Progress in Lipid Research 84 (2021) 101128

Contents lists available at ScienceDirect

Progress in Lipid Research

journal homepage: www.elsevier.com/locate/plipres

Review

Making extra room for carotenoids in plant cells: New opportunities for biofortification

Salvador Torres-Montilla, Manuel Rodriguez-Concepcion *

Institute for Plant Molecular and Cell Biology (IBMCP), Agencia Estatal Consejo Superior de Investigaciones Cientificas - Universitat Politècnica de València, 46022 Valencia, Spain

ARTICLE INFO

Keywords:
Biofortification
Biotechnology
Carotenoid
Chromoplast
Storage

ABSTRACT

Plant carotenoids are essential for photosynthesis and photoprotection and provide colors in the yellow to red range to non-photosynthetic organs such as petals and ripe fruits. They are also the precursors of biologically active molecules not only in plants (including hormones and retrograde signals) but also in animals (including retinoids such as vitamin A). A carotenoid-rich diet has been associated with improved health and cognitive capacity in humans, whereas the use of carotenoids as natural pigments is widespread in the agrofood and cosmetic industries. The nutritional and economic relevance of carotenoids has spurred a large number of biotechnological strategies to enrich plant tissues with carotenoids. Most of such approaches to alter carotenoid contents in plants have been focused on manipulating their biosynthesis or degradation, whereas improving carotenoid sink capacity in plant tissues has received much less attention. Our knowledge on the molecular mechanisms influencing carotenoid storage in plants has substantially grown in the last years, opening new opportunities for carotenoid biofortification. Here we will review these advances with a particular focus on those creating extra room for carotenoids in plant cells either by promoting the differentiation of carotenoid-sequestering structures within plastids or by transferring carotenoid production to the cytosol.

1. Introduction

Carotenoids are isoprenoid compounds produced by all photosynthetic organisms (including plants, algae and cyanobacteria) as well as some non-photosynthetic archaea, bacteria, fungi and animals [1]. In plants, carotenoids can be considered as both primary (essential) and secondary (specialized) metabolites. Carotenoids such as lutein, beta-carotene, violaxanthin, and neoxanthin are required for photosynthesis and photoprotection [2,3]. The absence of carotenoids in plants, due to a genetic or chemical inhibition of the pathway, hence results in a non-viable albino phenotype [4–6]. Also essential is their function as precursors of important phytohormones regulating plant development, growth or stress responses, such as abscisic acid (ABA) or strigolactones [7,8] (Fig. 1). Carotenoids can also be considered specialized metabolites due to their role as communication signals between plants and their environment. Most carotenoids are pigments that provide distinctive colors to the fruits and flowers of some plants for attracting animals for seed dispersal and pollination [1,9]. Additionally, carotenoid cleavage can generate volatile compounds that contribute to the flavor of some flowers and fruits. These properties as natural pigments and aromas make carotenoids an important economic target of cosmetic and food industries. However, their main interest for humans is their nutritional and health-promoting properties. Dietary carotenoids are used as precursors for the production of retinoids (including vitamin A). Additionally, carotenoid-enriched diets reduce the risk of several degenerative diseases, including age-related macular degeneration, cognitive malfunctioning, cardiovascular diseases, and some types of cancer [1,10].

Due to the importance of carotenoids for human (and animal) health and their economic value for the industry, a large number of biotechnological strategies have been developed to enrich plant tissues with carotenoids [11,12]. Carotenoid levels are the result of three interacting variables: (1) biosynthetic rate, (2) degradation rate and (3) storage capacity of the cell to accumulate and sequester them. Most biotechnological approaches to alter carotenoid contents in plants have been focused on manipulating their biosynthesis or degradation by over-expressing or silencing sequences encoding exogenous or endogenous enzymes or their regulators [11–17]. Improving the sink capacity of plant cells to accumulate enhanced levels of carotenoids, however, has received much less attention, in part because of our limited knowledge

* Corresponding author.
E-mail address: manuelrc@ibmcp.upv.es (M. Rodriguez-Concepcion).

https://doi.org/10.1016/j.plipres.2021.101128
Received 18 May 2021; Received in revised form 6 September 2021; Accepted 9 September 2021
Available online 13 September 2021

on how to manipulate this process. Here we will briefly review the general plant carotenoid metabolism (i.e. biosynthesis and degradation pathways) to provide some context but will focus on the storage mechanisms to then describe some recent accomplishments on creating new sites to produce and accumulate these health-promoting metabolites in plant cells.

2. Carotenoid metabolism

2.1. General features

The main structural feature of carotenoids is the system of conjugated double bonds known as the 'polyene chain' (Fig. 1). Carotenoids with a partially saturated polyene chain (such as phytoene) do not absorb light in the visible wavelength. However, they are rarities within the carotenoid family, as desaturation steps early in the biosynthetic pathway readily convert them to downstream products with colors in the visible (yellow to red) spectrum. Carotenoids can be either acyclic or cyclic attending to the absence or presence of end rings in their structure, respectively. They are also classified as carotenes (when they only contain carbon and hydrogen) and xanthophylls (when they also contain oxygen). Carotenoids can also have *trans* (*E*) or *cis* (*Z*) isomers which can markedly differ in shape. Furthermore, some carotenoids form optical isomers due to the presence of chiral centers in their molecules [1,18].

2.2. Biosynthetic pathway

Plants synthesize carotenoids in plastids from the isoprenoid precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), derived from the methylerythritol 4-phosphate (MEP) pathway [1,15,19]. Three IPP and one DMAPP molecules are condensed by geranylgeranyl diphosphate (GGPP) synthase to form GGPP, which is

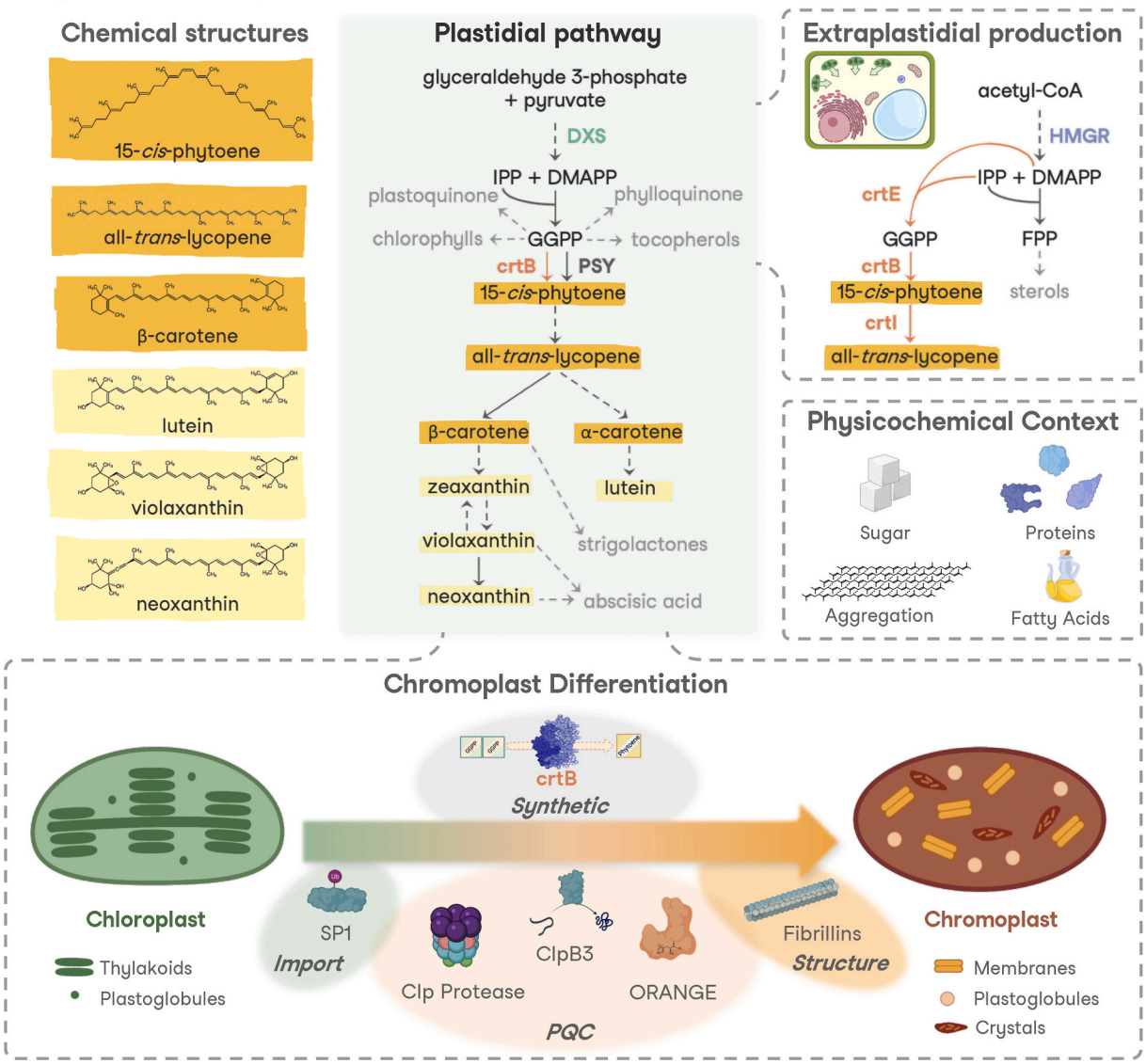

Fig. 1. Carotenoid biosynthesis and strategies to engineer new storage sites in plant cells. The endogenous plant carotenoid pathway is located in plastids and fed by precursors synthesized by the MEP pathway, whose flux is mainly controlled by DXS. The first and main rate-determining enzyme of the carotenoid pathway is PSY. Carotenes and xanthophylls produced by this pathway are boxed in orange and yellow, respectively. Extra room for their accumulation can be engineered by promoting the differentiation of carotenoid-sequestering structures within plastids (hence becoming chromoplasts) or by transferring carotenoid production to the cytosol using the indicated bacterial enzymes (in red) and precursors produced by the MVA pathway, whose flux is mainly controlled by HMGR. Processes and proteins influencing chromoplast differentiation are represented. Regardless the subcellular production site, storage of carotenoids is influenced by their physicochemical properties, including their aggregation state or the interaction with proteins, sugars and lipids (e.g. esterification). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the common precursor of carotenoids and other photosynthesis-related plastidial isoprenoids such as chlorophylls, plastoquinone, phylloquinones, and tocopherols, but also of the plant hormones gibberellins [6,20,21] (Fig. 1). The first reaction specific of the plant carotenoid pathway is the condensation of two molecules of GGPP for the synthesis of the non-colored carotenoid 15-*cis*-phytoene, catalyzed by the enzyme phytoene synthase (PSY). This is considered a bottleneck reaction and a major control step of the metabolic flux to carotenoids [15,22]. Different enzymes catalyze the sequential desaturation and isomerization reactions that convert 15-*cis*-phytoene into the red-colored all-*trans* lycopene [1,16]. Formation of β- or/and ε-ionone rings in the ends of the linear lycopene molecule represents a branch point of the pathway, leading to either α-carotene (β,ε branch) or β-carotene (β,β branch) [16]. Hydroxylation and epoxidation of the rings in these carotenes results in different types of xanthophylls, such as lutein from α-carotene or zeaxanthin, violaxanthin and neoxanthin from β-carotene (Fig. 1).

