Carotenoids in nature: insights from plants and beyond

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Abstract. Carotenoids are natural isoprenoid pigments that provide leaves, fruits, vegetables and flowers with distinctive yellow, orange and some reddish colours as well as several aromas in plants. Their bright colours serve as attractants for pollination and seed dispersal. Carotenoids comprise a large family of C40 polyenes and are synthesised by all photosynthetic organisms, aphids, some bacteria and fungi alike. In animals carotenoid derivatives promote health, improve sexual behaviour and are essential for reproduction. As such, carotenoids are commercially important in agriculture, food, health and the cosmetic industries. In plants, carotenoids are essential components required for photosynthesis, photoprotection and the production of carotenoid-derived phytohormones, including ABA and strigolactone. The carotenoid biosynthetic pathway has been extensively studied in a range of organisms providing an almost complete pathway for carotenogenesis. A new wave in carotenoid biology has revealed implications for epigenetic and metabolic feedback control of carotenogenesis. Developmental and environmental signals can regulate carotenoid gene expression thereby affecting carotenoid accumulation. This review highlights mechanisms controlling (1) the first committed step in phytoene biosynthesis, (2) flux through the branch to synthesis of α- and β-carotenes and (3) metabolic feedback signalling within and between the carotenoid, MEP and ABA pathways.

Additional keywords: abscisic acid, apocarotenoids, carotenoid, chloroplast, chromatin, epigenetic, hormones, isoprenoid, metabolic feedback, plant, photoisomerisation, regulation, signal molecule, strigolactone.

Functions for carotenoids in nature
Vitamins, antioxidants and spices

Animals are generally unable to synthesise carotenoids and require a dietary intake of plant products to meet daily health demands. There are exceptions such as the synthesis of carotenoids in human protist parasites (e.g. Plasmodium and Toxoplasma) and aphids, which can be explained by the existence of a remnant plastid and lateral gene transfer of carotenoid biosynthesis genes from a fungus, respectively (Tonhosolo et al. 2009; Moran and Jarvik 2010). In most animals, dietary carotenoids are cleaved to provide precursors for vitamin A biosynthesis (of which a deficiency leads to blindness) and are valuable for many physiological functions and thus promote human health (e.g. antioxidant activity, immunostimulants, yolk nourishment to embryos, photoprotection, visual tuning as well as limiting age-related macular degeneration of the eye) (Johnson 2002; Krinsky and Johnson 2005).

A deficiency in vitamin A is responsible for child (and maternal) mortality and it is estimated that a 23–34% reduction in preschool mortality can be expected from vitamin A program reaching children in undernourished settings (The State of the World’s Children, UNICEF, www.un.org/en/mdg/summit2010, accessed 19 August 2011). The development of high yielding β-carotene crops such as ‘golden rice’ could provide close to the recommended daily allowance of vitamin A for malnourished children and help combat vitamin A deficiency-induced mortality and morbidity (Fig. 1) (www.goldenrice.org, accessed 19 August 2011).

The nutraceutical industry synthetically manufactures five major carotenoids on an industrial scale (e.g. lycopene, β-carotene, canthaxanthin, zeaxanthin and astaxanthin) for use in a range of food products and cosmetics, such as vitamin supplements and health products and as feed additives for poultry, livestock, fish and crustaceans (reviewed by Del Campo et al. 2007; Jackson et al. 2008). One of the most commercially valuable pigments, astaxanthin, is primarily synthesised by marine microorganisms, such as the green alga Haematococcus pluvialis and accumulates in fish such as salmon, thus, colouring their flesh red. Astaxanthin has been implicated as a potential therapeutic agent treating cardiovascular disease and prostatic cancer (Fassett and Coombes 2011).
Carotenoids accumulate in light exposed tissues, such as skin and as such have gained increased value in the cosmetic industries as suitable compounds for photoprotection due to their scavenging action on reactive oxygen species (ROS) and anti-inflammatory properties (Stahl and Sies 2007). Photo-oxidative damage affects cellular lipids, proteins and DNA and is involved in the patho-biochemistry of erythema formation, premature aging of the skin, development of photodermatoses and skin cancer. Evidence shows that β-Carotene, lutein and perhaps even lycopene, can prevent UV-induced erythema formation and contribute to life-long protection against exposure to harmful affects of sunlight (Stahl and Sies 2007).

Apocarotenoids are also highly valued as additives in the food industry. Spices such as bixin (annatto), a red-collared, dicarboxylic monomethyl ester apocarotenoid is traditionally derived from the plant *Bixa orellana* (also known as achiote). Saffron comes from the thread-like reddish coloured female reproductive organs of the *Crocus sativa* flower (petals are coloured light purple), which is considered one of the worlds most expensive spices and widely used as a natural colourant. The

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### Fig. 1. The many essential functions for carotenoids in nature. Carotenoids play important roles in (1) promoting animal behaviour, reproduction and survival, (2) improving nutrition and human health, (3) assembly of photosystems, light capture and photoprotection and (4) providing substrates for the biosynthesis of plant hormones and signalling molecules.

![Diagram of carotenoid functions](image_url)
colour is due to the degradation of carotenoids (e.g. zeaxanthin to crocin and crocetin), whereas the flavour arises from the accumulation of carotenoid cleavage oxidation products from zeaxanthin (e.g. mainly safranal and the bitter glucoside, picrocrocin). Safranal (2,6,6-trimethylcyclohexa-1,3-dien-1-carboxaldehyde) is easily synthesised by de-glucosylation of picrocrocin and composes ~70% of total volatiles from crocin flowers (Fig. 1) (Leffingwell 2002; Bouvier et al. 2003a; Schwab et al. 2008). There is a growing interest in the impact of saffron carotenoids on human health due to their high antioxidant capacity (Verma and Borda 1998). Other carotenoid-derived volatiles, such as α- and β-ionone are predominant norisoprenoid volatiles in the mature stigma tissue that are also important components of aroma and taste produced during flower and fruit development (Goff and Klee 2006).

