Vitamin Synthesis in Plants: Tocopherols and Carotenoids

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Abstract

Carotenoids and tocopherols are the two most abundant groups of lipid-soluble antioxidants in chloroplasts. In addition to their many functional roles in photosynthetic organisms, these compounds are also essential components of animal diets, including humans. During the past decade, a near complete set of genes required for the synthesis of both classes of compounds in photosynthetic tissues has been identified, primarily as a result of molecular genetic and biochemical genomics-based approaches in the model organisms *Arabidopsis thaliana* and *Synechocystis* sp. PCC6803. Mutant analysis and transgenic studies in these and other systems have provided important insight into the regulation, activities, integration, and evolution of individual enzymes and are already providing a knowledge base for breeding and transgenic approaches to modify the types and levels of these important compounds in agricultural crops.

Contents

INTRODUCTION: PLASTIDIC ISOPRENOID SYNTHESIS

Plastids contain sophisticated biochemical machinery producing an enormous array of compounds that perform vital plastidic and cellular functions. Many of these compounds are also important for agriculture and human nutrition. Plastidic isoprenoid synthesis represents a major source of such compounds and includes the two major groups of lipid-soluble antioxidants in photosynthetic tissues, the tocochromanols and carotenoids. The tocochromanols are a group of eight tocopherols and tocotrienols that collectively constitute vitamin E, an essential nutrient in the diet of all mammals. Carotenoids constitute a much larger group of over 700 structures (17) that provide fruit and flowers with distinctive red, orange, and yellow coloring and are the dietary source of pigmentation in the tissues of many fish, crustaceans, and birds. In some cases specific carotenoids are essential components of mammalian diets as precursors for vitamin A synthesis. Vitamin A deficiency remains a significant global health problem (121, 147a).

Both carotenoids and tocochromanols are synthesized in whole or in part from the plastidic isoprenoid biosynthetic pathway. The biosynthesis of isoprenoid precursors is covered in detail elsewhere (67). Briefly, two distinct pathways exist for isopetenylpyrophosphate (IPP) production: the cytosolic mevalonic acid pathway and the plastidic mevalonate-independent, methylerythritol 4-phosphate (MEP) pathway. The methylerythritol 4-phosphate pathway combines glyceraldehyde-3-phosphate and pyruvate to form deoxy-D-xylulose 5-phosphate, and a number of steps are then required to form IPP and dimethylallylpyrophosphate (DMAPP) (67). IPP is subject to a sequential series of condensation reactions to form geranylgeranyl diphosphate (GGDP), a key intermediate in the synthesis of carotenoids, tocochromanols, and many other plastidic isoprenoids (**Figure 1**).

The tocochromanol and carotenoid biosynthetic pathways are typical of many plant compounds in that the enzymes from plant sources have historically proven extremely difficult to purify and analyze. This is a result of a combination of properties, including membrane association, low specific activity and poor stability of the enzymes