2.3. Breakdown pathways

The electron-rich polyene backbone of carotenoids makes them very susceptible to oxidative breakdown. Carotenoid degradation can be mediated by carotenoid cleavage dioxygenases (CCDs) that catalyze the oxidative breakdown of carbon–carbon double bonds in different locations of the polyene backbone to produce carotenoid cleavage products (CCPs) containing carbonyl groups (aldehyde or ketone groups) in the cleaving ends. While carotenoid levels are negatively correlated with the activity of these enzymes in some plants and tissues, CCD-independent degradation can be a main contributor to carotenoid loss in other cases [23–25]. For example, lipoxygenases (LOXs) that oxidize polyunsaturated fatty acids can produce hyperperoxides that cause the degradation (i.e. cooxidation) of carotenoids [26,27]. Carotenoids can also be oxidized non-enzymatically by reactive oxygen species (ROS) produced after photooxidative stress [28]. The CCPs resulting from either CCD activity or non-enzymatic oxidation are typically referred to as apocarotenoids. Many of them have signaling roles as pigments and flavors, and some have been shown to function as hormones and defense compounds [8,29]. The role of many other apocarotenoids, however, remains unknown.

3. Carotenoid storage

3.1. Physical-chemical considerations

Carotenoids can be found in plant tissues either free or associated with other molecules (proteins, fatty acids or sugars). They can also form aggregates as a result of weak and reversible bonding by hydrogen bonds, van der Waals interactions, dipole forces and the hydrophobic effects of hydrophobic molecules, their polar groups and the surrounding solvent [30–32]. With only some exceptions (e.g. carotenoids harboring carboxylic groups such as bixin and azafrin), free carotenoids are hydrophobic compounds usually found in lipid-rich environments. Carotenes are typically more lipophilic than xanthophylls [18]. The conjugated double bonds in their polyene chain causes rigidity of the carotenoid molecules. While *cis* isomers have an angular shape and are less susceptible to aggregation, those in all-*trans* configuration typically exhibit a rod-like shape (Fig. 1). Besides solubility, these differences influence the ability of carotenoids to fit into cellular structures or to interact with enzymes and other proteins, eventually impacting their bioaccessibility (i.e. the amount of carotenoid released from the food matrix during digestion and made available for absorption) and bioavailability (i.e. the fraction that is actually absorbed by our bodies and available for biological use).

Plant carotenoids can also be found associated to proteins (e.g., in photosynthetic complexes) or conjugated with sugar (e.g., glucose) or lipid (e.g. fatty acids) moieties (Fig. 1). Carotenoid glycosylation is most frequent in microorganisms as a natural mechanism to increase their hydrophilicity [33]. Glycosylated carotenoids appear to play important roles in maintaining cell wall structure and stabilizing thylakoid membranes in cyanobacteria [34]. In plants, the best known examples are crocins and picrocrocin, water-soluble glycosylated apocarotenoids that contribute to the color and taste of the saffron (*Crocus sativus*) spice. They are produced from precursors resulting from the cleavage of zeaxanthin by a specific plastid-localized CCD enzyme (CCD2) that leave the plastid to be eventually glycosylated by identified glycosyltransferases and then imported into vacuoles by specific ABC transporters [35–38]. While glycosylation increases water solubility and decreases reactivity, compartmentalization into vacuoles allows for long-term storage and higher stability [36]. Glycosylation of xanthophyll-derived apocarotenoids also occurs in fruits and leaves of several plant species, presumably as a compensatory mechanism for increased carotenoid flux [35,39–41].

The most frequent type of plant carotenoid modification is esterification with fatty acids [1,18]. Because the acylation reaction must necessarily be performed over a hydroxyl group, only hydroxyxanthophylls may become acyl esters. They are ubiquitous in foods of plant origin [42]. The ripening of many fruits is actually associated with the esterification of xanthophylls, supporting the conclusion that esterification is important to increase the carotenoid accumulation capacity of plant cells. While association with sugars makes carotenoids more water-soluble, esterification of xanthophylls renders them more lipophilic and promotes their sequestration in subplastidial structures [43]. The increase in the liposolubility compared to free xanthophylls likely improves the integration of esterified xanthophylls into membrane structures such as plastoglobules, which eventually results in higher stability and enhanced bioavailability [1,44]. Spatial configurations, however, prevent them from accumulating in standard bilayer membranes to appreciable amounts. As to the enzymes involved in xanthophyll esterification, recent works have identified several esterases or acyl transferases. XANTHOPHYLL ESTERASE (XES) enzymes with a conserved α/β hydrolase fold and acyltransferase domains have been identified in several plant species [45–48]. In tomato, the XES homolog PALE YELLOW PETAL 1 (PYP1) has been demonstrated to be required for the esterification of violaxanthin and neoxanthin and for carotenoid sequestration in petals [45]. A member of a different family, the GDSL esterase/lipase XANTHOPHYLL ACYL-TRANSFERASE (XAT) from wheat (*Triticum aestivum*), catalyzes the esterification of lutein but it can also use other xanthophylls as substrates [49]. It remains to be tested whether these genes can be used as biotechnological tools to improve the deposition, stability and bioavailability of xanthophylls.

3.2. Plastid types

Plant carotenoids are not only produced but also stored in plastids. While carotenoids are fairly sensitive to oxidation or isomerization by light and temperature when tissues are disrupted, they are relatively stable in their natural environment (i.e., inside plastids). In fact, the storage of carotenoids is heavily influenced by the presence of appropriate subplastidial structures that sequester them and promote their accumulation. Plastids are ubiquitous in plant cells, organs and species, but there are different types and they can fluctuate from one type to another depending on the developmental program and environmental factors [50,51]. Carotenoids are only absent in proplastids [52,53], which are undifferentiated plastids found in meristematic, reproductive and dedifferentiated tissues. They act as progenitors for all the other types of plastids [17,50].

Etioplasts are plastids that only occur in tissues that are not exposed to light (i.e., in seedlings that germinate in darkness). They develop when proplastids cannot be differentiated into chloroplasts. Etioplasts accumulate low amounts of carotenoids as well as the chlorophyll precursor protochlorophyllide in special structures called prolamellar bodies (PLBs) [54,55]. Carotenoids produced in etioplasts (such as violaxanthin and lutein) facilitate the transition to photosynthetic

development when they differentiate into chloroplasts during de-etiolation [56,57]. Carotenoid levels increase to protect plastids from photooxidative damage as protochlorophyllide is converted into chlorophylls and PLBs into thylakoids during the etioplast-to-chloroplast differentiation process [9,58].

Chloroplasts are photosynthetic plastids that are found in all green tissues. Chloroplasts accumulate high levels of carotenoids, with lutein and β-carotene being the most abundant ones (45% and 25–30% of the total, respectively) followed by violaxanthin and neoxanthin (10–15% each) in most plants [15]. These carotenoids are often associated with proteins in different complexes of the photosynthetic apparatus [2,3]. Xanthophylls are primarily found in the light-harvesting complexes, whereas β-carotene is usually located in both photosystems and the cytochrome *b6f* complex. Despite their high carotenoid content, chloroplast-containing tissues are green due to the presence of chlorophylls. Carotenoids and chlorophylls are actually maintained in a tightly controlled proportion to ensure correct functioning of the photosynthetic machinery. Carotenoids function as accessory light-harvesting pigments and contribute to the assembly, stabilization, and repair of the photosynthetic apparatus [2,3,59]. Another essential function of carotenoids in chloroplasts is photoprotection against photooxidative damage. Carotenoids prevent the formation of ROS in high light conditions via two complementary mechanisms: by dissipating excess energy as heat (through a mechanism called as non-photochemical quenching) and by quenching excited triplet chlorophyll and singlet oxygen (hence promoting free radical detoxification) [2,3,58,60,61]. Chloroplasts carotenoids can also be found in the envelope membranes, which are considered as major production site as most carotenoid biosynthetic enzymes are located there [15,62]. They are also present at low levels in plastoglobules, which are lipid bodies associated to thylakoids that may function as metabolite trafficking structures to transport carotenoids and other plastidial isoprenoids from their synthesis to their destination sites [17]. A hydrophobic ligand-binding domain protein named chloroplast Sec14-like 1 (CPSFL1), which binds carotenoids and occurs in both soluble and membrane-bound forms, has been recently proposed as another potential intraplastidial transporter [63].