Animal behaviour, reproduction and survival

Animals accumulate carotenoids where they are critical in determining sexual behaviour, reproduction and avoiding predation as well as parasitism. Fish and birds accumulate dietary carotenoids, which boost their immune system and advertise health, often leading to preferential selection by the sexual partner (McGraw et al. 2006b; Baron et al. 2008). Animals typically place different priorities on fitness-enhancing activities (e.g. gametic investment in females, sexual attraction in males) and carotenoid allocation appears to track such investment patterns in the two sexes (McGraw and Toomey 2010). For example, environmental and physiological factors influence colour expression in house finches (Fig. 1) and the type of dietary carotenoids is one determinant of their ability to become bright red rather than drab yellow (McGraw et al. 2006a). A particular primacy of higher levels of β-carotanthin was assigned to the coloration in the male house finches that become red and more sexually attractive. It is possible that the red house finches adopt selective foraging strategies for the most β-carotanthin-rich foods rather then grains and fruits, which typically contain the more common dietary yellow xanthophylls and carotenes (Fig. 2) (McGraw et al. 2006a). Finally, Hill (2000) demonstrated that birds fed diets rich in lutein and zeaxanthin turn only yellow, but grow red feathers when fed β-carotanthin-rich foods (e.g. tangerine juice). Perhaps, it is the metabolic precursors of β-carotanthin such as 3-hydroxycechinonone, which provide the red carotenoid pigmentation (Hill 2000).

Another intriguing example is the flamingo, whose feathers are coloured bright rosy pink (Fig. 1) from the carotenoid pigments (derivatives such as canthaxanthin, main pigment found in the secretions, as well as small quantities of β-carotanthin are likely to contribute to pink colouration) in the algae and various invertebrates (e.g. crayfish) that make up the bulk of a flamingo’s diet. Curiously, the crustaceans accumulate astaxanthan whose spectral properties become modified by the β-barrel protein, crustacyanin, resulting in blue pigmentation that shifts to red upon protein–pigment denaturation during cooking (Fig. 1; American lobster) (Krawczyk and Britton 2001). A dietary source of carotenoids by the flamingo was found in the oils of uropygial secretions, which deposit over the plumage and growing feathers more frequently during periods when in a group, contributing some colour and possibly indicating a cosmetic function for mate choice. The cosmetic nature of accumulating carotenoids by painting canthaxanthin-rich secretions onto their feathers to create a visually more attractive breeding partner appeared to enhance the frequency of their courting behaviour during mating seasons (Amat et al. 2010).

Carotenoids also play important roles in animals to avoid predation and reduce parasitism. For example, aphids (e.g. Acrithosiphon pisum) are the first known animal to have acquired the carotenoid biosynthetic machinery to produce carotenoids such as torulene and dehydro-γ, η-carotene, which provide a reddish colouration distinguished them from their green forms, which accumulate γ-carotene, β-carotene and α-carotene (Moran and Jarvis 2010). The colour polymorphism is maintained by frequency-dependent selection imposed by natural predators that preferentially prey upon the red morphs (Fig. 1) and higher rates of parasitism in the green forms (Losey et al. 1997).

Photosynthesis and photoprotection in plants

Carotenoids are essential for energy capture from the solar emission spectrum. Of the many naturally occurring carotenoids, less than 50 play a light-harvesting role in photosynthetic organisms (e.g. plants; protists such as the human parasites Plasmodium and Toxoplasma as well as the coral endosymbiotic Dinoflagellates; and the Symbiodinium marine algae which forms a relationship with the sea anemone, Aiptasia insignis) (Fig. 1) (Chidambaram Murthy et al. 2005; Polivka and Frank 2010). Carotenoid colours range from pale yellow to a reddish brown depending upon the number of conjugated double bonds along the C40 backbone, as well as other cyclic and oxygenic modifications. The distinctive yellow colour of light-harvesting carotenoids become more apparent in leaves during autumn when chlorophyll degrades revealing their strong colours. Carotenoids are required for the correct assembly of photosystems (Pogson et al. 2005; Li et al. 2009b). Here they absorb light across a broader range of the spectral region in which the sun irradiates maximally and transfer the energy to chlorophyll, initiating the photochemical events of photosynthesis (Polivka and Frank 2010). In plants, carotenoids are bound in discrete pigment–protein complexes referred to as the LHCII trimeric complex, which typically benefits from lutein, neoxanthin, as well as violaxanthin to transfer energy to chlorophyll (Pogson et al. 2005; Demmig-Adams and Adams 2006; Dall’Osto et al. 2010). A curious marine dinoflagellate, Amphidinium carterae alternatively evolved a peridinin–chlorophyll protein complex that contains more of the bound carotenoid (peridinin) when compared with chlorophyll (Hofmann et al. 1996; Polivka and Frank 2010) (Fig. 1).

Perhaps the roles that carotenoids play to protect the photosynthetic machinery from excessive light are among key biological functions of carotenoids relevant for life on earth. Plants are able to balance between absorbing sufficient light for photosynthetic processes while avoiding photo oxidative damage to membranes and proteins caused by excessive light. To meet this balance carotenoids (1) quench triplet chlorophyll, (2) scavenge ROS like singlet oxygen which damage membranes.
and proteins, thereby behaving as antioxidants (along with ascorbate and tocopherols) and (3) dissipate excess energy via xanthophyll-mediated non-photochemical quenching (NPQ) (e.g. xanthophyll carotenoids include zeaxanthin, antheraxanthin and lutein). The physiological relevance of xanthophylls to photosynthesis and plant biology in general is displayed by bleaching, delayed greening, viviparous and semi-lethal phenotypes observed in several carotenoid and NPQ-deficient mutants (Niyogi 1999; Pogson et al. 2005; Bailey and Grossman 2008; Alboresi et al. 2011).

