cascades (Ramel et al., 2012; Shumbe et al., 2014; Lv et al., 2015a; Shumbe et al., 2017). In addition, a carotenoid-derived signal was found to regulate lateral root branching in Arabidopsis (Van Norman et al., 2014). Since a large number of carotenoid derivatives exist in chloroplasts, some ambiguities include how to define a carotenoid-derived molecule as a signal molecule, and how plant cells perceive the signals. Are receptors like ABA receptors required or do the molecules pass signals by other means?

A number of regulatory mechanisms for carotenogenesis in chloroplasts are known and some are illustrated here (Figure 3). Transcriptional regulation of carotenoid biosynthetic genes plays a central role and has been investigated extensively in leaf tissues, although much remains to be revealed (Lu and Li, 2008; Ruiz-Sola and Rodríguez-Concepción, 2012; Nisar et al., 2015). Carotenogenic genes are activated/deactivated in response to both developmental and environmental signals (Ruiz-Sola and Rodríguez-Concepción, 2012; Nisar et al., 2015). Light signal and photoperiod are important for chloroplast function and affect transcript levels of carotenoid biosynthetic genes. Direct bindings of PIFs and HY5 to the G-box element in the *PSY* promoter provide a link between light signaling and carotenoid biosynthetic gene expression (Toledo-Ortiz et al., 2010; Bou-Torrent et al., 2015; Llorente et al., 2017) (Figure 3). Transcription factor RAP2.2 was found to bind to the ATCTA element of the *PSY* and *PDS* promoters to negatively regulate carotenogenesis in Arabidopsis (Welsch et al., 2007) (Figure 3). Epigenetic modification by a histone methyltransferase (SDG8) to specifically regulate *CrtISO* provides a fine-tuning mechanism in regulating carotenoid biosynthesis in chloroplasts (Cazzonelli et al., 2009; Cazzonelli et al., 2010) (Figure 3).

Post-translational regulation is critical to maintain functional forms of the carotenogenic enzymes in plants. Arabidopsis *PSY* has two alternative splice variants with different 5'UTRs. The different lengths of 5'UTRs exert different translation efficiency to regulate PSY enzyme activity (Álvarez et al., 2016) (Figure 3). This kind of translational regulation provides an alternative for the transcriptional control by using different copies of *PSY* found in other species. Deoxyxylulose 5-phosphate synthase (DXS), the first enzyme of plastidial isoprenoid pathway,

was found to be controlled by J-protein J20 via protein-protein interactions. J20 specifically targets misfolded or unfolded DXS, and delivers DXS to the HSP70 system for refolding and function (Pulido et al., 2013; Pulido et al., 2016; Pulido et al., 2017). GGPS11 interacts directly with PSY to mediate flux into carotenoid biosynthesis (Ruiz-Sola et al., 2016) (Figure 3). Moreover, the OR proteins were discovered to be the major post-translational regulators of PSY to regulate the PSY protein level and enzymatic activity in controlling carotenoid production (Zhou et al., 2015b; Park et al., 2016; Chayut et al., 2017) (Figure 3). Chloroplast protease systems are important for chloroplast proteome turnover (Nishimura et al., 2016). A recent study suggests that CCD4 could be a substrate of plastoglobule-localized metallopeptidase PGM48 (Bhuiyan and van Wijk, 2017) (Figure 3).

Carotenoid biosynthesis is also influenced by factors that affect carotenogenic enzyme activity (Figure 3). Redox state regulates carotene desaturases. PTOX serves as a redox regulator in phytoene desaturation to regulate carotenoid content in chloroplasts (Wu et al., 1999; Kambakam et al., 2016). Thylakoid localized VDE is sensitive to redox state and the disulfide bonds of VDE protein were found to be responsible for perceiving redox state (Hallin et al., 2015; Simionato et al., 2015). Z-ISO also controls carotenogenesis in a redox-regulated manner through a heme cofactor (Beltrán et al., 2015). These studies expand our knowledge on the regulation of carotenogenesis in plants.

Carotenogenic enzymes localize in the envelope membranes (Bonk et al., 1997; Gemmecker et al., 2015). However, only a few proteins are predicted to contain transmembrane domains, which suggests the formation of protein complexes to associate them with membranes (Ruiz-Sola and Rodríguez-Concepción, 2012). Indeed, some carotenoid biosynthetic enzymes were found in large protein complexes in chloroplasts (Bonk et al., 1997). PDS exists in two complexes in chloroplasts with one in the membrane fraction as a 360 kDa complex and the other in stroma as a 660 kDa complex in Arabidopsis (Lopez et al., 2008). *In vitro* experiments support the oligomeric assembly with membranes for the PDS protein (Gemmecker et al., 2015). GGPPS11 was found to serve as a hub isozyme for the formation of the membrane-associated carotenoid enzyme complex via physical interactions with PSY, GGR and SPS2 (Ruiz-Sola et al., 2016).

The formation of enzyme complexes has been suggested to facilitate metabolic channeling for carotenoid biosynthesis (Ruiz-Sola and Rodríguez-Concepción, 2012).