Developmental and environmental cues can promote the transformation of chloroplasts into other plastid types. For example, natural or dark-induced senescence transforms chloroplasts into gerontoplasts. Typically, gerontoplasts harbor disaggregated thylakoids and huge plastoglobules that accumulate carotenoids and their degradation products [50,64]. During senescence, the chlorophylls/carotenoids ratio decreases due to chlorophyll degradation and carotenoid retention, and leaves turn to typical yellow and orange autumn colors [65]. In many fleshy fruits, chloroplasts differentiate into carotenoid-overaccumulating plastids called chromoplasts. Chromoplasts, which can also derive from other plastid types such as leucoplasts or amyloplasts, are non-photosynthetic plastids specialized in the production and sequestering of carotenoids that provide color to organs such as fruits, flowers and storage roots [17,53,65–67]. In contrast to chloroplasts, the carotenoid composition of chromoplasts is very diverse. This composition often determines the storage structures that different types of chromoplasts develop to accommodate massive amounts of carotenoids [17,53,65,68].

3.3. Specialized storage structures

Chromoplast carotenoids can be stored in membrane systems, in plastoglobules, in lipoprotein complexes forming fibrils, or as crystals. These storage structures define the type of chromoplast, which can be classified as membranous, globular, fibrillar, or crystalline [17,53,65,68]. Chromoplasts harboring different carotenoid-bearing structures (i.e., chromoplasts of different types) can be found in different tissues of a single plant but also within the same organ [65,69,70]. Globular chromoplasts, found in saffron stigmas, mango, or citrus fruit, are enriched in plastoglobules. [70–72]. Besides carotenoids, plastoglobules may accumulate tocopherols [73], phylloquinone [74] and plastoquinone [75,76]. Plastoglobule morphology is very variable depending on the nature of polar lipids and proteins that compose them, but also on the content of carotenoids and their esterification. For example, tomato fruit chromoplasts show globular plastoglobules [77]) whereas fibrillar chromoplasts are characteristic of pepper fruit [78]. Membranous chromoplasts display concentrical multilayer membranes derived from a proliferation of the inner envelope membrane, where carotenoids are accumulated. They were reported in *Narcissus* (daffodil) flowers [79] and the orange curd of *Or* mutants of cauliflower [80]. Crystalline chromoplasts are abundant in tomato fruit and carrot root, where lycopene and β-carotene crystals, respectively, are encased in a bilayer of lipids [70,81]. This heterogeneity of types and structures illustrates the huge versatility of plastids to accumulate any kind of carotenoid, making chromoplasts the best-known machinery for carotenoid production and storage in plants. Indeed, promoting the differentiation of chromoplasts appears as a useful strategy for carotenoid biofortification as it improves both production and sink capacity [12,17,44,82]. Control of chromoplast differentiation has been a hot topic for decades, but the underlying mechanisms have only recently began to be unveiled [51,53,65,66,83,84]. In the next section we will review some of the processes impacting chromoplasts differentiation and the results of their manipulation for carotenoid biofortification.

4. Chromoplast differentiation

4.1. Protein import

Chromoplastogenesis is a complex process that requires an active production of carotenoids and hence catalytically active enzymes, but also other proteins required to reshape plastid ultrastructure in order to accommodate the newly made carotenoids. Building a chromoplast therefore implies major proteome changes [83,85–89]. The plastid genome only harbors about 100 genes, but its expression activity in chromoplasts appears to be maintained mainly to sustain the expression of only one of them, *accD*, which is involved in fatty acid metabolism [90]. The vast majority of the proteins required for chromoplastogenesis are hence encoded by nuclear genes, synthesized in the cytosol and then imported into plastids [51,91]. Most of these proteins are imported by translocons at the outer and inner chloroplast envelope known as TOC and TIC, respectively. In particular, the TOC machinery exists in multiple forms (i.e. with different protein import receptor isoforms) and it plays critical roles in precursor protein recognition. In *A. thaliana*, a plant that does not naturally differentiate chromoplasts, a reconfiguration of the protein import machinery takes place during the etioplast-to-chloroplast and chloroplast-to-gerontoplast transitions. This process is facilitated by selectively targeting TOC complexes for ubiquitination and degradation by the outer envelope-localized E3 ligase SP1 (*SUPPRESSOR OF ppi1*) [92]. The tomato homologues SP1 and SP1-like2 (SPL2) have been recently shown to also have an important role in the chloroplast-to-chromoplast transition [93] (Fig. 1). During tomato fruit ripening, the chloroplasts of mature green fruit lose their chlorophyll and gradually transform into chromoplasts that accumulate large amounts of the red carotenoid lycopene and the orange carotenoid β-carotene, the main precursor of vitamin A. Overexpression of SP1 promotes the chloroplast-to-chromoplast transformation, likely by accelerating the reconfiguration of the protein import machinery [93]. Interestingly, increased SP1 levels also impacted other ripening-associated processes such as fruit softening and metabolic changes, suggesting that improving chromoplast differentiation might speed up the whole ripening process. In agreement, knockdown of SP1 or SPL2 was found to delay chromoplast differentiation and fruit ripening [93]. The TIC machinery, which mediates the translocation of preproteins through the inner envelope membrane, also contains different components and isoforms. Their possible involvement in the differential import of specific subsets of precursor proteins into chromoplasts or any other

plastid type remains unclear [51].

4.2. Protein quality control

Following import, the transit peptide of nuclear-encoded plastidial proteins is cleaved by specific proteases and the mature proteins are folded, assembled into complexes and/or delivered to their subplastidial destination site by complex networks of plastidial chaperones. Chaperones and proteases also form the protein quality control (PQC) system that ensures stabilization, refolding, or degradation of mature proteins that lose their native conformation, become irreversibly damaged and/or need to be removed [94]. Plastid PQC components include several groups of prokaryotic-like chaperones such as Cpn60, Hsp70 and Hsp100 as well as small heat shock proteins and proteases (including Clp, Lon, Deg, and FstH). Most of our current knowledge of these components comes from studies in chloroplast-bearing model systems, but genetic manipulation of some of them in tomato and other crops has been recently shown to alter their carotenoid contents and to impact chromoplast differentiation.

Plastidial chaperones are up-regulated during natural chromoplast differentiation, presumably to deal with proteome changes and protein folding stress [87,88,94,95]. The small heat shock protein Hsp21 has been one of the first chaperones functionally associated to chromoplast differentiation [16,44,95–98]. Overexpression of Hsp21 in tomato showed a dual role, protecting photosystem II against oxidative stress in leaves (i.e. chloroplasts) and promoting the conversion of chloroplasts to chromoplasts during tomato fruit ripening [44,95]. How Hsp21 contributes to chromoplast differentiation is unknown. Small heat shock proteins such as Hsp21 provide fast protection against stress-induced protein aggregation in cooperation with Hsp70 and Hsp100 chaperones. Interestingly, plastidial isoforms of Hsp70 and Hsp100 (ClpB3) are also up-regulated during tomato fruit ripening and promote proper folding and hence enzymatic activity of deoxyxylulose 5-phosphate synthase (DXS), the first and main rate-determining enzyme of the MEP pathway [94,99–101] (Fig. 1). Overexpression of Hsp70 in tomato, however, led to increased levels of fruit carotenoids without altering chromoplast ultrastructure [94].

The chaperone most directly related to the induction of chromoplast differentiation is Orange (OR) (Fig. 1). Different OR versions were found to cause carotenoid overaccumulation in orange cultivars of cauliflower [102,103] and melon [104,105]. Transgene-mediated overexpression of OR has been demonstrated to induce carotenoid accumulation by promoting chromoplast differentiation in other crops [106–110]. The chaperone activity of OR ensures proper activity and prevent degradation of PSY, the enzyme catalyzing the first committed and main rate-determining step of the carotenoid pathway [111–113] (Fig. 1). However, OR appears to impact carotenoid accumulation and chromoplast differentiation by additional mechanisms. Recent results indicate that OR can physically interact with several components of the TIC machinery, including Tic40, and Tic110 [114]. OR reduces the turnover rate of Tic40, interferes with the interaction between Tic40 and Tic110, and reduces the binding of pre-proteins to Tic110 for translocation and processing. These results suggest that OR plays a role in protein import [114]. Additionally, OR proteins harboring the “golden SNP” responsible for the dark orange color of some melon cultivars (referred to as OR-His) prevent conversion of β-carotene into downstream products and promote the differentiation of membranous chromoplasts when overexpressed in tomato fruit tissues and dark-grown Arabidopsis calli [94,104,105,109,115,116]. OR-His has recently been reported to also restrict chromoplast number by interacting with ARC3 (*ACCUMULATION AND REPLICATION OF CHLOROPLASTS* 3) to interfere with its binding with PARC6 (*PARALOG OF ARC6*) [114]. Both ARC3 and PARC6 are key regulators of plastid division, suggesting an additional role of OR-His in regulating chromoplast number besides promoting carotenoid biosynthesis and chromoplast biogenesis. Interestingly, increasing chromoplast number and size has been shown to cause higher metabolic sink strength and improve total carotenoid levels in ripe fruits from several tomato *high pigment* mutants [13,51]. OR-His overexpression did not alter number or ultrastructure of chloroplasts [109,114,115]. However, direct interaction of Arabidopsis OR with the transcription factor TCP14 (*TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR 14*) in the nucleus represses its transactivation activity and eventually represses etioplast-to-chloroplast transition during seedling deetiolation [117]. Recent reviews are available with additional, comprehensive information on these and other potential roles of OR [13,17,118,119].

Regarding proteases, the Clp protease complex has been demonstrated to control the levels of DXS, PSY, and other enzymes involved in carotenoid biosynthesis [99,113,120–122]. The Clp proteolytic complex is the main machinery for protein degradation in plastids, removing proteins that are not properly folded and hampering the formation of toxic protein aggregates [100,123]. Two different and separated domains constitute the Clp complex: a proteolytic core consisting in two rings formed by ClpP and ClpR subunits and a chaperone ring formed by ClpC and ClpD subunits that unfolds the substrates for delivery into the proteolytic chamber [121,123]. Higher levels of carotenoid biosynthetic enzymes (but not their transcripts) have been observed in Arabidopsis mutants and tobacco transgenic lines defective in subunits of the Clp catalytic core (such as ClpR1) or the chaperone ring (such as ClpC1), suggesting that these enzymes are direct targets of the protease [99,113,120,122]. The levels of carotenoids in the leaves of these lines, however, were not increased as defective Clp protease function also impacts chloroplast development and hence alters carotenoid deposition sites. Repression of Clp protease activity by down-regulation of *ClpR1* gene expression specifically in tomato fruit also led to increased levels of DXS and PSY proteins (but not transcripts) in chromoplasts [101]. Most interestingly, ClpR1-defective tomato fruit showed increased expression of genes encoding plastidial chaperones, similar to that observed in Clp protease-deficient Arabidopsis mutants [100,101]. It was proposed that this might be a compensatory mechanism to mitigate protein folding stress resulting from defective turnover. The expression of OR-encoding genes was also induced in ClpR1-defective fruits, which showed phenotypes associated with OR overexpression such as the development of membranous chromoplasts enriched in β-carotene [94,101,109]. Together, chaperones such as OR and proteases such as Clp appear to be closely connected to ensure proper chromoplast differentiation (Fig. 1).