Fig. 2. Carotenoid biosynthesis and regulation in Arabidopsis. The light responsive enzyme, phytoene synthase (PSY) catalyses the first committed step in the synthesis phytoene from geranylgeranyl pyrophosphate and is a major bottleneck in the pathway. The branch in carotenoid synthesis is another key regulatory step. The isomerisation of tetra-cis-lycopene (pro-lycopene) to lycopene requires the carotenoid isomerase (CRTISO) and light-mediated photoisomerisation. Modification of chromatin surrounding the CRTISO locus by the histone lysine methyltransferase, SET DOMAIN GROUP8 (SDG8), is rate limiting for lutein production. Finally, lycopene undergoes modifications by εLCY (epsilon cyclase) and β-LCY (β-cyclase) to produce α- and β-carotene respectively. The carotenes serve as substrates for the production of xanthophylls including lutein, violaxanthin and neoxanthin. The xanthophyll cycle (interconversion between zeaxanthin, antheraxanthin and violaxanthin) is responsive to light and important in photoprotection. PDS, phytoene desaturase, ZDS, ζ-carotene desaturase; Z-ISO ζ-carotene isomerase, βOH, β-hydroxylase; εOH, ε-hydroxyase, NXS, neoxanthin synthase; VDE, violaxanthin de-epoxidase; ZEP, zeaxanthin epoxidase, CCD: carotenoid cleavage dioxygenase; NCED, 9-cis-epoxy-carotenoid dioxygenase.
Colour, volatiles, phytohormones and signalling molecules in plants

The bright colours of carotenoids serve as powerful visual attractants for birds and insects, which assist in the dispersal of pollen and seeds aiding plant reproduction. For example, a single locus, YELLOW UPPER5 (YUP), which was located in the petals of pink-flowered Mimulus lewissii (monkey flower), which is pollinated by bumblebees and its red-flowered sister species M. cardinalis, which is pollinated by hummingbirds (Schemske and Bradshaw 1999). Researchers creating near-isogenic lines (NILs) in which the YUP allele from each species was substituted altering flower colour and revealed an adaptive shift in pollinator preference as a result of a single mutation altering carotenoid composition (Bradshaw and Schemske 2003). The next question from a plant regulatory perspective is what is the underlying molecular nature or mutation associated with the YUP allele? The regulation of carotenoid colours in flowers has been reviewed recently (Zhu et al. 2010). In addition to providing colour to flowers and fruits, C40 carotenoid derivatives serve as substrates in the biosynthesis of plant volatile scents and aroma constituents (geranyl acetone and β-ionone) that attract insects and animals for pollination as well as seed dispersal (Simkin et al. 2004; Walter et al. 2010).

In plants, carotenoids serve as substrates for the production of phytohormones such as abscisic acid and strigolactone as well as other signalling molecules (e.g. blumenin and mycorradin) (Figs 1, 3). ABA is a cleavage product of 9-cis-violaxanthin and/or 9-cis-neoxanthin by 9-cis-epoxycarotenoid dioxygenase (NCED), which was first identified in the maize viviparous 14 (vp14) mutant (Schwartz et al. 1997; Seo and Koshiba 2002; Tan et al. 2003; Chinnusamy et al. 2008). In some situations, carotenoid biosynthetic genes, such as β-carotene hydroxylase from rice, were shown to be rate-limiting for ABA biosynthesis and can alter the plants resistance to drought and oxidative stress by modulating the levels of xanthophylls and abscisic acid synthesis (Du et al. 2010). ABA mediates responses to environmental stresses, notably the control of stomatal aperture and transpiration during drought, also the induction of many stress-related gene products. Finally, ABA promotes developmental processes such as seed maturation and dormancy (Seo and Koshiba 2002; Nambara and Marion-Poll 2005; Chinnusamy et al. 2008).

The strigolactone class of carotenoid-derived terpenoids functions to (1) inhibit shoot branching, presumably by inhibiting bud outgrowth (Gomez-Roldan et al. 2008; Umehara et al. 2008), (2) influence mycorrhizal hyphal branching in order to stimulate a symbiotic relationship in the root rhizosphere (Akiyama et al. 2005) and (3) encourage germination of parasitic plant seeds such as striga (Matusova et al. 2005). Indeed, strigolactone application of GR24 can restore wild-type branching phenotype in pea ccd8 mutants and also in the orthologous ccd8 Arabidopsis, in a dose-dependent manner (Gomez-Roldan et al. 2008). Beta-carotene has been proposed as the substrate for strigolactone biosynthesis (Matusova et al. 2005; Rani et al. 2008) and it has yet to be determined if changes in β-carotene levels perturb strigolactone production.

Other carotenoid-derived signalling molecules such as C13 cyclohexenone derivatives (e.g. blumenol) and C14 apocarotenoids (e.g. mycorradin) play important roles in controlling beneficial arbuscule turnover during arbuscular mycorrhizal (AM) fungi symbiosis as well as triggering hyphal branching in the rhizosphere (Fig. 1) (Giuliano et al. 2003; Akiyama et al. 2005; Walter et al. 2010). Further, evidence exists for the potential for a novel mobile carotenoid-derived signalling metabolite required for normal root and shoot development. The Arabidopsis BYPASS (BPSI) gene encodes an unknown protein and roots of bpsI mutants produce a graft transmissible signal arresting shoot development (Van Norman et al. 2004). That fluridone (blocks phytoene production) can partially rescue both leaf and root defects displayed by bpsI and CPTA (a lycopene cyclase inhibitor) treatment indicates that the signal is likely to require the biosynthesis of β-carotene and its derivatives (Fig. 2). The mobile signal is neither ABA- nor strigolactone related and the signal does not require the activity of any single carotenoid cleavage dioxygenase (Van Norman et al. 2004; Van Norman and Sieburth 2007). Unravelling the biosynthesis and regulation of carotenoid-derived signalling molecules will undoubtedly provide new insights into the essential roles that carotenoids play in nature.

Carotenoid biosynthesis and regulation

Carotenoid biosynthesis requires an available source of isoprenoid substrates derived from the plastid-localised 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway (Rodriguez-Concepcion 2010). Isoprenoids (or terpenoids) are naturally-occurring organic chemicals that serve as precursors to produce a diverse range of compounds such as tocopherols, chlorophylls, phylloquinone (PhQ), gibberellins, abscisic acid, monoterpens and plastoquinone (PQ). Glyceraldehyde-3-phosphate and pyruvate (generated from the Calvin cycle or glycolysis) act as initial substrates leading to five-carbon isoprene isomers, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), which are then condensed to synthesise geranylgeranyl diphosphate (GGPP) (Fig. 3). The first steps in the MEP pathway are catalysed by 1-deoxyxylulose-5-phosphate synthase (DXS) and 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR). 1-Hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate reductase (HDR) then catalyses the production of IPP (isopentenyl diphosphate) and DMAPP (dimethylallyl diphosphate). Abiotic and biotic factors are likely to influence the production of isoprenoid substrates for carotenogenesis. Most importantly, light and circadian oscillations appear to regulate the expression of most MEP and several carotenoid biosynthetic genes. The biosynthesis and regulation of the MEP pathway and isoprenoid substrates required for carotenogenesis has been covered elsewhere (Cordoba et al. 2009).