As being indispensable components of the photosynthetic apparatus, carotenoids are synthesized in a coordinated manner with chlorophyll biosynthesis in chloroplasts. Thus, defects in chlorophyll biosynthesis and chloroplast formation usually result in reduced carotenoid biosynthesis. Concomitantly, the coordinated biosynthesis of carotenoids and chlorophylls in chloroplasts also restrains further carotenoid accumulation in green leaf tissues following up-regulation of carotenoid biosynthetic and regulatory genes (Maass et al., 2009; Yuan et al., 2015b). Currently, the genes that directly coordinate carotenoid and chlorophyll biosynthesis in chloroplasts remain to be unknown.

CHROMOPLASTS 'A COLORFUL WORLD'

As the main plastids that synthesize and store carotenoids in flowers, fruits, and roots, chromoplasts contain diverse kinds and levels of carotenoids in different organs and species, unlike other types of plastids. The diversity of carotenoids in chromoplasts produces colorful plant organs to attract insects and animals for pollination and seed dispersal (Lu and Li, 2008; Cazzonelli, 2011; Nisar et al., 2015).

Chromoplasts are fully developed and usually terminated plastids. They are derived from all other plastids, including proplastids, amyloplasts, and chloroplasts, although the molecular basis underlying chromoplast differentiation remains unknown (Egea et al., 2010; Li and Yuan, 2013; Li et al., 2016). Chromoplasts are commonly seen to be derived from chloroplasts in fruits and vegetables that undergo green to yellow or red color change during ripening, such as in tomato (Egea et al., 2011; Suzuki et al., 2015), bell pepper (Spurr and Harris, 1968), and grapefruit peel (Lado et al., 2015a). Chromoplasts also evolve from proplastids and amyloplasts in non-photosynthetic tissues, such as in orange cauliflower mutant (Li et al., 2001), carrot root (Kim et al., 2010), papaya (Schweiggert et al., 2011), and citrus pulp (Lado et al., 2015b; Zeng et al., 2015b). The conversion into chromoplasts is marked by massive carotenoid accumulation

following dramatic membrane remodeling and the formation of carotenoid-lipoprotein sequestration substructures within chromoplasts.

Based on the pigment-bearing sequestration substructures, chromoplasts are classified into five major types as globular, crystalline, membranous, fibrillar, and tubular (Egea et al., 2010; Li and Yuan, 2013; Schweiggert and Carle, 2017). Usually more than one kind of chromoplasts coexist in a species. Globular chromoplasts are characterized by abundant plastoglobules, which are found in many plants, such as in mango, yellow papaya, tomato (Jeffery et al., 2012), Bixa seed (Louro and Santiago, 2016), saffron stigma (Gómez-Gómez et al., 2017), and citrus (Lado et al., 2015a; Lado et al., 2015b; Zeng et al., 2015b; Lu et al., 2017). Crystalline chromoplasts typically overaccumulate lycopene and β-carotene as red/orange crystals, and are abundant in tomato (Jeffery et al., 2012), watermelon (Jeffery et al., 2012; Zhang et al., 2017), and carrot (Kim et al., 2010). Interestingly, while all-*trans*-β-carotene is deposited as crystalline type, its *cis* isomers are in globular chromoplasts (Vasquez-Caicedo et al., 2006). Membranous chromoplasts are typified by the large amount of condensed whirly multi-layer membrane structures, as shown in daffodil (Hansmann et al., 1987) and orange curd cauliflower (Paolillo et al., 2004). Fibrillar chromoplasts contain spindle-shaped fibrils and are best known in red pepper (Kilcrease et al., 2013). Tubular chromoplasts display an elongated tube-shaped appearance with numerous tubules arranged in bundles, as shown in yellow-fleshed papaya (Schweiggert et al., 2011). In addition, unique storage membrane structures for specific apocarotenoids, such as “crocinoplast” for crocins and osmiophilic bodies and vesicles for bixin and norbixin, were noted recently in the vacuoles of carotenoid storage cells in saffron stigma and Bixa seed arils, respectively (Louro and Santiago, 2016; Gómez-Gómez et al., 2017). Large amounts of lipophilic droplets connected by stromules, the stroma-filled tubular extensions, for C_{13} apocarotenoid accumulation were also observed in the vacuoles of root cells during late stages of arbuscular mycorrhizal symbiosis and arbuscule senescence (Strack and Fester, 2006). The highly heterogeneous nature or plasticity of carotenoid sequestering substructures in chromoplasts likely contributes to the diverse kinds and variable levels of carotenoids in fruits, vegetables, and roots. This has been documented from examining chromoplasts from various citrus fruits (Lado et al., 2015b) and pepper fruits (Kilcrease et al., 2015).