4.3. Structural proteins

Fibrillins and other proteins associated to carotenoid-sequestering structures such as fibrils and plastoglobules have been linked to the differentiation of fruit chromoplasts [43,77,78,124]. In particular, the fibrillin FBN1 is a major component of the pepper fruit chromoplast fibrils, forming a protein shell surrounding a layer of polar lipids and a central core of carotenoids [78]. These fibrils are self-assembled structures that likely contribute to prevent toxic effects associated to high carotenoid levels while protecting the degradation of their carotenoid content. Related structural proteins associated with chromoplast differentiation and carotenoid accumulation include the chromoplast-specific carotenoid-associated protein (CHRC) and chromoplast protein D (CHRD) [125–127]. Transgene-mediated overexpression of some of these proteins was shown to alter carotenoid levels in tomato fruit, often through changes in chromoplast development [77,128,129]. The potential of manipulating structural proteins for carotenoid biofortification, however, remains poorly explored.

4.4. Engineered carotenoid production

It was generally reported that enhanced production of carotenoids was necessary and sufficient to promote the differentiation of chromoplasts in tissues that are naturally prone to accumulate carotenoids. For example, activating metabolic flux towards the carotenoid pathway, e.g.

through upregulation of plant PSY or plastid-targeted versions of bacterial phytoene synthase (known as crtB), stimulated the development of chromoplasts in carrot roots or tomato fruits [81,130]. The same strategy was found to produce chromoplast-like plastids in dark-grown calli and roots from Arabidopsis, a plant species that does not naturally develop chromoplasts [25,81,131,132]. By contrast, chromoplast differentiation could not be triggered in leaves until recently [84,133]. Delivering the *Pantoea ananatis* (previously called *Erwinia uredovora*) crtB enzyme to leaf chloroplasts led to the differentiation of chromoplast-like plastids in all plants tested, despite this is something that only a few species do in nature [84]. The *crtB*-expressing leaf sections accumulated 2-fold more carotenoids than control sections of the same leaf [84]. Following a crtB-mediated massive production of phytoene, photosynthesis was impaired and chloroplasts eventually transformed their thylakoids and grana into highly packed membrane stacks with a concomitant proliferation of plastoglobules. The newly created membrane structures likely contribute to reaching high carotenoid levels by accommodating increasing amounts of these lipophilic pigments (hence preventing their degradation) but also by stimulating the activity of endogenous carotenoid biosynthetic enzymes, many of which are membrane-associated [15]. The transcriptomic and protein profiles of the newly formed plastids also resembled those of typical chromoplasts. This synthetic system (Fig. 1) has allowed to propose a two-step model for chloroplast-to-chromoplast transformation that also applies to natural systems. In the first step of the proposed model [84], chloroplasts must be preconditioned by lowering their photosynthetic capacity. This step is not necessary for non-photosynthetic plastids or chloroplasts with low photosynthetic competence (such as those of green fruits). In the second step, increased production of carotenoids is all that it takes for chromoplasts to differentiate in a process that involves a massive reprogramming of nuclear gene expression and ultrastructural changes but does not require OR activity [84].

Differentiation of chloroplasts into chromoplasts with disordered membrane systems and proliferated plastoglobules has also been reported when a full transplastomic pathway for the production of the non-plant carotenoid astaxanthin was introduced in tobacco leaves [133]. It is proposed that engineered accumulation of high levels of carotenoids normally absent from chloroplasts (such as astaxanthin or crtB-produced phytoene) likely alters the properties of thylakoids and grana membranes and interferes with the photosynthetic machinery, hence preconditioning chloroplasts to become chromoplasts as carotenoids are produced. In nature, developmental cues are likely responsible for making chloroplasts competent (first step) and for upregulating the expression of carotenoid biosynthetic genes (second step). A corollary of this model is that the plant PSY enzymes cannot produce enough phytoene by themselves in leaf chloroplasts to disrupt photosynthesis and hence allow chromoplastogenesis to proceed, suggesting that they are under strict regulatory controls that prevent a too high activity (likely because it would be detrimental for photosynthesis). Such regulatory controls appear to be more relaxed in green fruits and non-photosynthetic organs such as roots or calli, allowing the differentiation of chromoplasts when PSY activity increases either by upregulating the expression of genes encoding this enzyme or its positive regulators such as OR [22,81,94,109,111,130,132,134,135]. Non-plant crtB is most likely free of such regulatory control and produces very high levels of phytoene in chloroplasts, explaining why OR is not required for crtB-dependent chromoplastogenesis in leaves [84]. The identification of specific signals triggering and sustaining the different stages of the crtB-mediated chromoplast differentiation process, the genes and molecules that respond to phytoene accumulation, and the identification of the regulatory mechanisms that control endogenous PSY activity in chloroplasts await further investigation.

5. Extraplastidial storage

While the activity of crtB in plastids can lead to the differentiation of chromoplasts, its combination with other bacterial enzymes in the cytosol has been shown to successfully produce carotenoids in extraplastidial locations [136,137]. Isoprenoid precursors IPP and DMAPP are produced in the cytosol by the activity of the mevalonic acid (MVA) pathway and then used for the production of sterols and other isoprenoids derived from farnesyl diphosphate (FPP). MVA-derived IPP and DMAPP can be used to produce GGPP using the *P. ananatis crtE* gene, encoding GGPP synthase, and then diverted to the production of the red carotenoid lycopene using the bacterial genes *crtB* and *crtI* (Fig. 1). Plastid-targeted versions of these microbial enzymes have been extensively used for plant carotenoid biotechnology, generating biofortified products such as Golden Rice [138,139]. Virus-mediated expression of *crtE*, *crtB* and *crtI* was successful to produce lycopene in the cytosol of tobacco (*Nicotiana tabacum*) cells, even though the extraplastidial accumulation of this pigment achieved was relatively modest (about 10% of the total leaf carotenoid content) and unstable [137]. The delivery of constructs by agroinfiltration together with other improvements resulted in much higher and more stable carotenoid titers in *N. benthamiana* leaves [136]. Specifically, the upregulation of the MVA pathway flux using a deregulated version of the hydroxymethylglutaryl-CoA reductase (HMGR) (Fig. 1) led to an increased supply of carotenoid precursors, whereas the use of a crtB protein with a blocked N-terminus ensured cytosolic localization of this phytoene-producing enzyme [84]. Interestingly, the extraplastidial phytoene that accumulated in these leaves showed an improved bioaccessibility compared to plastidial phytoene [136], suggesting a differential association to membranes or lipidic structures. Lycopene accumulated to levels similar to those found in ripe tomatoes and formed crystals of similar physical-chemical properties as those formed in fruit chromoplasts. The extraplastidial accumulation of these carotenoids prevented direct interference with photosynthesis, even though a likely secondary cell damage-associated effect did cause photosynthesis to slow down [136]. Attempts to improve extraplastidial carotenoid storage by enhancing the development of endomembrane structures and lipid bodies were not successful.

6. Future perspectives

Our knowledge on the molecular mechanisms influencing carotenoid sink strength in plants has substantially grown in the last years, opening new opportunities to create carotenoid-enriched plants with high nutritional values and profitable for agrofood, pharmaceutic and cosmetic industries. The use of different OR versions to promote chromoplast differentiation in non-leaf systems, the crtB-mediated induction of chromoplastogenesis in plant leaves, or the use of synthetic pathways to stably accumulate carotenoids in extraplastidial locations represent startling advances. Combination of different approaches, including push (i.e. promote biosynthesis, even in different cellular locations) and pull (i.e. improve sink capacity and storage) strategies or even different pull strategies (e.g. triggering chromoplast differentiation and xanthophyll esterification) remains to reveal all its potential. An often overlooked but yet important point that should be kept in mind when targeting biofortification is bioavailability. As an example, widely diverse bioavailability values are obtained for β-carotene in leaves (where it is mostly associated with photosynthetic complexes), carrots (where it forms crystals) or Golden Rice (where it is stored in lipid droplets) [140–142]. Improving storage of carotenoids in membrane environments or promoting esterification will likely lead to higher bioavailability.

Current genome editing technologies should target the genes identified to date in different plant systems to provide solutions to the challenge of creating new carotenoid-biofortified varieties with minimal interference at the genetic level. However, it is important to take into account that one of the main lessons that we have learned is that different organs or plant species often show specific mechanism regulating carotenoid biosynthesis, storage and degradation [1,9,44]. The integration of published data related to carotenoids in different plant

species should contribute to obtain transversal conclusions about what genes, proteins, metabolites or processes are directly connected to carotenoids, generating not particular but general answers for plants.

Acknowledgements

We greatly thank Carmen and Pilar Torres-Montilla for their collaboration and help in the design and execution of the figures. We also thank Luca Morelli and BioRender.com for some of the images. Work in our lab is funded by Spanish grants BIO2017-84041-P and PID2020-115810GB-I00 from the Agencia Estatal de Investigacion (AEI) and 202040E299 from Consejo Superior de Investigaciones Científicas (CSIC) to MRC. STM was supported by PhD fellowship FPU16/04054 from the Spanish Ministerio de Educacion y Cultura.

References

[1] Rodriguez-Concepcion M, Avalos J, Bonet ML, Boronat A, Gomez-Gomez L, Hornero-Mendez D, et al. A global perspective on carotenoids: metabolism, biotechnology, and benefits for nutrition and health. Prog. Lipid Res. 2018;70:62–93. https://doi.org/10.1016/j.plipres.2018.04.004.