The condensation of two GGPP molecules catalysed by the phytoene synthase (PSY) enzyme produces 15-cis-phytoene, the first colourless carotenoid (Fig. 2). PSY is an important rate-limiting regulatory enzyme in the pathway, forming the first committed step and a bottleneck in carotenogenesis. In addition to high light, PSY can be transcriptionally responsive to ABA, salt, drought, temperature, photoperiod, development cues and post-transcriptional feedback regulation (Cazzonelli and Pogson 2010). Carotenoid pathway flux can be controlled by
multiple PSYs, as is the case for tomato, rice and maize, whereas only a single allele exists in Arabidopsis (Welsch et al. 2000, 2008; Giorio et al. 2008; Li et al. 2008a). Multiple homologues of PSY are likely to be somewhat redundant; however, individual PSY alleles are expressed in a tissue-specific manner and only some alleles show a unique response to abiotic stress-induced ABA signalling of PSY gene expression (Li et al. 2008b; Welsch et al. 2008; Qin et al. 2011). Alternative splicing as well as allelic variation are mechanisms by which to titrate PSY enzyme activity and are likely to underlie a major QTL determinant of flour colour.
in bread and durum wheats (Howitt et al. 2009; Rodriguez-Suárez et al. 2011). The expression of PSY is highly co-regulated with photosynthesis-related genes and isoprenoid biosynthesis pathway genes insuring an optimum photosynthetic metabolism (Meier et al. 2011). Environmental, developmental and metabolic cues are major regulators of PSY gene expression and will be considered later in the review.

The production of all-trans-lycopene from 15-cis-phytoene involves four enzymes, phytoene desaturase (PDS), \( \xi \)-carotene isomerase (\( \xi \)-ISO), \( \xi \)-carotene desaturase (ZDS) and carotenoid isomerase (CRTISO) as well as a light-mediated photoisomeration (Fig. 2) (Bartley et al. 1999; Park et al. 2002; Isaacscon et al. 2004; Breitenbach and Sandmann 2005; Dong et al. 2007; Chen et al. 2010). PDS mRNA transcript levels are slightly upregulated during photomorphogenesis via the phytochrome-mediated pathway, which could imply that PDS plays a rate-limiting role in the generation of 9,15,9'-tri-cis-\( \xi \)-carotene (\( \xi \)-carotene) (Welsch et al. 2000; Qin et al. 2007). The plastid-targeted alternative oxidase (PTOX) protein was required for phytoene desaturase (PDS) activity and links desaturation to chloroplast electron transport (Yu et al. 2007). Regulatory roles for ZDS and Z-ISO in the catalysis of \( \xi \)-carotene to tetra-cis-lycopene (pro-lycopene), the substrate for CRTISO are beginning to emerge. ZISO gene expression appears to be regulated by an extended the dark period and the loss-of-function of ZDS affects carotenoid accumulation as well as plastid-to-nucleus communications (referred to as retrograde signalling; Pogson et al. 2008), most likely due to impaired plastid development (Dong et al. 2007; Chen et al. 2010).

CRTISO catalyses the cis–trans reactions to isomerise the four cis-bonds introduced by the desaturases and emerges as a major regulatory node in the pathway (Cazzonelli et al. 2009c).\( \xi \) iso Mutants accumulate poly-cis-isomers (e.g. phytoene, phytofluene, \( \xi \)-carotene and neurosporene in addition to prolycopene) instead of the typical xanthophyll profile in non-photosynthetic tissues such as flowers (e.g. petals), the inner pericarp of green fruit and etiolated seedlings (Isaacson et al. 2002; Park et al. 2002). The function of these cis-carotenoids remains largely unknown and it is easy to speculate that they could play a role as novel signalling molecules. Most recently, CRTISO was shown to be essential for establishing an equilibrium between cis and trans carotenoid isomers (Yu et al. 2011). Perhaps CRTISO has other functions, such as to control the production of apocarotenoids precursors required for phytohormone biosynthesis (e.g. ABA or strigolactone) or to establish an equilibrium between cis-to trans-isomerisation and, hence, control the accumulation of cis-carotenoids. A recent insight revealed that the OsCRTISO may function to regulate the expression of the PSII core proteins (CP43 and CP47), thereby affecting the redox state of the PQ to stabilise oxygen-evolving extrinsic proteins and activity of photosynthetic oxygen evolution in rice (Wei et al. 2010).

Despite the block from cis- to an all-trans-configuration in \textit{crtiso} mutants, carotenogenesis can proceed in chloroplasts via photoisomeration, but there is a delay in the greening of etiolated seedlings and a substantial reduction in lutein in \textit{Arabidopsis} as well as some degree of chlorosis in tomato and rice (Isaacscon et al. 2002; Park et al. 2002; Fang et al. 2008; Wei et al. 2010; Chai et al. 2011). Perhaps, CRTISO plays important roles in non-green tissues (e.g. fruit and root appear to be protected from photoisomerisation) and in germinating seeds as they grow through the soil towards the light (Isaacscon et al. 2002; Park et al. 2002). The role of light in substituting for the absence of CRTISO activity in green tissues is under debate and now emerges as an exciting area of intense carotenoid research to unravel the elusive chemical mechanisms and regulatory nature associated with photoisomisation (Fig. 2) (Cunningham and Schiff 1985; Sandmann 1991; Park et al. 2002; Isaacscon et al. 2004; Yu et al. 2011).