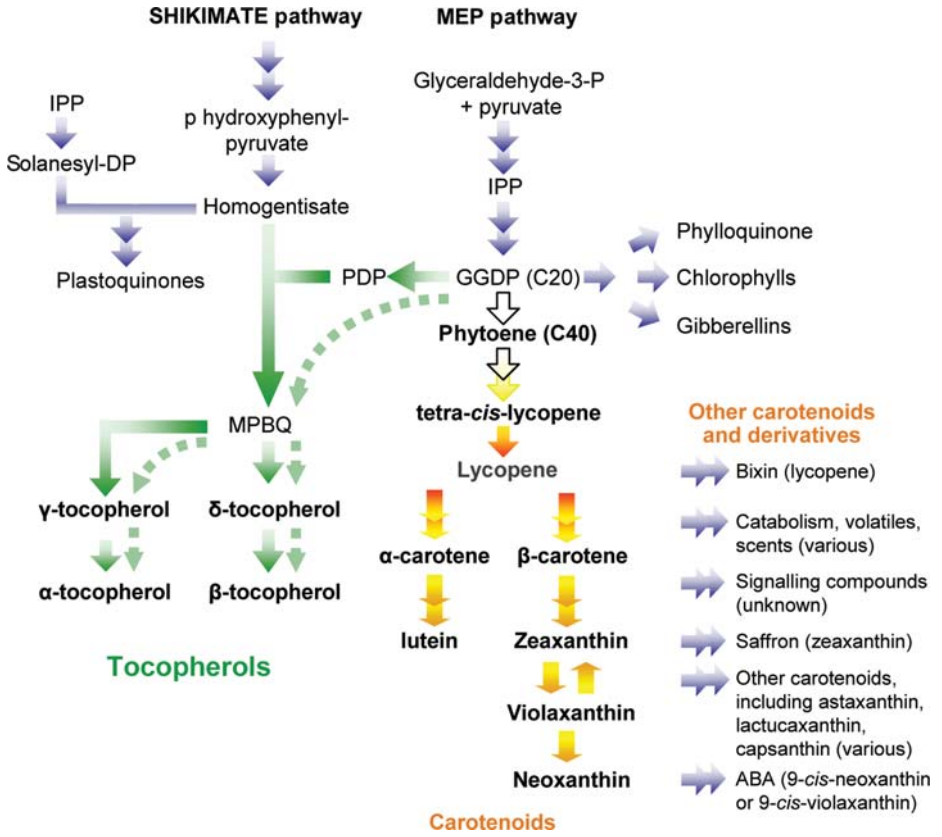

Figure 1

Overview of carotenoid and tocopherol biosynthesis in plants. The 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway provides isopetenylpyrophosphate (IPP) for synthesis of the central intermediate geranylgeranyl diphosphate (GGDP). GGDP can be used for synthesis of phytoene, chlorophylls, and tocotrienols or reduced to phytyl-diphosphate (PDP) used for phylloquinone, chlorophyll, and tocopherol synthesis. Phytol released from chlorophyll degradation is also used for tocopherol synthesis (not shown, see text). The pathway shown by orange arrows provides the carotenoids found in leaves of most species. Other carotenoids and carotenoid cleavage/modification products are produced in certain species and/or particular tissues, and when known, the primary substrate for cleavage is given in parentheses. The pathway shown by green arrows is the synthesis of tocopherols from homogentisate, a product of the shikimate pathway. For clarity, only tocopherols are shown, but when GGDP is condensed with homogentisate, the corresponding tocotrienols are produced by the same pathway. ABA, abscisic acid; MPBQ, methyl-6-phytyl-1,4-benzoquinone.

during isolation as well as limitations in synthesizing or obtaining commercially available substrates for assays. Thus, much of our understanding of the genes and enzymes of tocochromanol and carotenoid biosynthesis has resulted from the increasing ease of integrating complementary comparative genomics, biochemical genetics, and molecular approaches. This review focuses on progress since the last reviews on these subjects in this series (30, 45). However, several recent reviews on aspects of the biosynthesis, function, and catabolism of these compounds are available, and readers are directed to these sources for additional information (29, 33, 38, 49, 74, 82–84, 104, 140, 144).

CAROTENOID BIOSYNTHESIS IN PLANTS

General Considerations and Early Steps in Biosynthesis

Carotenoids comprise a large isoprenoid family and most are C_{40} tetraterpenoids derived from phytoene. The carotenoid backbone is either linear or contains one or more cyclic β-ionone or ε-ionone rings or, less frequently, the unusual cyclopentane ring of capsanthin and capsorubin that imparts the distinct red color to peppers. Nonoxygenated carotenoids are referred to as carotenes, whereas their oxygenated derivatives are designated as xanthophylls. The most commonly occurring carotenes are β-carotene in chloroplasts and lycopene in chromoplasts of some flowers and fruits, e.g., tomatoes. The most abundant xanthophylls in photosynthetic plant tissues (lutein, violaxanthin, and neoxanthin) are key components of the light-harvesting complexes. Carotenoids are involved in photosystem assembly, light harvesting and photoprotection, photomorphogenesis, nonphotochemical quenching, lipid peroxidation and affect the size and function of the light-harvesting antenna and seed set (35, 47, 52, 62, 69, 93). As these topics have been reviewed extensively elsewhere (33, 51, 84), the roles of carotenoids in photosynthesis are not considered here.

The initial steps of plant carotenoid synthesis and their chemical properties have been thoroughly discussed in prior reviews, and readers are referred to these for more detail (28, 29, 33, 49, 104). Briefly, the first committed step in plant carotenoid synthesis is the condensation of two molecules of GGDP to produce phytoene (**Figure 2**) by the enzyme phytoene synthase (PSY). Phytoene is produced as a 15-*cis* isomer, which is subsequently converted to all-*trans* isomer derivatives. Two plant desaturases, phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS), catalyze similar dehydrogenation reactions by introducing four double bonds to form lycopene. Desaturation requires a plastid terminal oxidase and plastoquinone in photosynthetic tissues (8, 21, 85). Bacterial desaturation differs from plants in that a single enzyme, *crtI* (phytoene desaturase), introduces four double bonds into phytoene to yield all-*trans*-lycopene (28).