[2] Domonkos I, Kis M, Gombos Z, Ughy B. Carotenoids, versatile components of oxygenic photosynthesis. Prog. Lipid Res. 2013;52:539–61. https://doi.org/10.1016/j.plipres.2013.07.001.

[3] Hashimoto H, Uragami C, Cogdell RJ. Carotenoids and photosynthesis. In: Stange C, editor. Carotenoids Nat; 2016. https://doi.org/10.1007/978-3-319-39126-7.

[4] Qin G, Gu H, Ma L, Peng Y, Deng XW, Chen Z, et al. Disruption of phytoene desaturase gene results in albino and dwarf phenotypes in Arabidopsis by impairing chlorophyll , carotenoid , and gibberellin biosynthesis. Cell Res. 2007;17:471–82. https://doi.org/10.1038/cr.2007.40.

[5] Pokhilko A, Bou-Torrent J, Pulido P, Rodr M. Mathematical modelling of the diurnal regulation of the MEP pathway in Arabidopsis. New Phytol. 2015;206:1075–85.

[6] Ruiz-Sola MÁ, Barja MV, Manzano D, Llorente B, Schipper B, Beekwilder J, et al. A single Arabidopsis gene encodes two differentially targeted Geranylgeranyl Diphosphate synthase isoforms. Plant Physiol. 2016;172:1393–402. https://doi.org/10.1104/pp.16.01392.

[7] Hou X, Rivers J, León P, Mcquinn RP, Pogson BJ. Synthesis and function of Apocarotenoid signals in plants. Trends Plant Sci. 2016;21:792–803. https://doi.org/10.1016/j.tplants.2016.06.001.

[8] Moreno JC, Mi J, Alagoz Y, Al-Babili S. Plant apocarotenoids : from retrograde signaling to interspecific communication. Plant J. 2020:1–25. https://doi.org/10.1111/tpj.15102.

[9] Llorente B, Martinez-Garcia JF, Stange C, Rodriguez-Concepcion M. Illuminating colors: regulation of carotenoid biosynthesis and accumulation by light. Curr. Opin. Plant Biol. 2017;37:49–55. https://doi.org/10.1016/j.pbi.2017.03.011.

[10] Eggersdorfer M, Wyss A. Carotenoids in human nutrition and health. Arch. Biochem. Biophys. 2018;652:18–26. https://doi.org/10.1016/j.abb.2018.06.001.

[11] Giuliano G. Provitamin A biofortification of crop plants: a gold rush with many miners. Curr. Opin. Biotechnol. 2017;44:169–80. https://doi.org/10.1016/j.copbio.2017.02.001.

[12] Zheng X, Giuliano G, Al-Babili S. Carotenoid biofortification in crop plants: citius, altius, fortius. Biochim Biophys Acta - Mol Cell Biol Lipids 1865;2020:158664. https://doi.org/10.1016/j.bbalip.2020.158664.

[13] Sun T, Li L. Toward the 'golden' era: the status in uncovering the regulatory control of carotenoid accumulation in plants. Plant Sci. 2020;290. https://doi.org/10.1016/j.plantsci.2019.110331.

[14] Shumskaya M, Wurtzel ET. The carotenoid biosynthetic pathway: thinking in all dimensions. Plant Sci. 2013;208:58–63. https://doi.org/10.1016/j.plantsci.2013.03.012.

[15] Ruiz-Sola MÁ, Rodríguez-Concepción M. Carotenoid biosynthesis in Arabidopsis: a colorful pathway. Arab B 2012;10:e0158. https://doi.org/10.1199/tab. 0158.

[16] Nisar N, Li L, Lu S, Khin NC, Pogson BJ. Carotenoid metabolism in plants. Mol. Plant 2015;8:68–82. https://doi.org/10.1016/j.molp.2014.12.007.

[17] Sun T, Yuan H, Cao H, Yazdani M, Tadmor Y, Li L. Carotenoid metabolism in plants: the role of plastids. Mol. Plant 2017:1–17. https://doi.org/10.1016/j.molp.2017.09.010.

[18] Britton G. Structure and properties of carotenoids in relation to function. FASEB J. 1995;9:1551–8. https://doi.org/10.1096/fasebj.9.15.8529834.

[19] Phillips MA, León P, Boronat A, Rodríguez-Concepción M. The plastidial MEP pathway: unified nomenclature and resources. Trends Plant Sci. 2008;13:619–23. https://doi.org/10.1016/j.tplants.2008.09.003.

[20] Ruiz-Sola MÁ, Coman D, Beck G, Barja MV, Colinas M, Graf A, et al. Arabidopsis geranylgeranyl diphosphate synthase 11 is a hub isozyme required for the production of most photosynthesis-related isoprenoids. New Phytol. 2016;209:252–64.

[21] Zhou F, Wang CY, Gutensohn M, Jiang L, Zhang P, Zhang D, et al. A recruiting protein of geranylgeranyl diphosphate synthase controls metabolic flux toward chlorophyll biosynthesis in rice. Proc. Natl. Acad. Sci. U. S. A. 2017;114:6866–71. https://doi.org/10.1073/pnas.1705689114.

[22] Fraser PD, Romer S, Shipton CA, Mills PB, Kiano JW, Misawa N, et al. Evaluation of transgenic tomato plants expressing an additional phytoene synthase in a fruit-specific manner. Proc. Natl. Acad. Sci. U. S. A. 2002;99:1092–7. https://doi.org/10.1073/pnas.241374598.

[23] Gonzalez-Jorge S, Ha S-H, Magallanes-Lundback M, Gilliland LU, Zhou A, Lipka AE, et al. Carotenoid cleavage dioxygenase4 is a negative regulator of β-carotene content in Arabidopsis seeds. Plant Cell 2013;25:4812–26. https://doi.org/10.1105/tpc.113.119677.

[24] Adami M, De Franceschi P, Brandi F, Liverani A, Giovannini D, Rosati C, et al. Identifying a carotenoid cleavage Dioxygenase (ccd4) gene controlling yellow / white fruit flesh color of peach. Plant Mol. Biol. Report. 2013;31:1166–75. https://doi.org/10.1007/s11105-013-0628-6.

[25] Schaub P, Rodriguez-Franco M, Cazzonelli CI, Alvarez D, Wüst F, Welsch R. Establishment of an Arabidopsis callus system to study the interrelations of biosynthesis, degradation and accumulation of carotenoids. PLoS One 2018;13:1–28. https://doi.org/10.1371/journal.pone.0192158.

[26] Chedea VS, Jisaka M. Lipoxygenase and carotenoids: a co-oxidation story. African J Biotechnol 2013;12:2786–91. https://doi.org/10.5897/AJB12.2944.

[27] Gao L, Gonda I, Sun H, Ma Q, Bao K, Tieman DM, et al. The tomato pan-genome uncovers new genes and a rare allele regulating fruit flavor. Nat. Genet. 2019;51:1044–51. https://doi.org/10.1038/s41588-019-0410-2.

[28] Havaux M. Carotenoid oxidation products as stress signals in plants. Plant J. 2014;79:597–606. https://doi.org/10.1111/tpj.12386.

[29] Dhar MK, Mishra S, Bhat A, Chib S, Kaul S. Plant carotenoid cleavage oxygenases : structure-function relationships and role in development and metabolism. Brief Funct Genomics 2020;19:1–9. https://doi.org/10.1093/bfgp/elz037.

[30] Gruszecki WI. Carotenoids in membranes. In: Frank HA, Young AJ, Britton G, Cogdell RJ, editors. Photochem. Carotenoids; 1999. p. 363–79. https://doi.org/10.1007/0-306-48209-6_20.

[31] Simonyi M, Bikádi Z, Zsila F, Deli J. Supramolecular exciton chirality of carotenoid aggregates. Chirality 2003;15:680–98. https://doi.org/10.1002/chir.10282.

[32] Popova AV, Andreeva AS. Carotenoid-Lipid Interactions. Adv. Planar Lipid Bilayers Liposomes 2013;17:215–36. https://doi.org/10.1016/B978-0-12-411516-3.00008-5. 1st ed., Copyright © 2013 Elsevier Inc. All rights reserved.

[33] Wurtzel ET. Changing form and function through carotenoids and synthetic biology. Plant Physiol. 2019;179:830–43. https://doi.org/10.1104/pp.18.01122.

[34] Mohamed HE, Van De Meene AML, Roberson RW, Vermaas WFJ. Myxoxanthophyll is required for normal cell wall structure and thylakoid organization in the cyanobacterium Synechocystis sp. strain PCC 6803. J. Bacteriol. 2005;187:6883–92. https://doi.org/10.1128/JB.187.20.6883-6892.2005.

[35] Demurtas OC, Frusciante S, Ferrante P, Diretto G, Azad NH, Pietrella M, et al. Candidate enzymes for saffron crocin biosynthesis are localized in multiple cellular compartments. Plant Physiol. 2018;177:990–1006. https://doi.org/10.1104/pp.17.01815.

[36] Demurtas OC, de Brito Francisco R, Diretto G, Ferrante P, Frusciante S, Pietrella M, et al. ABCC transporters mediate the vacuolar accumulation of Crocins in saffron stigmas. Plant Cell 2019;31:2789–804. https://doi.org/10.1105/tpc.19.00193.

[37] Diretto G, Ahrazem O, Rubio-Moraga Á, Fiore A, Sevi F, Argandoña J, et al. UGT709G1: a novel uridine diphosphate glycosyltransferase involved in the biosynthesis of picrocrocin, the precursor of safranal in saffron (Crocus sativus). New Phytol. 2019;224:725–40. https://doi.org/10.1111/nph.16079.

[38] López AJ, Frusciante S, Niza E, Ahrazem O, Rubio-Moraga Á, Diretto G, et al. A new glycosyltransferase enzyme from family 91, ugt91p3, is responsible for the final glucosylation step of crocins in saffron (Crocus sativus l.). Int. J. Mol. Sci. 2021;22:1–17. https://doi.org/10.3390/ijms22168815.