Carotenoid biosynthesis bifurcates after lycopene to produce epsilon- and \( \beta \)-carotenoids by enzymatic activity of two lycopene cyclases, \( \epsilon \)LCY and \( \beta \)LCY (Fig. 2). Regulation by \( \epsilon \)LCY is the first committed step coordinating carotenoid flux through the \( \beta \)-\( \epsilon \) branch and modulates the ratio of the most abundant carotenoid, lutein, to the \( \beta \)-carotenoids leading to changes in lutein content (Cuttriss et al. 2007; Harjes et al. 2008; Cazzonelli et al. 2009a; Howitt et al. 2009). A molecular synergism between \( \epsilon \)LCY and \( \beta \)LCY activities is an overall major determinant of flux through the branch leading to production of lutein, \( \beta \)-carotene and other xanthophylls (Fig. 1) (Yu et al. 2008; Bai et al. 2009). Investigations have yielded mutants perturbing lutein biosynthesis including \( \lambda \)lut1, \( \epsilon \)-hydroxylase (Tian et al. 2004); \( \lambda \)lut2, \( \epsilon \)LCY (Cunningham et al. 1996; Pogson et al. 1996); \( \epsilon \)cr2, CRTISO (Park et al. 2002); and \( \lambda \)lut5, an additional \( \beta \)-hydroxylase (Kim and DellaPenna 2006) as well as the SDG8 chromatin regulator mutant, \( \epsilon \)cr1 (Cazzonelli et al. 2009a). Implications for the epigenetic regulation of carotenoid flux through the \( \beta \)-\( \epsilon \) branch are discussed below.

The xanthophyll carotenoids are major carotenoid sink metabolites accumulating in plant leaf tissues produced by the oxidative functionalisation of either \( \alpha \)-carotene (lutein) or \( \beta \)-carotenes (zeaxanthin, violaxanthin and neoxanthin) (Fig. 2). Modulation of xanthophyll composition can greatly affect photosystem assembly light harvesting, photoprotection and impact a plant’s response to stressful conditions (Pogson et al. 1998; Dall’Osto et al. 2006). Zeaxanthin can be epoxidised to produce violaxanthin by zeaxanthin epoxidase (ZEP) (Marin et al. 1996). Under high-light stress, this reaction is reversed by violaxanthin de-epoxidase (VDE) (Pfundel et al. 1994). Violaxanthin is converted to neoxanthin by neoxanthin synthase (NXS) (Al-Babili et al. 2000). Neoxanthin is the final carotenoid of the \( \beta \)-\( \beta \) branch of the classical biosynthetic pathway (Fig. 2). Many other xanthophyll carotenoids have been identified in some plants tissues and these include the highly valued ketocarotenoids (e.g. astaxanthin, capsanthin, capsorubin and canthaxanthin) and their biosynthesis has been reviewed elsewhere (Giuliano et al. 2008; Jackson et al. 2008).

\section*{Turnover and degradation of carotenoids}

Carotenoids are relatively stable compounds that accumulate in diverse types of tissues (photosynthetic and non-photosynthetic). Recently, it was demonstrated by \( ^{13} \)CO\textsubscript{2} uptake experiments that carotenoid turnover appears to be much greater than expected (Beisel et al. 2010). Given the continued synthesis in mature leaves the active degradation of carotenoids by CCD (carotenoid cleavage dioxygenases) and NCED (9-cis-epoxycarotenoid dioxygenase) enzymatic turnover has emerged an exciting area
of discovery (Bouvier et al. 2005; Walter et al. 2010; Lewinsohn et al. 2005). Members of these gene families are involved in the biosynthesis of the phytohormone ABA (NCEDs), which controls abiotic stress signalling pathways and strigolactone (CCDs), which controls shoot growth and root-myccorrhizal symbiosis (Figs 1, 3).

The NCED gene family (NCED2, 3, 5, 6, 9) show different substrate specificity as well as tissue distribution and changes in NCED transcript levels regulate carotenoid composition and abundance (Schwartz et al. 1997, 2003; Tan et al. 1997, 2003). Further, NCED3 gene expression appears to be controlled by chromatin modifying protein, ATX1 (homologue of the Drosophila Trithorax, TRX protein also referred to as SDG27), which is implicated in diverse dehydration stress-response mechanisms in Arabidopsis (Ding et al. 2011; Thorstensen et al. 2011). ATX1 was shown to affect the quantity of mRNA polymerase II bound to the NCED3 locus and is necessary for the increased levels of NCED3 transcripts and nucleosomal histone-3-lysine-4 trimethylation (H3K4 me3) that occur during dehydration stress. In the axtl mutant the NCED3 transcript levels decline and this in part reduces ABA production. The loss of function of axtl mutants shows a reduced germination rate, enlarged stomatal aperture, increased transpiration rates and a lower tolerance to dehydration stress (Ding et al. 2011).

The CCD gene family (CCD1, 4, 7 and 8) play essential roles in synthesis of colour, apocarotenoid flavour, aroma volatiles and phytohormones such as strigolactone. The active degradation of the xanthophylls by CCD activity can reduce lutein content in strawberries as well as cause changes in the pigmentation in chrysanthemums from white to yellow (Oomiya et al. 2006; García-Limones et al. 2008). In maturing Arabidopsis seeds a loss of function of CCD1 activity leads to higher carotenoid levels and may have a role in synthesis of apocarotenoid flavour and aroma volatiles (Auldridge et al. 2006). Similarly, in tomato (Lycopersicon esculentum) LeCCD1 activity contributes to the formation of the flavour volatiles β-ionone, pseudoionone and geranylacetone (Sinikin et al. 2004). The Crocus, zeaxanthin 7,8(9′,8′)-cleavage dioxygenase (CsZCD) and 9,10(9′,10′)-cleavage dioxygenase (CsCCD) initiate the biosynthesis of carotenoid derivatives such as crocetin glycosides, picrocrocin and safranal (saffron). Indeed, the expression of CsZCD mRNA was mostly restricted to the style branch tissues and transcript levels were inducible under dehydration stress (Bouvier et al. 2003b).

Two of the genes that affect shoot branching encoding CCD7 and CCD8 can sequentially cleave β-carotene to form the C18 compound 13-apo-carotenone (Schwartz et al. 2004). CCD7 appears to be a biosynthetic cross point, controlling both strigolactone and AM-induced C13 cyclohexenone and C14 mycorradicin apocarotenoids (Vogel et al. 2010). Finally, there are examples of cross talk where inhibition of ABA biosynthesis reduces CCD7 and CCD8 transcript abundance as well as strigolactone levels (López-Ráez et al. 2010).