The various carotenoid-lipoprotein sequestering substructures serve two distinctive roles for massive accumulation in chromoplasts (Vishnevetsky et al., 1999; Li et al., 2016). One role is to sequester the newly synthesized carotenoids into pigment-lipoprotein substructures within chromoplasts for stable storage. For example, a study reveals that the sequestered carotenoids exhibit remarkable photostability *in situ* (Merzlyak and Solovchenko, 2002). The other role is to stimulate continuous biosynthesis by removing the newly synthesized carotenoids from plastid envelope membranes to avoid overloading of end-products at the site of carotenoid biosynthesis. As such, it is not surprising to find that formation of pigment-lipoprotein sequestering substructure or increase of chromoplast compartment size/number strongly correlates with elevated carotenoid accumulation (Li and Yuan, 2013). Dramatic remodeling of membrane system, including increased number and size of plastoglobules, high frequency of membranous formation, and increased stromules were noticed during chromoplast formation with carotenoid overproduction (Bian et al., 2011; Nogueira et al., 2013; Lado et al., 2015b). Studies of tomato *high pigment* mutants provides evidence for a direct link between carotenoid levels and plastid compartment size and number (Liu et al., 2004; Kolotilin et al., 2007; Galpaz et al., 2008). Esterification of carotenoids also modulates carotenoid content because of its role in facilitating sequestration and enhancing carotenoid stability (Ariizumi et al., 2014). Unlike chloroplasts with restrained sequestration of carotenoids to maintain proper carotenoid/chlorophyll ratios for optimal photosynthesis and photoprotection, chromoplasts possess much higher capacities with great metabolic sink strength for massive carotenoid accumulation.

Chromoplasts are the main organelles for carotenoid accumulation. Thus, regulation of chromoplast biogenesis exerts profound influence on total carotenoid accumulation. In cauliflower, the transcript levels of carotenoid metabolic pathway genes are similar in white and orange curd of the *Or* mutant (Li et al., 2001; Lu et al., 2006). Similarly, no significant differences in the expression of carotenoid pathway genes are noted in orange and non-orange flesh melon fruits (Chayut et al., 2015). The metabolic flux rates are also comparable between orange and non-orange tissues in both cauliflower and melon fruit (Li et al., 2006; Chayut et al., 2017). A single 'golden SNP' in melon *Or* gene alters a highly-conserved arginine to histidine in the OR protein and is responsible for β-carotene accumulation in chromoplasts from a broad germplasm collection (Tzuri et al., 2015). While OR regulates PSY protein level and enzymatic

activity (Zhou et al., 2015b; Park et al., 2016), the 'golden SNP'-induced carotenoid accumulation in orange tissues does not directly correlate with the biosynthetic activity. Further investigation of the underlying mechanism reveals that the high level of carotenoid accumulation is due to the unique ability of the 'golden SNP' in mediating chromoplast biogenesis or plastid fate change (Yuan et al., 2015a; Chayut et al., 2017). Another example is in a red-flesh sweet orange mutant, in which the massive accumulation of carotenes is not explained by carotenogenic activity, but by distinct crystalline chromoplast development (Lu et al., 2017). These studies demonstrate the crucial role of chromoplast biogenesis for carotenoid accumulation.

Chromoplast differentiation represents a complicated process that is not understood (Egea et al., 2010; Li et al., 2016). Several non-carotenogenic genes and proteins have been shown to be associated with chromoplast formation and development. They include a small molecular chaperone and a plastid encoded fatty acid biosynthetic enzyme accD in tomato fruit (Neta-Sharir et al., 2005; Kahlau and Bock, 2008), a plastid fusion and/or translocation factor (Pftf) in pepper fruit (Hugueney et al., 1995), and fibrillin proteins (Deruère et al., 1994; Kilambi et al., 2013; Kilcrease et al., 2015). However, the genes and pathways that control chromoplast biogenesis remain largely unknown. *Or* represents the only known gene as a *bona fide* molecular switch to trigger chromoplast differentiation in cauliflower (Li et al., 2001; Lu et al., 2006) and orange melon fruit (Tzuri et al., 2015; Chayut et al., 2017). Its specific role in initiating chromoplast biogenesis is documented by ectopic expression of the *Or* transgene in potato tubers and Arabidopsis calli (Lopez et al., 2008; Yuan et al., 2015a). In the latter case, typical membranous chromoplasts as found in orange cauliflower mutant (Paolillo et al., 2004) were clearly observed under TEM in the *Or*-induced orange calli (Yuan et al., 2015a). Although *Or* is known to have dual functions in mediating carotenoid accumulation, the mechanism by which *Or* governs chromoplast biogenesis remains unknown.

A mechanism of carotenoid metabolite-induced chromoplast biogenesis was postulated following the observation of plastid transition to form chromoplast-like structures in the premature fruits of tomato overexpressing *PSY1* (Fraser et al., 2007). Indeed, chromoplast differentiation is strongly associated with carotenoid production. Moreover, the formation of new

and distinctive types of chromoplasts has been found to correlate with the accumulation of specific carotenoids. The accumulation of lycopene in red-flesh citrus fruits causes the formation of crystalline chromoplasts (Lado et al., 2015b; Lu et al., 2017). Similarly, the 2-(4-Chlorophenylthio)-triethylamine hydrochloride (CPTA)-induced lycopene production leads to the development of crystalline chromoplasts instead of globular chromoplasts in the normal citrus fruits (Lu et al., 2017). While it is clear that carotenoid metabolites induce chromoplast substructure changes, whether and how the metabolites by themselves initiate chromoplast biogenesis remain to be elucidated.