Isomerizations During Carotenoid Desaturation

Until recently, the higher plant desaturases were assumed sufficient for the production of all-*trans*-lycopene. This conclusion was reached despite the accumulation of tetra-*cis*-lycopene in *tangerine* tomato and algal mutants (27, 135) and biochemical evidence to the contrary from daffodil (9). Recently, the carotenoid isomerase gene, *CRTISO*, was identified in Arabidopsis and tomato (55, 89). Intriguingly, the protein shows 20%–30% identity to the bacterial carotenoid desaturases; however it has no desaturase activity (89). Rather, the pathway to all-*trans*-lycopene proceeds via *cis* intermediates (16, 54): The PDS and ZDS enzymes introducing *cis*-carbon-carbon double bonds (5) (**Figure 2**) and CRTISO catalyze *cis-trans* isomerizations resulting in all-*trans*-lycopene (54, 89). There is evidence the desaturation and isomerization reactions can occur sequentially (16) or concurrently (54).

Mutant plants deficient in CRTISO activity accumulate various *cis*-isomer biosynthetic intermediates when dark-grown, but these intermediates can be photoisomerized in the light and yield viable plants, albeit with reduced lutein levels (89). If photoisomerization is sufficient, why are three genes required for the synthesis of *trans*-lycopene (*PDS*, *ZDS*, and *CRTISO*) in plants but only one (*crtI*) in bacteria? One possible explanation is CRTISO contributes to the regulation of the pathway, in that there is a delay in greening and a reduction in lutein in leaves of *crtISO* mutants (89). Consistent with this, expression of bacterial *crtI* in tobacco, Arabidopsis,