### Accumulation, storage and insights from biofortification

The storage of carotenoids requires a lipophilic environment, usually within the membranes (envelope and in some cases the thylakoid membranes) of plastid organelles, which behave as a sink for carotenoid accumulation. The biogenesis of organelles is a major determinant of the storage compartment size of plastids and can affect the accumulation of carotenoids (reviewed by Pogson and Albrecht 2011) The colourless pluripotent progenitor proplastid differentiates into specialised plastids that can store chlorophylls and carotenoids (Kirk and Tinline-Bassett 1978). Carotenoids are usually synthesised de novo in differentiated plastids of roots, flowers, fruits and seeds, accumulating mostly in chloroplasts (green photosynthetic plastids) and chromoplasts (coloured plastids), but also in amyloplasts (starch-storing plastids), leucoplasts (colourless plastids), etioplasts (dark-grown precursors of the chloroplast) and elaioplasts (lipid-storing plastids) (Cazzonelli and Pogson 2010). There are links between changes in carotenoid composition and plastid biogenesis, morphology and protein translocation, in particular, it is noteworthy that carotenoids (e.g. lutein) are necessary for the differentiation of an etioplast into a chloroplast (Fig. 1) (Park et al. 2002).

The regulation of carotenoid targeting, storage and sequestration within various plastid types is a process by which to modulate a sink for carotenoid accumulation (Lu et al. 2006; Cuttriss et al. 2007; Cazzonelli and Pogson 2010). For example, the high-pigment 1 tomato mutant, hp1, displays an increased pigmentation because of increased chromoplast compartment size (Cookson et al. 2003). A naturally occurring mutation in the Brassica oleracea orange-curd (or) gene changes a normally white cauliflower curd into an orange Or mutant, which accumulates high levels of β-carotene (Lu and Li 2008). The OR gene encodes a DnaJ cysteine-rich domain-containing protein and plays a role in coordinating the differentiation of proplastids in the shoot and inflorescence meristems of the curd into chromoplasts (Li et al. 2001; Lu et al. 2006). Carotenoids accumulate in lipoprotein structures within the chromoplast (Bartley and Scolnik 1995; Vishnevetsky et al. 1999), which might allow for additional carotenoid biosynthesis. Therefore, chromoplasts serve as a metabolic sink to control carotenoid accumulation in plants and reveals the importance of plastid differentiation in controlling carotenoid accumulation in plants (Lu et al. 2006; Li and Van Eck 2007). The Crocus chromoplast localised enzymes, CsZCD and CsCCD control the accumulation of saffron metabolites as well as the differentiation of amyloplasts and chromoplasts (Bouvier et al. 2003b). The oxidative cleavage of zeaxanthin in chromoplasts is intriguingly followed by the sequestration of modified water-soluble derivatives into the central vacuole (Bouvier et al. 2003b).

The introduction of foreign exogenous carotenoid biosynthetic genes into crops is another means by which to modulate the sink for carotenoid storage. Carotenoid accumulation has been achieved in oil seeds of canola (Brassica napus) through the overexpression of PSY, which resulted in 43–50-fold increase in total seed carotenoid content (Shewmaker et al. 1999; Lindgren et al. 2003). ‘Golden rice 2’ is a high β-carotene accumulating line overexpressing PSY from maize as well as bacterial CrtI (from Erwinia uredovora) and accumulates 23-fold more carotenoids (37 μg g⁻¹) than was the case for the initial ‘golden rice’, which overexpressed PSY from daffodil (Beyer et al. 2002; Paine et al. 2005). Underground food crops, such as potato and carrot have also been engineered to have increased carotenoid content, in particular β-carotene and...
ketocarotenoids (e.g. astaxanthin), which produce a deep yellow (‘golden’) potato tuber phenotype and a distinct pink to deep red colour compared with the typical orange colour in wild-type carrot roots (Taylor and Ramsay 2005; Diretto et al. 2007; Jayaraj et al. 2008). Finally, the overexpression of PSY in Arabidopsis increased carotenoid content by 10- to 100-fold in nonphotosynthetic calli and roots, predominantly β-carotene and its derivatives, which were deposited as crystals in storage plastids (Maass et al. 2009).

**Photostimulation of carotenoid gene expression**

Light regulates the key rate-limiting step in phytovene biosynthesis. PSY transcript abundance increases during photomorphogenesis via a phytochrome-mediated (red-light) pathway and this response was abolished in the phyA mutant (Welsch et al. 2000, 2008). Phytochrome-interacting factor 1 (PIF1) has been shown to bind to the PSY promoter and represses PSY mRNA expression. The authors show that light triggers the degradation of PIF1 after interacting with photoactivated phytochromes during de-etiolation and that a rapid derepression of PSY gene expression enhances carotenoid production during the optimal transition to photosynthetic metabolism (Toledo-Ortiz et al. 2010). The light-triggered degradation of PIFs causes a rapid accumulation of carotenoids, which is coordinated with chlorophyll biosynthesis to enable the rapid assembly of the photosynthetic machinery (Toledo-Ortiz et al. 2010). Very recently, the DELLA proteins were shown to function in part through the regulation of PIF activity, by a DELLA-PIF complex that coordinates the levels of POR, chlorophyll precursors and carotenoids in cotyledons of dark-grown seedlings (Cheminant et al. 2011). PSY is strongly light-induced in greening seedlings (Welsch et al. 2000) and a transcription factor, RAP2.2, (AP2/EREBP family) which binds to the PSY promoter was able to slightly perturb pigment alterations in Arabidopsis root calli (Welsch et al. 2007).

High light stress also alters the synthesis of β-xanthophylls, such as the synthesis of zeaxanthin from β-carotene (Depka et al. 1998). Plants with reduced zeaxanthin exhibit increased sensitivity to light stress (Havaux and Niyogi 1999). The amount of zeaxanthin can be modulated by regulating the expression of the β-hydroxylase (β-OH) gene, which is photo-inducible and violaxanthin de-epoxidase (VDE), whose transcript levels are slightly downregulated by light (Rossel et al. 2002). In addition, the luminal pH and ascorbate content appear to affect post-translation modulation of VDE activity and is critical for determining the levels of zeaxanthin upon exposure to excessive light (Rockholm and Yamamoto 1996). Further, the BLCY mutant (suppressor of zeaxanthin-less1, szl1) lacks zeaxanthin, yet accumulates higher levels of lutein and α-carotene, which partially restores quenching efficiency, revealing that lutein could substitute for zeaxanthin (Li et al. 2009a). The modulation of zeaxanthin levels by the violaxanthin de-epoxidase (VDE) is thought to be required for the thermal dissipation of excess light energy and the protection of photosynthetic membranes against lipid peroxidation, a process known as the xanthophyll cycle (Havaux and Niyogi 1999).