Multifaceted regulation of carotenoid metabolism has been revealed in chromoplast-containing tissues (Ruiz-Sola and Rodríguez-Concepción, 2012; Nisar et al., 2015; Yuan et al., 2015b). Transcriptional regulation of the pathway genes prevails in chromoplasts during fruit ripening from green to red/yellow in response to developmental signals, as shown in tomato (Ronen et al., 1999) and pepper (Hugueney et al., 1996). Transcriptional regulation also provides the first layer control of carotenoid production in many other species during ripening (Wei et al., 2014; Chayut et al., 2015) and flowering (Sagawa et al., 2016). Suppression of downstream gene expression by mutations or other unidentified mechanisms results in the accumulation of various specific upstream products in chromoplasts (Blas et al., 2010; Galpaz et al., 2013; Lv et al., 2015b; Zhang et al., 2015). Post-translational regulation to modulate pathway enzyme activities provides another layer of control for carotenoid production. This regulation includes membrane association (Al-Babili et al., 1996; Schledz et al., 1996; Ahrazem et al., 2016) and protein-protein interactions (Luo et al., 2013; Park et al., 2016; Chayut et al., 2017). The formation of enzyme complexes also facilitates metabolite channeling to drive flux toward completion (Ruiz-Sola et al., 2016). Other layers of regulation include feedback regulation of pathway genes and enzymes by carotenoid metabolites as shown in tomato fruits (Kachanovsky et al., 2012) and in carrot (Arango et al., 2014), as well as a negative feedback and feed-forward mechanism as revealed by investigating *CrtI* in the tomato *tangerine* and *old gold crimson* mutants (Enfissi et al., 2017). Epigenetic regulation of gene expression has been shown to affect tomato fruit ripening and carotenoid accumulation (Zhong et al., 2013). The multifaceted regulatory mechanisms also contribute to the diverse kinds and levels of carotenoids in chromoplasts that give the colorful organs.

Carotenogenesis in chromoplasts is controlled by developmental and environmental signals (Figure 3) (Ruiz-Sola and Rodríguez-Concepción, 2012; Fanciullino et al., 2014; Liu et al., 2015; Nisar et al., 2015). During tomato fruit ripening, developmentally-regulated chlorophyll degradation reduces the self-shading effect, which promotes PIF1a turnover and results in specific activation of PSY expression and carotenoid biosynthesis (Llorente et al., 2016) (Fig. 3). Bursts of carotenoid levels in chromoplasts are tightly linked with the ripening process in many fruits. A large number of ripening related transcription factors, particularly those that regulate ethylene production, were found to affect carotenoid levels in tomato fruits (Liu et al., 2015). However, only a few of them (RIN and SGR1) are documented to directly modulate transcription of the carotenogenic genes (Martel et al., 2011; Fujisawa et al., 2013; Luo et al., 2013) (Figure 3). In addition, phytohormones and light signaling all impact carotenoid levels in chromoplasts, and their influences on carotenoid metabolism in tomato fruits are reviewed recently (Liu et al., 2015). Chromoplast differentiation and carotenoid accumulation are promoted by shade in grapefruit (Lado et al., 2015a) and by dark in carrot roots (Fuentes et al., 2012). A putative repressor of photomorphogenesis encoded by the *Y* locus is proposed to be genetically associated with carotenogenesis in carrot root (Iorizzo et al., 2016). While various phytohormones such as ethylene, ABA, auxin, gibberellic acid, and jasmonic acid influence carotenoid levels (Liu et al., 2015), whether they directly regulate carotenoid metabolic pathway genes and enzymes is unclear.

MANIPULATION OF CAROTENOIDS IN PLASTIDS

Carotenoids are essential in human diets. Plant foods with low levels of β-carotene contribute to the worldwide prevalence of vitamin A deficiency, which has prompted global efforts to produce provitamin A enriched ‘golden’ staple crops (Bai et al., 2011; Wurtzel et al., 2012; Giuliano, 2017). Some best examples include orange maize that contains favorable alleles of lycopene ε-cyclase and β-carotene hydroxylase 1 generated by conventional breeding (Harjes et al., 2008; Yan et al., 2010), and Golden Rice generated by transforming *PSY* and the bacterial *CrtI* through biotechnology approach (Ye et al., 2000; Paine et al., 2005). Consumption of provitamin A

carotenoid–biofortified crops has been shown to significantly improve vitamin A status in humans (http://www.harvestplus.org/; http://www.goldenrice.org/).

Metabolic engineering of carotenoid metabolic pathway has generated a large number of crops with substantial alteration of carotenoids (Farré et al., 2011; Giuliano, 2017). Since the levels of carotenoids in plants are determined by the rate of biosynthesis, the rate of degradation, and the plastid sink strength (Cazzonelli and Pogson, 2010; Li and Yuan, 2013) (Figure 1), strategies to manipulate these processes are utilized in promoting carotenoid production in crops.