[39] Lätari K, Wüst F, Hübner M, Schaub P, Beisel KG, Matsubara S, et al. Tissue-specific apocarotenoid glycosylation contributes to carotenoid homeostasis in arabidopsis leaves. Plant Physiol. 2015;168:1550–62. https://doi.org/10.1104/pp.15.00243.

[40] Martí M, Diretto G, Aragonés V, Frusciante S, Ahrazem O, Gómez-Gómez L, et al. Efficient production of saffron crocins and picrocrocin in Nicotiana benthamiana using a virus-driven system. Metab. Eng. 2020;61:238–50. https://doi.org/10.1016/j.ymben.2020.06.009.

[41] Koschmieder J, Wüst F, Schaub P, Álvarez D, Trautmann D, Krischke M, et al. Plant apocarotenoid metabolism utilizes defense mechanisms against reactive carbonyl species and xenobiotics. Plant Physiol. 2021;185:331–51. https://doi.org/10.1093/PLPHYS/KIAA033.

[42] Mercadante AZ, Rodrigues DB, Petry FC, Mariutti LRB. Carotenoid esters in foods - a review and practical directions on analysis and occurrence. Food Res. Int. 2017;99:830–50. https://doi.org/10.1016/j.foodres.2016.12.018.

[43] Van Wijk KJ, Kessler F. Plastoglobuli: plastid microcompartments with integrated functions in metabolism, plastid developmental transitions, and environmental adaptation. Annu. Rev. Plant Biol. 2017;68:253–89. https://doi.org/10.1146/annurev-arplant-043015-111737.

[44] Watkins JL, Pogson BJ. Prospects for carotenoid biofortification targeting retention and catabolism. Trends Plant Sci. 2020;25:501–12. https://doi.org/10.1016/j.tplants.2019.12.021.

[45] Ariizumi T, Kishimoto S, Kakami R, Maoka T, Hirakawa H, Suzuki Y, et al. Identification of the carotenoid modifying gene PALE YELLOW PETAL 1 as an essential factor in xanthophyll esterification and yellow flower pigmentation in tomato (Solanum lycopersicum). Plant J. 2014;79:453–65. https://doi.org/10.1111/tpj.12570.

[46] Zacarías-García J, Lux PE, Carle R, Schweiggert RM, Steingass CB, Zacarías L, et al. Characterization of the Pale yellow Petal/xanthophyll esterase gene family in citrus as candidates for carotenoid esterification in fruits. Food Chem. 2021; 342:128322. https://doi.org/10.1016/j.foodchem.2020.128322.
[47] Berry HM, Rickett DV, Baxter CJ, Enfissi EMA, Fraser PD. Carotenoid biosynthesis and sequestration in red chilli pepper fruit and its impact on colour intensity traits. J. Exp. Bot. 2019;70:2637–50. https://doi.org/10.1093/jxb/erz086.
[48] Kishimoto S, Oda-Yamamizo C, Ohmiya A. Heterologous expression of xanthophyll esterase genes affects carotenoid accumulation in petunia corollas. Sci. Rep. 2020;10:1–13. https://doi.org/10.1038/s41598-020-58313-y.
[49] Watkins JL, Li M, Mcquinn RP, Chan KX, Mcfarlane HE, Ermakova M, et al. A GDSL esterase/lipase catalyzes the esterification of lutein in bread wheat. Plant Cell 2019;31:3092–112. https://doi.org/10.1105/tpc.19.00272.
[50] Solymosi K, Lethin J, Aronsson H. Diversity and plasticity of plastids in land plants. In: Maréchal E, editor. Plastids, Springer Protocols; 2018.
[51] Sadali NM, Sowden RG, Ling Q, Jarvis RP. Differentiation of chromoplasts and other plastids in plants. Plant Cell Rep. 2019;38:803–18. https://doi.org/10.1007/s00299-019-02420-2.
[52] Howitt CA, Pogson BJ. Carotenoid accumulation and function in seeds and non-green tissues. Plant Cell Environ. 2006;29:435–45. https://doi.org/10.1111/j.1365-3040.2005.01492.x.
[53] Li L, Yuan H, Zeng Y, Xu Q. Plastids and carotenoid accumulation. In: Stange C, editor. Carotenoids Nat. biosynthesis, Regul. Funct. vol. 79; 2016. p. 273–93. https://doi.org/10.1007/978-3-319-39126-7.
[54] Sundqvist C, Dahlin C. With chlorophyll pigments from prolamellar bodies to light-harvesting complexes. Physiol. Plant. 1997;100:748–59. https://doi.org/10.1034/j.1399-3054.1997.1000402.x.
[55] Solymosi K, Schoefs B. Etioplast and etio-chloroplast formation under natural conditions: the dark side of chlorophyll biosynthesis in angiosperms. Photosynth. Res. 2010;105:143–66. https://doi.org/10.1007/s11120-010-9568-2.
[56] Rodríguez-Villalón A, Gas E, Rodríguez-Concepción M. Phytoene synthase activity controls the biosynthesis of carotenoids and the supply of their metabolic precursors in dark-grown Arabidopsis seedlings. Plant J. 2009;60:424–35. https://doi.org/10.1111/j.1365-313X.2009.03966.x.
[57] Park H, Kreunen SS, Cuttriss AJ, Dellapenna D, Pogson BJ. Identification of the carotenoid isomerase provides insight into carotenoid biosynthesis, prolamellar body formation, and photomorphogenesis. Plant Cell 2002;14:321–32. https://doi.org/10.1105/tpc.010302.
[58] Quian-Ulloa R, Stange C. Carotenoid biosynthesis and plastid development in plants : the role of light. Int. J. Mol. Sci. 2021;22.
[59] Tóth TN, Chukhutsina V, Domonkos I, Knoppová J, Komenda J, Kis M, et al. Carotenoids are essential for the assembly of cyanobacterial photosynthetic complexes. Biochim. Biophys. Acta Bioenerg. 1847;2015:1153–65. https://doi.org/10.1016/j.bbabio.2015.05.020.
[60] Ruban AV, Johnson MP, Duffy CDP. The photoprotective molecular switch in the photosystem II antenna. Biochim. Biophys. Acta 1817;2012:167–81. https://doi.org/10.1016/j.bbabio.2011.04.007.
[61] Ramel F, Birtic S, Cuiné S, Triantaphylidès C, Ravanat JL, Havaux M. Chemical quenching of singlet oxygen by carotenoids in plants. Plant Physiol. 2012;158: 1267–78. https://doi.org/10.1104/pp.111.182394.
[62] Joyard J, Ferro M, Masselon C, Seigneurin-Berny D, Salvi D, Garin J, et al. Chloroplast proteomics and the compartmentation of plastidial isoprenoid biosynthetic pathways. Mol. Plant 2009;2:1154–80. https://doi.org/10.1093/mp/ssp088.
[63] García-Cerdán JG, Schmid EM, Takeuchi T, Mcrae I, Mcdonald KL, Yordduangjun N, et al. Chloroplast Sec14-like 1 (CPSFL1) is essential for normal chloroplast development and affects carotenoid accumulation in Chlamydomonas. Proc. Natl. Acad. Sci. U. S. A. 2020;117:12452–63. https://doi.org/10.1073/pnas.1916948117.
[64] Mulisch M, Krupinska K. Ultrastructural analysese of senescence associated dismantling of chloroplasts revisited. In: Briswal B, Krupinska K, Biswal UC, editors. Plast. Dev. Leaves Dur. Growth Senescence; 2013. p. 307–35. https://doi.org/10.1007/978-94-007-5724-0.
[65] Egea I, Barsan C, Bian W, Purgatto E, Latché A, Chervin C, et al. Chromoplast differentiation : current status and perspectives Mini review. Plant Cell Physiol 2010;51:1601–11. https://doi.org/10.1093/pcp/pcq136.
[66] Li L, Yuan H. Chromoplast biogenesis and carotenoid accumulation. Arch. Biochem. Biophys. 2013;539:102–9. https://doi.org/10.1016/j.abb.2013.07.002.
[67] Lopez-Juez E, Pyke KA. Plastids unleashed: their development and their integration in plant development. Int J Dev Biol 2005;49:557–77. https://doi.org/10.1387/ijdb.051997el.