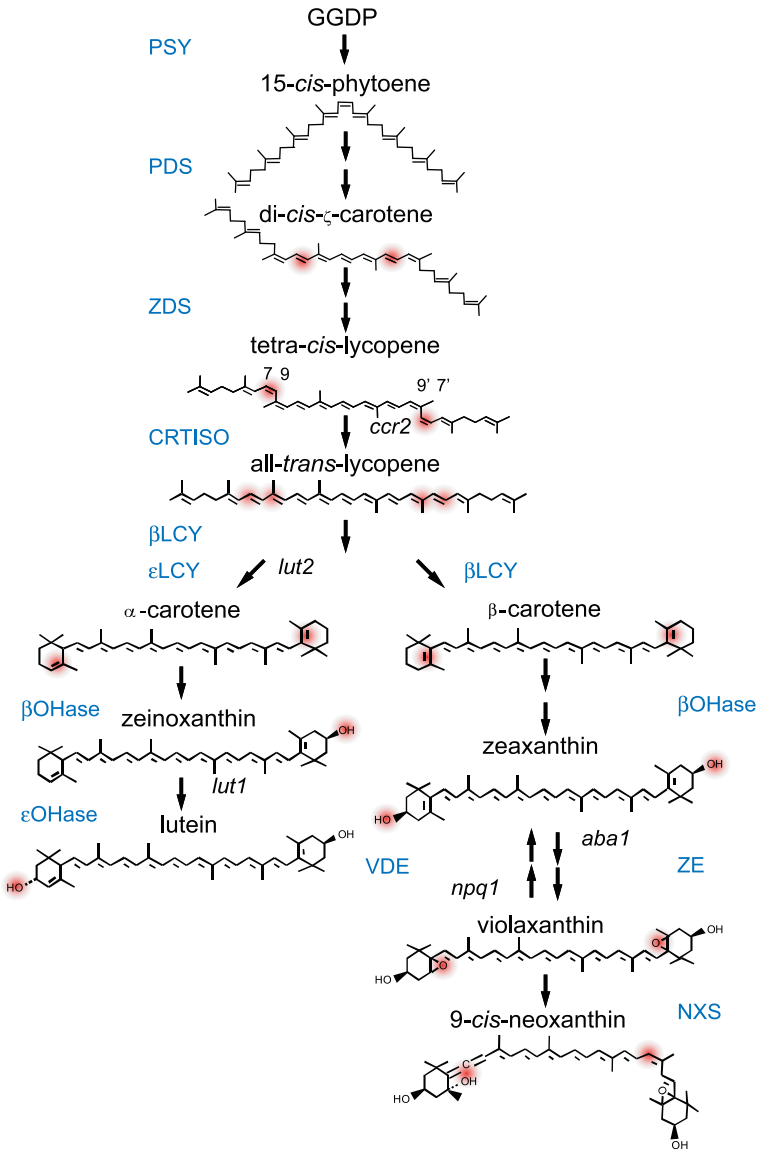

Figure 2

Carotenoid biosynthetic pathway in land plants. The pathway shows the primary steps found in nearly all plant species. The desaturases introduce a series of four double bonds in a *cis* configuration, which are isomerized to the all-*trans* conformations by the carotenoid isomerase. Although shown sequentially, there is evidence the carotenoid isomerase enzyme (CRTISO) may act in concert with the ζ–carotene desaturase (ZDS). Lycopene is cyclized to form α-carotene and β-carotene, which are subject to a series of oxygenation reactions to produce the xanthophylls typically found in chloroplasts. The abbreviation for the biosynthetic enzymes is given next to each step and Arabidopsis mutations are shown in italics (see **Table 1**). β-LCY, β-carotene cyclase; βOHase, β-carotene hydroxylase; εLCY, ε-cyclase; εOHase, ε-carotene hydroxylase; NXS, neoxanthin synthase; PDS, phytoene desaturase; PSY, phytoene synthase; VDE, violaxanthin deepoxidase; ZE, zeaxanthin epoxidase.

Table 1 Carotenoid biosynthetic enzymes in Arabidopsis and other selected plants

Enzyme (Genera)[a]	Abbreviation	Locus[b]	Gene ID[c]	Reference(s)
Early steps				
Phytoene synthase	PSY		At5g17230	(8)
Phytoene desaturase	PDS	*PDS3*	At4g14210	(28)
ζ-carotene desaturase	ZDS		At3g04870	(28)
Carotenoid isomerase	CRTISO	*CCR2*	At1g06820	(54, 89)
Cyclases				
β-carotene cyclase	βLCY1		At3g10230	(28)
β-carotene cyclase (*Lycopersicum*)	βLCY2	*BETA*	AF254793*	(102)
ε-cyclase	εLCY	*LUT2*	At5g57030	(28)
Xanthophyll enzymes				
β-carotene hydroxylase	βOHase1		At4g25700	(125)
	βOHase2		At5g52570	(132)
ε-carotene hydroxylase	εOHase	*LUT1*	At3g53130	(134)
Zeaxanthin epoxidase	ZE	*ABA1*	At5g67030	(28)
Violaxanthin deepoxidase	VDE	*NPQ1*	At1g08550	(28)
Neoxanthin synthase	NXS			
Other xanthophyll enzymes				
β-carotene 4-ketolase (*Adonis*)	AdKeto1		AAV85452*	(32)
	AdKeto2		AAV85453*	
Capsanthin/capsorubin synthase (*Capsicum*)	CCS		S71511*	(28)
Carotenoid cleavage and modifying enzymes				
9-*cis* epoxycarotenoid dioxygenase	NCED2	*CCD2*	At4g18350	(126)
	NCED3	*CCD3*	At3g14440	
	NCED5	*CCD4*	At1g30100	
	NCED6	*CCD6*	At3g24220	
	NCED9	*CCD9*	At1g78390	
Carotenoid cleavage dioxygenase	CCD1		At3g63520	(126)
	CCD4		At4g19170	
	CCD7	*MAX3*	At2g44990	(10)
	CCD8	*MAX4*	At4g32810	(122)
MORE AUXILLARY BRANCHING 1	MAX1		At2g26170	(11)
Carotenoid cleavage dioxygenase (*Crocus*)	CsCCD		AJ132927*	(15)
Zeaxanthin cleavage dioxygenase (*Crocus*)	CsZCD		AJ489276*	(15)
Crocin glucosyltransferase (*Crocus*)	CsGTase	*UGTCs2*	AY262037	(78)
Lycopene cleavage dioxygenase (*Bixa*)	BoLCD		CAD71148*	(13)

[a]Enzyme and genera in brackets if not *Arabidopsis*.
[b]Locus or alternate name. *CCR* = *CHLOROPLAST* and *CAROTENOID REGULATION*; *BETA* = *BETA-CAROTENE*; *LUT* = *LUTEIN*; *ABA* = *ABSCISIC ACID*; *NPQ* = *NONPHOTOCHEMICAL QUENCHING*; *UGTCs* = *UDP-GLUCOSYL TRANSFERASE* from *Crocus sativuss*.
[c]*Arabidopsis thaliana* (At) gene identifier or * GenInfo identifier from Genbank.

and rice bypasses the native CRTISO activity and also results in reduced leaf lutein levels (76, 87, 149). Also in tomato fruit chromoplasts, CRTISO activity is needed for all-*trans*-lycopene accumulation as in the tomato *crtISO* mutant (*tangerine*) *cis*-lycopene is accumulated and appears resistant to photoisomerization (55). Thus, CRTISO is required

for optimal carotenoid synthesis in etioplasts, chromoplasts, and chloroplasts.