Most efforts have been focused on increasing metabolic flux in plastids by overexpression of one or more flux control enzymes as a 'push' strategy (Giuliano et al., 2008; Zhu et al., 2013; Zhai et al., 2016; Giuliano, 2017). PSY is the major rate-limiting enzyme and directs metabolic flux into carotenoid biosynthesis to define the size of carotenoid pool. Thus, it is the main target for modification. Selection of PSY variants with high activity was found to profoundly affect total carotenoid accumulation as documented in Golden Rice II (Paine et al., 2005). Overexpression of *PSY* with or without a versatile bacterial desaturase/isomerase gene *CrtI* successfully increases β-carotene and total carotenoids in many staple crops including rice, potato, maize, wheat and banana as well as canola (Shewmaker et al., 1999; Ye et al., 2000; Ducreux et al., 2005; Paine et al., 2005; Diretto et al., 2007a; Zhu et al., 2008; Wang et al., 2014; Paul et al., 2016).

Reducing metabolic flux downstream or reducing the degradation rate of carotenoids is used as a 'block' strategy (Giuliano, 2017). Down-regulation of specific pathway enzymes typically results in the accumulation of upstream metabolites as shown by silencing of β-carotene hydroxylase for β-carotene accumulation in potato and sweet potato (Diretto et al., 2007b; Van Eck et al., 2007; Kang et al., 2017). Carotenoid cleavage dioxygenase enzymes, CCD1, CCD2 and CCD4, are responsible for carotenoid degradation and depletion of the carotenoid pool (Ohmiya et al., 2006; Falchi et al., 2013; Frusciante et al., 2014; Ahrazem et al., 2016; Gonzalez-Jorge et al., 2016). They are potential targets for engineering as shown in potato (Campbell et al., 2010).

Increasing the carotenoid sink capacity is another major determinant for carotenoid accumulation in plastids as a 'pull' strategy (Li and Van Eck, 2007; Lu and Li, 2008; Giuliano, 2017). Plastid type, number, size and specific storage substructure all govern the sink capacity to affect carotenoid sequestration and storage. Various types of plastids display different capacities in synthesizing and sequestering carotenoids with etioplast, amyloplast, chloroplast, and chromoplast from low to high levels (Ruiz-Sola and Rodríguez-Concepción, 2012; Li et al., 2016) (Figure 2). Thus, engineering plant carotenoids in different plastids results in different carotenoid content and stability (Li et al., 2012; Schaub et al., 2017). *Or*, the *bona fide* molecular switch for chromoplast biogenesis, exerts dual functions in posttranslational regulation of PSY activity and initiating chromoplast differentiation (Lu et al., 2006; Yuan et al., 2015a; Zhou et al., 2015b; Chayut et al., 2017). Manipulation of wild-type *Or* has been shown to increase carotenoid levels in several plant species (Park et al., 2015; Wang et al., 2015; Bai et al., 2016; Berman et al., 2017), likely due to its function in regulating PSY and plastid development. Overexpression of the mutant allele results in chromoplast formation with substantially enhanced carotenoid levels (Lu et al., 2006; Lopez et al., 2008; Li et al., 2012; Yuan et al., 2015a), owing to the specific characteristics of chromoplasts in providing high capacity for carotenoid biosynthesis and stable storage. Indeed, biochemical and genetic studies of *Or* in melon reveals its role in stabilizing and inhibiting β-carotene turnover (Chayut et al., 2017). Currently, although there are limited genetic tools available to manipulate the plastid sink strength, the successful demonstration of the effectiveness of *Or* shows the importance of this strategy in promoting carotenoid accumulation. However, it should be noted that alteration of sink capacity alone is not enough without adequate biosynthetic activity.

Since carotenoid accumulation reflects a dynamics of multiple processes, a combination of various strategies is likely to result in further carotenoid enhancement. This has been documented in β-carotene enrichment in wheat endosperm, ketocarotenoid production in maize endosperm, and carotenoid improvement in rice endosperm (Zeng et al., 2015a; Farré et al., 2016; Bai et al., 2016). Because biosynthesis provides the driving force to increase the metabolic flux pool, it is critically important for archiving high levels of carotenoid production. Alteration of the sink strength, particularly by inducing chromoplast biogenesis, provides the plastids with high potential and capacity for continuous biosynthesis and stable accumulation.

Metabolic engineering of carotenoids in staple crops by the 'push' strategy usually results in carotenoid accumulation in amyloplasts (Paine et al., 2005; Bai et al., 2016; Mortimer et al., 2016). The accumulated carotenoids are prone to degradation during final stage of maturation and post-harvest storage (Farré et al., 2013; De Moura et al., 2015; Che et al., 2016; Schaub et al., 2017). Both enzymatic and non-enzymatic oxidation of carotenoids lead to carotenoid turnover (Gayen et al., 2015; Gonzalez-Jorge et al., 2016; Qin et al., 2016). By inducing chromoplast biogenesis in staple crops, it likely further facilitates carotenoid biosynthesis and possibly enhances storage stability due to the specific characteristics of chromoplasts (Li et al., 2012). The discovery of the 'golden SNP' of *Or* in melon and proof of its effectiveness in promoting chromoplast formation and carotenoid accumulation (Tzuri et al., 2015; Yuan et al., 2015a) provide an opportunity for genome editing to create functional alleles of *Or* in manipulating plastid sink strength in staple crops. Further identification of the genes and elucidation of the molecular mechanisms underlying chromoplast biogenesis may create additional genetic tools and strategies to facilitate carotenoid biosynthesis and enable carotenoid stable storage in plastids of the staple crops.