[68] Ljubesic N, Wrischer M, Devide Z. Chromoplasts - the last stages in plastid development. Int J Dev Biol 1991;35:251–8.
[69] Ljubesic N, Wrischer M, Devidé Z. Chromoplast structures in Thunbergia flowers. Protoplasma 1996;193:174–80.
[70] Jeffery J, Holzenburg A, King S. Physical barriers to carotenoid bioaccessibility . Ultrastructure survey of chromoplast and cell wall morphology in nine carotenoid-containing fruits and vegetables. J. Sci. Food Agric. 2012:2594–602. https://doi.org/10.1002/jsfa.5767.
[71] Lado J, Zacarías L, Gurrea A, Page A, Stead A, Rodrigo MJ. Exploring the diversity in Citrus fruit colouration to decipher the relationship between plastid ultrastructure and carotenoid composition. Planta 2015;242:645–61. https://doi.org/10.1007/s00425-015-2370-9.
[72] Gómez-Gómez L, Parra-Vega V, Rivas-Sendra A, Seguí-Simarro JM, Prieto A, Ahrazem O. Unraveling massive Crocins transport and accumulation through proteome and microscopy tools during the development of saffron stigma. Int. J. Mol. Sci. 2017;18:1–22. https://doi.org/10.3390/ijms18010076.
[73] Vidi P, Kanwischer M, Baginsky S, Austin JR, Csucs G, Dörmann P, et al. Tocopherol Cyclase (VTE1) Localization and Vitamin E Accumulation in Chloroplast Plastoglobule Lipoprotein Particles. J. Biol. Chem. 2006;281: 11225–34. https://doi.org/10.1074/jbc.M511939200.
[74] Lohmann A, Scho MA, Bréhélin C, Kessler F, Bock R, Cahoon EB. Deficiency in Phylloquinone (Vitamin K1) Methylation Affects Prenyl Quinone Distribution , Photosystem I Abundance , and Anthocyanin Accumulation in the Arabidopsis AtmenG Mutant. J. Biol. Chem. 2006;281:40461–72. https://doi.org/10.1074/jbc.M609412200.
[75] Zbierzak AM, Kanwischer M, Wille C, Vidi PA, Giavalisco P, Lohmann A, et al. Intersection of the tocopherol and plastoquinol metabolic pathways at the plastoglobule. Biochem. J. 2010;425:389–99. https://doi.org/10.1042/BJ20090704.
[76] Havaux M. Plastoquinone in and beyond photosynthesis. Trends Plant Sci. 2020; 25:1252–65. https://doi.org/10.1016/j.tplants.2020.06.011.
[77] Simkin AJ, Gaffé J, Alcaraz JP, Carde JP, Bramley PM, Fraser PD, et al. Fibrillin influence on plastid ultrastructure and pigment content in tomato fruit. Phytochemistry 2007;68:1545–56. https://doi.org/10.1016/j.phytochem.2007.03.014.
[78] Deruere J, Romer S, D'harlingue A, Backhaus RA, Kuntz M, Camara B. Fibril assembly and carotenoid overaccumulation in chromoplasts: a model for supramolecular lipoprotein structures. Plant Cell 1994;6:119–33. https://doi.org/10.1105/tpc.6.1.119.
[79] Hansmann P, Junker R, Sauter H, Sitte P. Chromoplast development in daffodil coronae during Anthesis. J. Plant Physiol. 1987;131:133–43. https://doi.org/10.1016/S0176-1617(87)80274-2.
[80] Paolillo DJ, Garvin DF, Parthasarathy MV. The chromoplasts of or mutants of cauliflower (Brassica oleracea L. var. botrytis). Protoplasma 2004;224:245–53. https://doi.org/10.1007/s00709-004-0059-1.
[81] Maass D, Arango J, Wüst F, Beyer P, Welsch R. Carotenoid crystal formation in Arabidopsis and carrot roots caused by increased Phytoene synthase protein levels. PLoS One 2009;4. https://doi.org/10.1371/journal.pone.0006373.
[82] Dhami N. Chloroplast-to-chromoplast transition envisions provitamin A biofortification in green vegetables. Plant Cell Rep. 2021;40:799–804. https://doi.org/10.1007/s00299-021-02684-7.
[83] Bian W, Barsan C, Egea I, Purgatto E, Chervin C, Zouine M, et al. Metabolic and molecular events occurring during Chromoplast biogenesis. J Bot 2011;2011: 1–13. https://doi.org/10.1155/2011/289859.
[84] Llorente B, Torres-Montilla S, Morelli L, Florez-Sarasa I, Matus JT, Ezquerro M, et al. Synthetic conversion of leaf chloroplasts into carotenoid-rich plastids reveals mechanistic basis of natural chromoplast development. Proc. Natl. Acad. Sci. U. S. A. 2020;117:21796–803. https://doi.org/10.1073/pnas.2004405117.
[85] Shinozaki Y, Nicolas P, Fernandez-Pozo N, Ma Q, Evanich DJ, Shi Y, et al. High-resolution spatiotemporal transcriptome mapping of tomato fruit development and ripening. Nat. Commun. 2018;9. https://doi.org/10.1038/s41467-017-02782-9.
[86] Egea I, Bian W, Barsan C, Jauneau A, Pech JC, Latché A, et al. Chloroplast to chromoplast transition in tomato fruit: spectral confocal microscopy analyses of carotenoids and chlorophylls in isolated plastids and time-lapse recording on intact live tissue. Ann. Bot. 2011;108:291–7. https://doi.org/10.1093/aob/mcr140.
[87] Barsan C, Sanchez-Bel P, Rombaldi C, Egea I, Rossignol M, Kuntz M, et al. Characteristics of the tomato chromoplast revealed by proteomic analysis. J. Exp. Bot. 2010;61:2413–31. https://doi.org/10.1093/jxb/erq070.
[88] Suzuki M, Takahashi S, Kondo T, Dohra H, Ito Y, Kiriiwa Y, et al. Plastid proteomic analysis in tomato fruit development. PLoS One 2015;10:1–25. https://doi.org/10.1371/journal.pone.0137266.
[89] Rödiger A, Agne B, Dobritzsch D, Helm S, Müller F, Pötzsch N, et al. Chromoplast differentiation in bell pepper (Capsicum annuum) fruits. Plant J. 2020:1–12. https://doi.org/10.1111/tpj.15104.
[90] Kahlau S, Bock R. Plastid transcriptomics and translatomics of tomato fruit development and chloroplast-to-chromoplast differentiation: Chromoplast gene expression largely serves the production of a single protein. Plant Cell 2008;20: 856–74. https://doi.org/10.1105/tpc.107.055202.
[91] Sato S, Nakamura Y, Kaneko T, Asamizu E, Tabata S. Complete structure of the chloroplast genome of Arabidopsis thaliana. DNA Res. 1999;6:283–90.
[92] Ling Q, Huang W, Baldwin A, Jarvis P. Chloroplast biogenesis is regulated by direct action of the ubiquitin-proteasome system. Science (80-) 2012;338: 655–60.
[93] Ling Q, Sadali NM, Soufi Z, Zhou Y, Huang B, Zeng Y, et al. The chloroplast-associated protein degradation pathway controls chromoplast development and fruit ripening in tomato. Nat Plants 2021;7. https://doi.org/10.1038/s41477-021-00916-y.
[94] D'andrea L, Rodriguez-Concepcion M. Manipulation of Plastidial protein quality control components as a new strategy to improve carotenoid contents in tomato fruit. Front. Plant Sci. 2019;10:1–7. https://doi.org/10.3389/fpls.2019.01071.
[95] Neta-Sharir I, Isaacson T, Lurie S, Weiss D. Dual role for tomato heat shock protein 21: protecting photosystem II from oxidative stress and promoting color changes during fruit maturation. Plant Cell 2005;17:1829–38. https://doi.org/10.1105/tpc.105.031914.
[96] Luo Z, Zhang J, Li J, Yang C, Wang T, Ouyang B, et al. A STAY-GREEN protein SlSGR1 regulates lycopene and b -caro- tene accumulation by interacting directly with SlPSY1 during rip- ening processes in tomato. New Phytol. 2013;198: 442–52.