**Epigenetic maintenance of carotenoid composition**

Beyond the heavy demands from developmental and environmental cues to coordinate carotenoid gene expression (Cazzonelli and Pogson 2010), there are further implications for the epigenetic control over apocarotenoid formation (described above) and especially carotenoid biosynthesis (Cazzonelli et al. 2009c; Ding et al. 2011). A chromatin modifying protein was found to regulate CRTISO expression and carotenoid flow through the branch to α and β-carotenoids, thereby limiting lutein production. The SET DOMAIN GROUP (SDG) family of histone lysine methyltransferases are chromatin modifying proteins that methylate lysine residues (chromatin marks) on the tails of histone proteins in order to recruit other regulatory factors and make the DNA either more or less accessible to RNA polymerase II transcriptional complex (Cazzonelli et al. 2009b; Thorstensen et al. 2011). The methylation of lysine residues can be maintained through cell division (mitosis) establishing long-term memory required for adaptation and these marks are usually reset during gamete formation (meiosis) (Szate 2008).

SDG8 (homologue of the Drosophila absent, small or homoeotic disks 2 often referred to as ASHH2 or in plants as carotenoid and chloroplast regulation, ccrl, or early flowering in short days, efs) maintains a transcriptionally permissive chromatin state surrounding the CRTISO locus and thus is able to regulate carotenoid composition, particularly accumulation of the most abundant carotenoid, lutein (Fig. 2) (Cazzonelli et al. 2009a). In non-photosynthetic tissues, SDG8 is required for CRTISO activity and in the absence of SDG8 activity, etiolated tissues accumulate cis-isomers (Park et al. 2002). SDG8 maintains H3K4 trimethylation of regions surrounding the CRTISO promoter and SDG8 is necessary for regulating the CRTISO promoter in rapidly dividing tissues during epigenetic programming (e.g. mitosis in the shoot apex) and reprogramming (e.g. meiosis in pollen) events (Cazzonelli et al. 2010). The reporter gene expression patterns enabled by SDG8 and CRTISO promoters overlap considerably, with the strongest expression being apparent in many tissues essential for defining plant architecture and development, including germinating seedlings, vasculature, meristems, shoot apices, floral anthers and pollen (Cazzonelli et al. 2010).

The sdg8 mutant shows several pleiotropic phenotypes, such as early flowering, altered shoot and root architecture, altered pathogen resistance, male sterility, abnormal sporophyte and gametophyte development (Kim et al. 2005; Cazzonelli et al. 2009a; Grini et al. 2009; Berr et al. 2010). A few putative target genes responsible for these phenotypes have been described and target gene loci show a lower abundance of H3K4 or H3K36 methylation marks, as well as reduced mRNA levels in the sdg8 mutant. However, only the early flowering and lutein deficient phenotypes have been sufficiently complemented by overexpression of the corresponding target genes, FLOWERING LOCUS C (FLC) and CRTISO, respectively (Kim et al. 2005; Cazzonelli et al. 2009a). The effect of the chromatin marks can spread and appear to only have a slight affect on the relative transcript abundance of genes neighbouring CRTISO and FLC, suggesting that indeed both CRTISO and FLC are likely to be direct targets of SDG8 activity (Kim et al. 2005; Cazzonelli et al. 2009a).
The chromatin regulatory proteins required to recruit and or interact with SDG8 in order to post-transcriptionally modify the CRTISO locus with permissive chromatin marks is yet to be discovered. The epigenetic regulation of FLC by vernalisation has been extensively studied and should pave the way to discover new insights into the epigenetic regulatory mechanisms controlling CRTISO expression and perhaps link carotenoids with the developmental regulation of floral initiation (Dennis and Peacock 2007). It was recently demonstrated that SDG8 interacts with FRIGIDA (FRI), a protein that delays flowering by activating FLC expression and in the absence of a prolonged cold winter period (a process known as vernalisation) FRI promotes a vegetative state of growth (Choi et al. 2011). SDG8 is essential for FRI function and SDG8 was recently demonstrated to have a novel dual substrate specificity for H3K4 and H3K36 specific histone lysine methylation activity (Ko et al. 2010). The effect of FRI on carotenoid accumulation, if any, remains to be determined.

Theoretically more questions that remain to be answered. First, does SDG8 also affect H3K36 chromatin marks surrounding the CRTISO locus? Second, what is the molecular nature of the mechanisms used to target SDG8 to key target genes like CRTISO and FLC? Third, is carotenoid composition regulated in response to an epigenetic phenomena? Finally, what novel roles might SDG8 and CRTISO play in fine tuning plant development, architecture and the production of precursors for phytohormone biosynthesis? It is possible to speculate the role, if any, for photoisomeration in overriding SDG8 regulation in photosynthetic tissues (Corona et al. 1996). OsPSY3 gene expression is upregulated in rice during increased abscisic acid (ABA) formation as well as upon salt treatment and drought, especially in roots indicating that the third PSY allele in rice plays a specialised role in abiotic stress-induced ABA formation (Welsch et al. 2008). A positive feedback regulation mediated by the application of ABA induces OsPSY3 as well as OsNCED gene expression. An ABA-response element as well as a coupling element was identified in the promoter region for OsPSY3, but its function remains to be validated (Welsch et al. 2008). Therefore, the regulation of phytoene biosynthesis in plants appears to involve both negative and positive feedback regulatory mechanisms. The control of carotenoid flux through the branch from all-trans-lycopene to synthesis of α- and β-carotenoids is also under negative metabolite feedback regulation and mediated through the transcription control of εLCY gene expression (Cuttriss et al. 2007). Investigations have indicated that both CRTISO (ccr2) and SDG8 (ccr1) mutants have an inhibitory effect on εLCY transcript levels (Fig. 3), revealing that feedback may partially explain the reduced lutein observed in ccr2 and ccr1 mutants (Cuttriss et al. 2007; Cazzonelli et al. 2009a). Further, a reduction in seed εLCY mRNA levels show higher total carotenoid content, specifically increased levels of β-carotene, zeaxanthin, violaxanthin and unexpectedly, lutein (Yu et al. 2008). However, another report showed that tuber specific silencing of εLCY resulted in an expected increase in β-carotene levels in potato (Solanum tuberosum) (Diretto et al. 2006). In B. napus, total carotenoids