CONCLUSIONS

Although the genes and enzymes in the carotenoid metabolic pathway have been extensively studied, the regulation of carotenoid accumulation remains to be fully elucidated. Since plastids are the sites for carotenoid metabolism, plastid types profoundly impact carotenoid biosynthesis and accumulation. The unique functions of various plastids govern the regulatory networks of carotenoid metabolism and define their capacities for carotenoid biosynthesis and sequestration, which results in diverse amounts and kinds of carotenoids in plant organs. Increasing evidence supports the critical role of sequestration substructures in carotenoid biosynthesis and final accumulation in plastids.

Both opportunities and challenges exist in unraveling the regulatory mechanisms of carotenoid metabolism in different types of plastids and in manipulation of carotenoids in staple crops. The advanced "omics" technologies and genome wide association analyses could facilitate our

understanding of the complex carotenoid metabolic networks and expedite the identification of novel genes involved in carotenoid metabolism. Insights generated from characterization of new regulators and understanding the intrinsic regulatory mechanisms could contribute greatly to advance our current knowledge on the regulatory control of carotenoid metabolism and generation of novel strategies for carotenoid enrichment in crops. Enhancing metabolic sink strength for carotenoids in plastids, particularly by induction of chromoplast formation from amyloplasts along with elevating biosynthetic activity could hold enormous promise for biofortification of staple crops with increased carotenoid content and stability to improve human nutrition and health.

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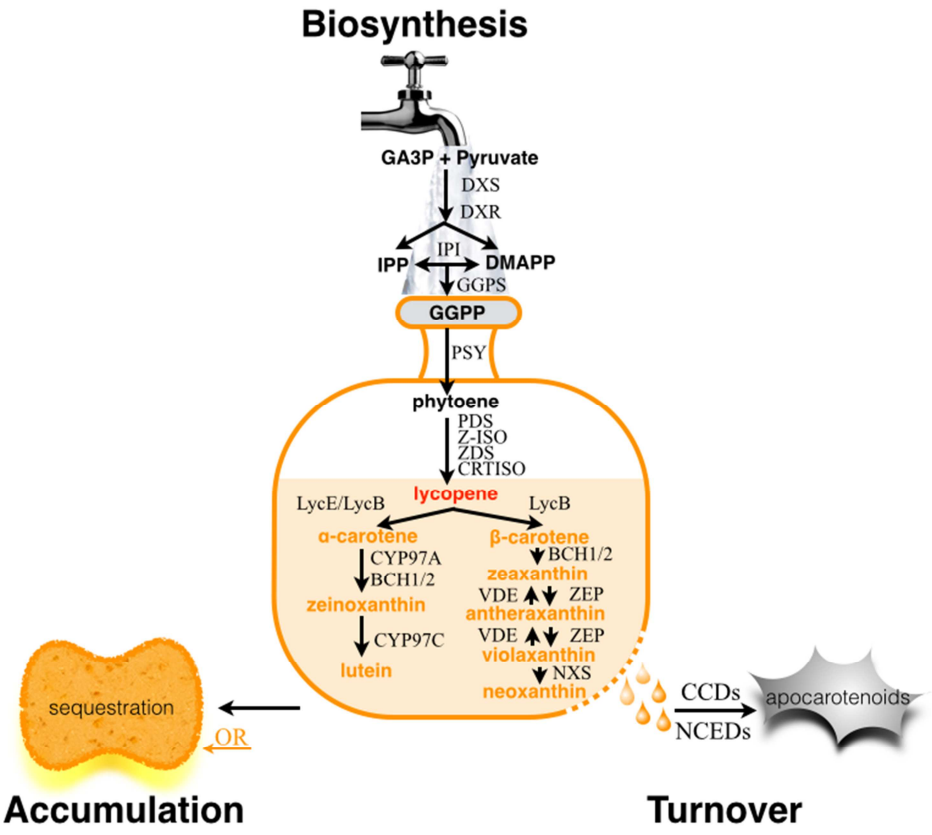
FIGURE LEGENDS