[97] Yuan H, Zhang J, Nageswaran D, Li L. Carotenoid metabolism and regulation in horticultural crops. Hortic Res 2015;2. https://doi.org/10.1038/hortres.2015.36.
[98] Carvalho LJCB, Chen JLS, Souza CRB, Vieira EA, Anderson JV. Characterization of carotenoid-protein complexes and gene expression analysis associated with carotenoid sequestration in pigmented cassava (Manihot Esculenta Crantz) storage root. Open Biochem J 2012;6:116–30. https://doi.org/10.2174/1874091x01206010116.
[99] Pulido P, Llamas E, Llorente B, Ventura S, Wright LP, Rodríguez-Concepción M. Specific Hsp100 chaperones determine the fate of the first enzyme of the Plastidial Isoprenoid pathway for either refolding or degradation by the stromal Clp protease in Arabidopsis. PLoS Genet. 2016;12:1–19. https://doi.org/10.1371/journal.pgen.1005824.
[100] Llamas E, Pulido P, Rodriguez-Concepcion M. Interference with plastome gene expression and Clp protease activity in Arabidopsis triggers a chloroplast unfolded protein response to restore protein homeostasisvol. 13; 2017. https://doi.org/10.1371/journal.pgen.1007022.
[101] D'andrea L, Simon-Moya M, Llorente B, Llamas E, Marro M, Loza-Alvarez P, et al. Interference with Clp protease impairs carotenoid accumulation during tomato fruit ripening. J. Exp. Bot. 2018;69:1557–67. https://doi.org/10.1093/jxb/erx491.
[102] Li L, Paolillo DJ, Parthasarathy MV, Dimuzio EM, Garvin DF, Plant US, et al. A novel gene mutation that confers abnormal patterns of b-carotene accumulation in cauli ¯ ower (*Brassica oleracea* var. botrytis). Plant J. 2001;26:59–67.
[103] Lu S, Van Eck J, Zhou X, Lopez AB, O'halloran DM, Cosman KM, et al. The cauliflower or gene encodes a DnaJ cysteine-rich domain-containing protein that mediates high levels of b -carotene accumulation. Plant Cell 2006;18:3594–605. https://doi.org/10.1105/tpc.106.046417.
[104] Tzuri G, Zhou X, Chayut N, Yuan H, Portnoy V, Meir A, et al. A 'golden' SNP in CmOr governs the fruit flesh color of melon (Cucumis melo). Plant J. 2015;82:267–79. https://doi.org/10.1111/tpj.12814.
[105] Chayut N, Yuan H, Ohali S, Meir A, Sa'ar U, Tzuri G, et al. Distinct mechanisms of the ORANGE protein in controlling carotenoid flux. Plant Physiol. 2017;173:376–89. https://doi.org/10.1104/pp.16.01256.
[106] Park SC, Kim SH, Park S, Lee HU, Lee JS, Park WS, et al. Enhanced accumulation of carotenoids in sweetpotato plants overexpressing IbOr-ins gene in purple-fleshed sweetpotato cultivar. Plant Physiol. Biochem. 2015;86:82–90. https://doi.org/10.1016/j.plaphy.2014.11.017.
[107] Bai C, Capell T, Berman J, Medina V, Sandmann G, Christou P, et al. Bottlenecks in carotenoid biosynthesis and accumulation in rice endosperm are influenced by the precursor-product balance. Plant Biotechnol. J. 2016;14:195–205. https://doi.org/10.1111/pbi.12373.
[108] Berman J, Zorrilla-López U, Medina V, Farré G, Sandmann G, Capell T, et al. The Arabidopsis ORANGE (AtOR) gene promotes carotenoid accumulation in transgenic corn hybrids derived from parental lines with limited carotenoid pools. Plant Cell Rep. 2017;36:933–45. https://doi.org/10.1007/s00299-017-2126-z.
[109] Yazdani M, Sun Z, Yuan H, Zeng S, Thannhauser TW, Vrebalov J, et al. Ectopic expression of ORANGE promotes carotenoid accumulation and fruit development in tomato. Plant Biotechnol. J. 2019;17:33–49. https://doi.org/10.1111/pbi.12945.
[110] Lopez AB, Van Eck J, Conlin BJ, Paolillo DJ, O'neill J, Li L. Effect of the cauliflower or transgene on carotenoid accumulation and chromoplast formation in transgenic potato tubers. J. Exp. Bot. 2008;59:213–23. https://doi.org/10.1093/jxb/erm299.
[111] Zhou X, Welsch R, Yang Y, Álvarez D, Riediger M, Yuan H, et al. Arabidopsis OR proteins are the major posttranscriptional regulators of phytoene synthase in controlling carotenoid biosynthesis. Proc. Natl. Acad. Sci. U. S. A. 2015;112:3558–63. https://doi.org/10.1073/pnas.1420831112.
[112] Park S, Kim HS, Jung YJ, Kim SH, Ji CY, Wang Z, et al. Orange protein has a role in phytoene synthase stabilization in sweetpotato. Sci. Rep. 2016:1–12. https://doi.org/10.1038/srep33563.
[113] Welsch R, Zhou X, Yuan H, Álvarez D, Sun T, Schlossarek D, et al. Clp protease and OR directly control the Proteostasis of Phytoene synthase, the crucial enzyme for carotenoid biosynthesis in Arabidopsis. Mol. Plant 2018;11:149–62. https://doi.org/10.1016/j.molp.2017.11.003.
[114] Sun T, Yuan H, Chen C, Kadirjan-Kalbach DK, Mazourek M, Osteryoung KW, et al. ORHis, a natural variant of OR, specifically interacts with plastid division factor ARC3 to regulate Chromoplast number and carotenoid accumulation. Mol. Plant 2020;13:864–78. https://doi.org/10.1016/j.molp.2020.03.007.
[115] Yuan H, Owsiany K, Sheeja TE, Zhou X, Rodriguez C, Li Y, et al. A single amino acid substitution in an ORANGE protein promotes carotenoid overaccumulation in arabidopsis. Plant Physiol. 2015;169:421–31. https://doi.org/10.1104/pp.15.00971.
[116] Feder A, Chayut N, Gur A, Freiman Z, Tzuri G, Meir A, et al. The role of Carotenogenic metabolic flux in carotenoid accumulation and Chromoplast differentiation: lessons from the melon fruit. Front. Plant Sci. 2019;10:1–7. https://doi.org/10.3389/fpls.2019.01250.
[117] Sun T, Huang X-Q, Chen W-C, Kong M, Zhou C-F, Zhuang Z, et al. ORANGE represses chloroplast biogenesis in etiolated arabidopsis cotyledons via interaction with TCP14[OPEN]. Plant Cell 2019;31:2996–3014. https://doi.org/10.1105/tpc.18.00290.
[118] Kim HS, Ji CY, Lee CJ, Kim SE, Park SC, Kwak SS. Orange: a target gene for regulating carotenoid homeostasis and increasing plant tolerance to environmental stress in marginal lands. J. Exp. Bot. 2018;69:3393–400. https://doi.org/10.1093/jxb/ery023.
[119] Osorio CE. The role of Orange gene in carotenoid accumulation: manipulating Chromoplasts toward a colored future. Front. Plant Sci. 2019;10. https://doi.org/10.3389/fpls.2019.01235.
[120] Pulido P, Toledo-Ortiz G, Phillips MA, Wright LP, Rodríguez-Concepción M. Arabidopsis J-protein J20 delivers the first enzyme of the plastidial isoprenoid pathway to protein quality control. Plant Cell 2013;25:4183–94. https://doi.org/10.1105/tpc.113.113001.
[121] Rodriguez-Concepcion M, D'andrea L, Pulido P. Control of plastidial metabolism by the Clp protease complex. J. Exp. Bot. 2019;70:2049–58. https://doi.org/10.1093/jxb/ery441.
[122] Moreno JC, Martínez-Jaime S, Schwartzmann J, Karcher D, Tillich M, Graf A, et al. Temporal proteomics of inducible RNAi lines of Clp protease subunits identifies putative protease substrates. Plant Physiol. 2018;176:1485–508. https://doi.org/10.1104/pp.17.01635.
[123] Nishimura K, Van Wijk KJ. Organization , function and substrates of the essential Clp protease system in plastids. Biochim. Biophys. Acta 1847;2015:915–30. https://doi.org/10.1016/j.bbabio.2014.11.012.
[124] Ytterberg AJ, Peltier J-B, Van Wijk KJ. Protein profiling of plastoglobules in chloroplasts and chromoplasts . a surprising site for differential accumulation of metabolic enzymes. Plant Physiol. 2006;140:984–97. https://doi.org/10.1104/pp.105.076083.984.
[125] Kilambi HV, Kumar R, Sharma R, Sreelakshmi Y. Chromoplast-specific carotenoid-associated protein appears to be important for enhanced accumulation of carotenoids in hp1 tomato fruits. Plant Physiol. 2013;161:2085–101. https://doi.org/10.1104/pp.112.212191.
[126] Leitner-Dagan Y, Ovadis M, Shklarman E, Elad Y, David DR, Vainstein A. Expression and functional analyses of the plastid lipid-associated protein CHRC suggest its role in chromoplastogenesis and stress. Plant Physiol. 2006;142:233–44. https://doi.org/10.1104/pp.106.082404.
[127] Leitner-Dagan Y, Ovadis M, Zuker A, Shklarman E, Ohad I, Tzfira T, et al. CHRD, a plant member of the evolutionarily conserved YjgF family, influences photosynthesis and chromoplastogenesis. Planta 2006;225:89–102. https://doi.org/10.1007/s00425-006-0332-y.
[128] Rey P, Gillet B, Römer S, Eymery F, Massimino J, Peltier G, et al. Over-expression of a pepper plastid lipid-associated protein in tobacco leads to changes in plastid ultrastructure and plant development upon stress. Plant J. 2000;21:483–94. https://doi.org/10.1046/j.1365-313X.2000.00699.x.
[129] Li J, Yang J, Zhu B, Xie G. Overexpressing OsFBN1 enhances plastoglobule formation, reduces grain-filling percent and jasmonate levels under heat stress in rice. Plant Sci. 2019;285:230–8. https://doi.org/10.1016/j.plantsci.2019.05.007.
[130] Fraser PD, Enfissi EMA, Halket JM, Truesdale MR, Yu D, Gerrish C, et al. Manipulation of phytoene levels in tomato fruit: effects on isoprenoids, plastids, and intermediary metabolism. Plant Cell 2007;19:3194–211. https://doi.org/10.1105/tpc.106.049817.
[131] Camagna M, Grundmann A, Bar C, Koschmieder J, Beyer P, Welsch R. Enzyme fusion removes competition for geranylgeranyl diphosphate in carotenogenesis. Plant Physiol. 2019;179:1013–27. https://doi.org/10.1104/pp.18.01026.
[132] Cao H, Zhang J, Xu J, Ye J, Yun Z, Xu Q, et al. Comprehending crystalline -carotene accumulation by comparing engineered cell models and the natural carotenoid-rich system of citrus. J. Exp. Bot. 2012;63:4403–17. https://doi.org/10.1093/jxb/err115.
[133] Lu Y, Stegemann S, Agrawal S, Karcher D, Ruf S, Bock R. Horizontal transfer of a synthetic metabolic pathway between plant species. Curr Biol 2017;27. https://doi.org/10.1016/j.cub.2017.08.044. 3034–3041.e3.
[134] Iorizzo M, Ellison S, Senalik D, Zeng P, Satapoomin P, Huang J, et al. A high-quality carrot genome assembly provides new insights into carotenoid accumulation and asterid genome evolution. Nat. Genet. 2016;48:657–66. https://doi.org/10.1038/ng.3565.
[135] Welsch R, Beyer P, Hugueney P, Kleinig H, Von Lintig J. Regulation and activation of phytoene synthase, a key enzyme in carotenoid biosynthesis, during photomorphogenesis. Planta 2000;211:846–54. https://doi.org/10.1007/s004250000352.
[136] Andersen TB, Llorente B, Morelli L, Torres-Montilla S, Bordanaba-Florit G, Espinosa FA, et al. An engineered extraplastidial pathway for carotenoid biofortification of leaves. Plant Biotechnol. J. 2021:0–2. https://doi.org/10.1111/pbi.13526.
[137] Majer E, Llorente B, Rodríguez-Concepción M, Daròs J-A. Rewiring carotenoid biosynthesis in plants using a viral vector. Sci. Rep. 2017:1–10. https://doi.org/10.1038/srep41645.
[138] Ye X, Al-Babili S, Klöti A, Zhang J, Lucca P, Beyer P, et al. Engineering the Provitamin A (β-Carotene) Biosynthetic Pathway into (Carotenoid-Free) Rice Endosperm. Science (80-) 2000;287:303–6.
[139] Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, et al. Improving the nutritional value of Golden Rice through increased pro-vitamin a content. Nat. Biotechnol. 2005;23:482–7. https://doi.org/10.1038/nbt1082.
[140] Schweiggert RM, Mezger D, Schimpf F, Steingass CB, Carle R. Influence of chromoplast morphology on carotenoid bioaccessibility of carrot, mango, papaya, and tomato. Food Chem. 2012;135:2736–42. https://doi.org/10.1016/j.foodchem.2012.07.035.
[141] Tang G, Qin J, Dolnikowski GG, Russell RM, Grusak MA. Spinach or carrots can supply significant amounts of vitamin a as assessed by feeding with intrinsically deuterated vegetables. Am. J. Clin. Nutr. 2005;82:821–8. https://doi.org/10.1093/ajcn/82.4.821.
[142] Tang G, Qin J, Dolnikowski GG, Russell RM, Grusak MA. Golden Rice is an effective source of vitamin a. Am. J. Clin. Nutr. 2009;89:1776–83. https://doi.org/10.1097/HP.0b013e318292b9fc.