Carotenoid signals and metabolite feedback control

Several genes encoding enzymes in isoprenoid and carotenoid biosynthesis are the subject of negative and positive feedback regulatory processes and are likely to be mediated by a carotenoid or a molecule derived from a carotenoid (Römer et al. 2000; Beyer et al. 2002; Cuttriss et al. 2007; Qin et al. 2007; Bai et al. 2009). There is strong evidence to show carotenoid metabolite feedback regulation requires post-transcriptional and transcriptional processes to regulate specific genes that (1) supply isoprenoid precursors for carotenogenesis, (2) control activity of the rate-limiting phytoene synthase step and (3) control carotenoid flux through the branch to synthesis of α- and β-carotenones. Essentially, positive and negative feedback metabolic mechanisms exist within the carotenoid pathway and among carotenoids, MEP and ABA (Fig. 3).

The overexpression of PSY transcript levels enhanced carotenoid levels in etiolated Arabidopsis seedlings via the concomitant post-transcriptional accumulation of DXS protein, which initiated feedback stimulating the supply of MEP substrates (Fig. 3) (Rodríguez-Villalón et al. 2009). Additional evidence that PSY controls metabolic flux was obtained through the paclobutrazol treatment of Arabidopsis seedlings, which not only inhibits gibberellin synthesis but stimulates de-etiolation despite the absence of light. PSY activity and carotenoid levels were shown to increase in the dark following paclobutrazol treatment and this increase was supported by feedback regulation of DXS and DXR protein abundance (Rodríguez-Villalón et al. 2009). Surprisingly, overexpression of DXS mRNA in dark-grown seedlings does not affect carotenoid accumulation (Rodríguez-Villalón et al. 2009). An increase in DXS protein activity (but not gene expression levels) was also detected in tomato fruit containing increased PSY transcript levels and it was speculated that this could occur through the enhanced accumulation of active DXS enzymes in chromoplasts (Fraser et al. 2007; Rodríguez-Villalón et al. 2009). Finally, the establishment of plant root arbuscular mycorrhizal (AM) fungi in the rhizosphere activates the MEP pathway enhancing transcript levels of the first two enzymes of the MEP pathway (e.g. DXS and DXR), the two desaturases in the carotenoid pathway (e.g. PDS and ZDS) as well as the carotenoid-cleaving dioxygenases in AM roots (Strack and Fester 2006). The molecular nature of the metabolite feedback regulatory mechanisms controlling the accumulation of MEP precursors and DXS at the protein or gene expression levels, remain open for discovery.

PSY regulates the first committed step in carotenogenesis (e.g. phytoene production) and appears to be controlled by source and sink metabolites (Fig. 3). For example, in the pds3 mutant, downstream (e.g. ZDS and BLCY) and upstream (IP1, GGPS and PSY) pathway genes were downregulated, but also those related to the biosynthesis of gibberellins as well as chlorophylls (Qin et al. 2007). The absence of downstream carotenoids in pds3 mutants could provide the signal as evidence from the analysis of a tomato PDS promoter::GUS fusion demonstrated end product regulation in photosynthetic tissues (Corona et al. 1996). OsPSY3 gene expression is upregulated in rice during increased abscisic acid (ABA) formation as well as upon salt treatment and drought, especially in roots indicating that the third PSY allele in rice plays a specialised role in abiotic stress-induced ABA formation (Welsch et al. 2008). A positive feedback regulation mediated by the application of ABA induces OsPSY3 as well as OsNCED gene expression. An ABA-response element as well as a coupling element was identified in the promoter region for OsPSY3, but its function remains to be validated (Welsch et al. 2008). Therefore, the regulation of phytoene biosynthesis in plants appears to involve both negative and positive feedback regulatory mechanisms.
increased in the βLCY (lcyB-m2.1) mutant by nearly 200 and 40% in mutant embryo and endosperm tissues of maize respectively (Bai et al. 2009). Lycopene was the primary carotenoid that accumulated in lcyB-m2.1 embryo tissues, together with a small amount of δ-carotene (Bai et al. 2009) and perhaps these molecules are candidates for degradation and subsequent feedback regulation. Together, these lines of evidence support a hypothesis that lutein composition is largely rate-determined by eLCY expression and that metabolite feedback might be another key regulator of the branch node in carotenogenesis (Fig. 3).

Future prospects

Carotenoids are everywhere and are essential in nature, clearly serving numerous functions during animal and plant development (Fig. 1). A deeper investigation into the control of carotenoid biosynthesis will undoubtedly provide new regulatory mechanisms by which to facilitate improvements to crop nutrition. Understanding how carotenoids are sequestered within plastid types is an area ripe for discovery and has the potential to further improve metabolic engineering attempts to enhance the composition of essential dietary micronutrients, such as the xanthophylls and carotenes. The discovery of new regulators of carotenoid biosynthesis that modulate the production of strigolactones and ABA will really highlight the important roles that carotenoids play in plants, especially in response to developmental and environmental signals. Two major nodes in the pathway play essential roles in regulating carotenoid composition and flux. One node affects the production of phytoene at the bottleneck in the pathway and the other modulates the accumulation of cis-carotenoids and lutein at the branch to α- and β-carotenoids. The cross-talk between and within the MEP, carotenoid and ABA biosynthetic pathways is an intriguing example of metabolite feedback control. This could involve a signalling protein bound to specific carotenoids or their cis-trans-isomers and perhaps even soluble or volatile carotenoid cleavage products could mediate signalling between the plastid and nucleus. For now the nature of the different forms of metabolic feedback regulation remain unknown. It will be necessary to establish an evolutionary importance for some of the carotenoid regulatory processes described in this review using non-model plant species. Finally, a deeper understanding of the molecular nature by which metabolite feedback and chromatin regulatory mechanisms control carotenoid abundance and composition is paramount to determine if indeed there is a memory forming process epigenetically regulating carotenogenesis in plants.

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