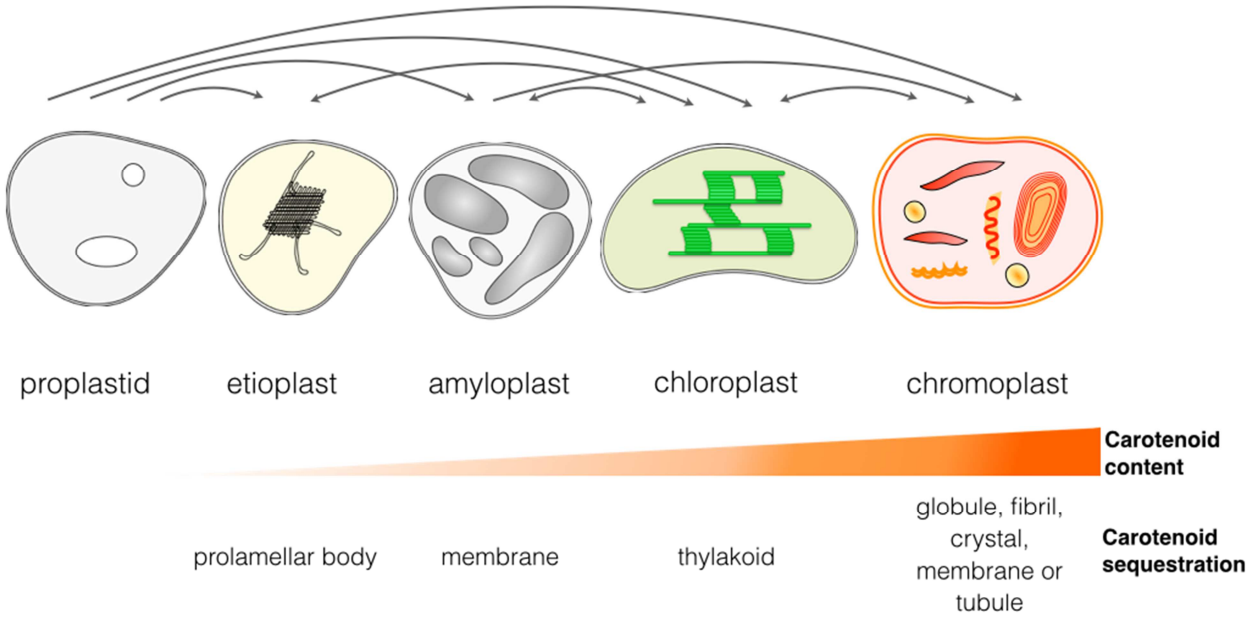
Figure 1. Schematic Representation of Plant Carotenoid Metabolism Pathway in Plastids. Carotenoid biosynthesis begins with the formation of C5 IPP and DMAPP via MEP pathway. The committed step for carotenoid biosynthesis involves the condensation of two C20 GGPPs into C40 carotenoid phytoene catalyzed by PSY, the "bottleneck" enzyme that affects carotenoid pool size. Lycopene cyclization yields α- and β-branch of the pathway. Enzymatic oxidative cleavages of carotenoids by CCDs or NCEDs produce apocarotenoids and control carotenoid turnover. Sequestration mechanisms influence carotenoid stable storage in plastids. OR represents a molecular switch to initiate chromoplast biogenesis. Genetic engineering includes the 'push' strategy for biosynthesis, 'block' strategy for reducing metabolic flux toward downstream or reducing turnover, and 'pull' strategy for sequestration (Giuliano, 2017).

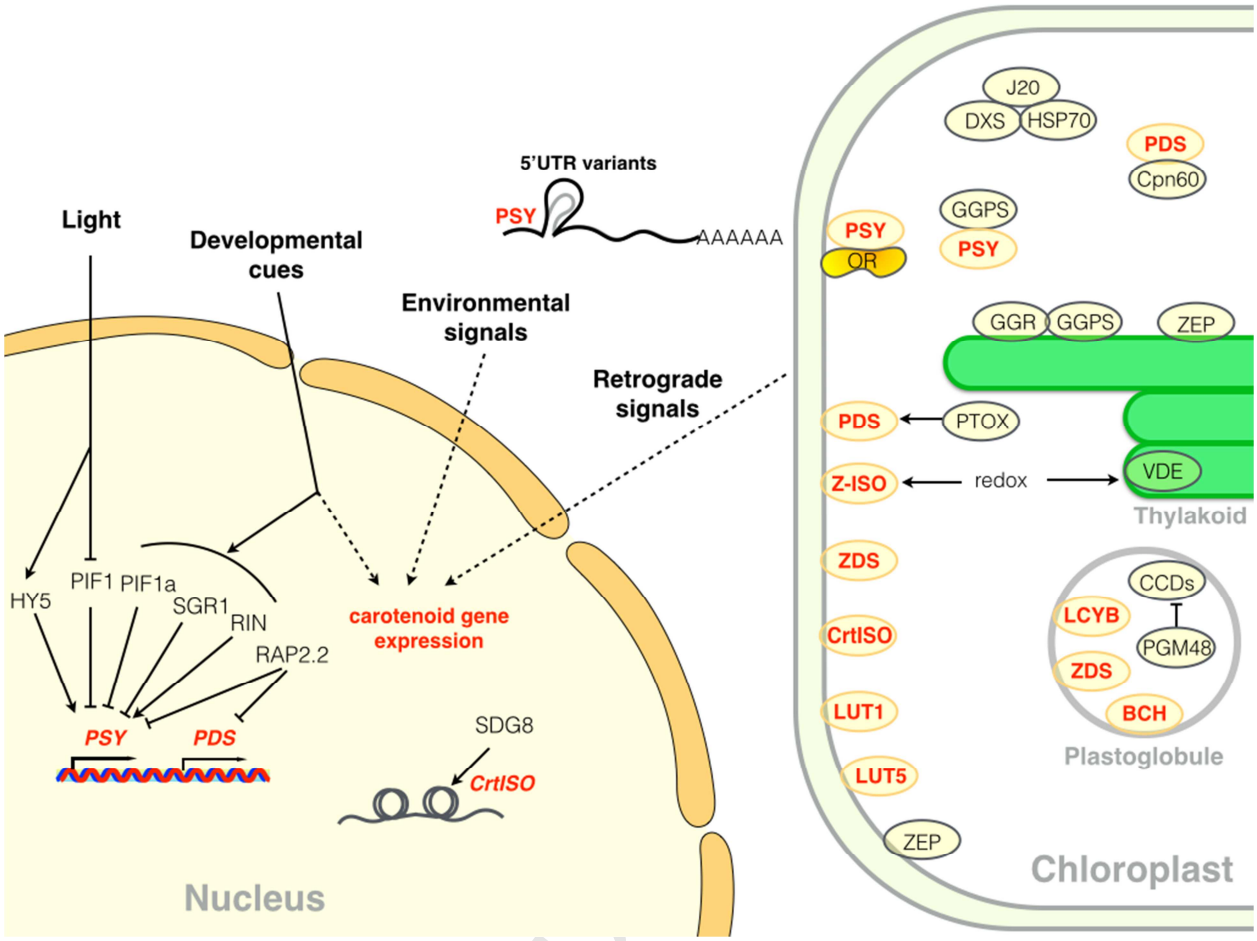
GA3P, glyceraldehyde 3-phosphate; DXS, 1-deoxy-d-xylulose 5-phosphate synthase; DXR, 1-deoxy-d-xylulose 5-phosphate reductoisomerase; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GGPP, geranylgeranyl diphosphate; GGPPS, geranylgeranyl pyrophosphate synthase; PSY, phytoene synthase; PDS, phytoene desaturase; Z-ISO, ζ-carotene isomerase; CrtISO, carotene isomerase; LycB, lycopene β-cyclase; LycE, lycopene ε-cyclase; BCH, β-carotene hydrolase; ZEP, zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase; NXS, neoxanthin synthase; CCD, carotenoid cleavage dioxygenases; NCED, 9-cis-epoxycarotenoid dioxygenase; OR, ORANGE protein.

Figure 2. Plastid Types and Sequestration Structures in Relation to Carotenoid Accumulation. Proplastid is the progenitor of all other types of plastids and not considered as a carotenogenic plastid. Etioplast is a dark developed plastid with limited activity of carotenoid biosynthesis. Amyloplast is a starch-containing plastid with low to mediate range of carotenoid accumulation. Chloroplast defines plants with coordinated biosynthesis of carotenoids and chlorophylls. Chromoplast is a carotenoid-accumulating plastid that contributes to the diverse color of plant organs. Interconversions of various types of plastids are indicated by fine pointed lines.

Figure 3. Overview of the Multifaceted Regulation of Carotenogenesis. Transcriptional regulation of the carotenoid pathway genes is controlled by internal factors such as histone modification, developmental programming, retrograde signals from organelles, as well as external cues such as light signaling and other environmental signals. Transcription factors that are known to directly control carotenogenic gene expression are indicated. Other layers of regulation to affect pathway enzyme activities are not well understood although multiple mechanisms are known, which are exemplified in chloroplasts as depicted. They include protein-protein interactions, membrane associations, enzyme complexes for metabolic channeling, and redox regulation. The localizations of carotenoid pathway enzymes within chloroplasts are shown based on subplastidial proteomic identifications (Ytterberg et al., 2006; Joyard et al., 2009; Bruley et al., 2012).


Biosynthesis
GA3P + Pyruvate
DXS
DXR
IPP
IPI
DMAPP
GGPS
GGPP
PSY
phytoene
PDS
Z-ISO
ZDS
CRTISO
lycopene
LycE/LycB
LycB
α-carotene
CYP97A
BCH1/2
zeinoxanthin
CYP97C
lutein
β-carotene
BCH1/2
zeaxanthin
VDE
ZEP
antheraxanthin
VDE
ZEP
violaxanthin
NXS
neoxanthin
sequestration
OR
CCDs
NCEDs
apocarotenoids
Accumulation
Turnover


proplastid
etioplast
amyloplast
chloroplast
chromoplast
Carotenoid content
prolamellar body
membrane
thylakoid
globule, fibril, crystal, membrane or tubule
Carotenoid sequestration


Light
Developmental cues
Environmental signals
Retrograde signals
5'UTR variants
PSY
AAAAAA
HY5
PIF1
PIF1a
SGR1
RIN
RAP2.2
carotenoid gene expression
PSY
PDS
SDG8
CrtISO
Nucleus
J20
DXS
HSP70
PDS
Cpn60
GGPS
PSY
PSY
OR
GGR
GGPS
ZEP
PDS
PTOX
Z-ISO
redox
VDE
Thylakoid
ZDS
CrtISO
LUT1
LUT5
ZEP
CCDs
LCYB
PGM48
ZDS
BCH
Plastoglobule
Chloroplast