β-Carotene Derived Xanthophyll Biosynthesis

β-carotene and zeaxanthin. The plant carotenoid biosynthetic pathway has two main branches after lycopene, distinguished by different cyclic end-groups. Two beta rings lead to the β,β branch (β-carotene and its derivatives: zeaxanthin, violaxanthin, antheraxanthin, and neoxanthin) whereas one beta and one epsilon ring define the β,ε branch (α-carotene and its derivatives). Although there is but a single *βLCY* gene in Arabidopsis, a second lycopene β-cyclase was identified in tomato (102). Expression of the second *βLCY* is low during wild-type fruit ripening but is dramatically elevated in the high β-carotene *Beta* mutant, demonstrating this gene's importance in determining fruit pigment composition (102). Interestingly, the second *βLCY* is only 53% identical to the first *βLCY* yet 86% identical to the capsanthin-capsorubin synthase from pepper (*Capsicum annum*), suggesting capsanthin-capsorubin synthase diverged from an ortholog of the second tomato *βLCY*.

Nearly all xanthophylls in higher plants have hydroxyl moieties on the carbon 3 of the cyclic β-ionone end-group. Although most other carotenoid pathway reactions are encoded by a single gene in Arabidopsis, multiple hydroxylase genes occur in Arabidopsis and tomato with distinct evolutionary backgrounds. Plant β-ring hydroxylases (β-OHs) share significant identity with bacterial β-ring hydroxylases (125) and are ferredoxin-dependent, nonheme dioxygenases with an iron-coordinating histidine cluster (14). The two Arabidopsis *β-OHs* are expressed in all tissues, albeit at different levels (132), whereas in tomato one *β-OH* is expressed in chloroplasts and the other in flowers (49). *β-OH* gene expression is strongly induced by excess light in Arabidopsis leaves (103) and is modulated by different intensities of white light during photomorphogenesis (147).

Violaxanthin and neoxanthin. An epoxide group is introduced into both rings of zeaxanthin by zeaxanthin epoxidase to form violaxanthin. Under high light stress, which acidifies the lumen, violaxanthin deepoxidase is activated, resulting in increased levels of zeaxanthin (84). Violaxanthin deepoxidase and zeaxanthin epoxidase were the first identified plant lipocalins, a class of β-barrel proteins that bind small hydrophobic molecules but are not usually catalytic (18).

Conversion of violaxanthin to neoxanthin is performed by the enzyme neoxanthin synthase (NXS). Genes encoding enzymes with limited NXS activity were identified in tomato and potato (1, 12), but whether they are the primary NXS in vivo remains a matter of debate, especially considering Arabidopsis lacks an ortholog for the potato and tomato enzymes but has NXS activity. The recent identification of mutants that lack neoxanthin in Arabidopsis (*Ataba4*) and tomato may lead to the resolution of this issue (A. Marion-Poll & J. Hirschberg, personal communication).

Lutein Biosynthesis

Both the β-cyclase and ε-cyclase enzymes (β*LCY* and ε*LCY*, respectively) are required to form α-carotene and lutein (30, 92). Increasing or decreasing ε*LCY* expression resulted in lutein levels ranging from 10% to 180% of wild type (94). Lettuce (*Lactuca sativa*) appears unique among higher plants in that its εLCY enzyme can catalyze formation of the bicyclic ε,ε-carotene (31, 90). In fact, a single amino-acid substitution, H457L in lettuce, was found sufficient for bicyclic to monocyclic ε-ring formation, with the converse occurring for L448H in Arabidopsis (31). Another interesting organism is the marine cyanobacterium *Prochlorococcus marinus* MED4, which contains a *βLCY* gene and an additional novel cyclase capable of forming both β- and ε-end-groups (124).

In contrast to the nonheme β-ring hydroxylases, the recently identified ε-ring hydroxylase was found to be a member of the

cytochrome P450-type monooxygenase superfamily, and thus has a distinctly different enzymatic mechanism from the plant β-ring OHases described above (134). Arabidopsis mutant genotypes deficient in all three of the known carotene ring hydroxylases (β-hydroxylases 1 and 2 and the ε-ring hydroxylase) still produced at least 50% of the wild-type level of hydroxylated β-rings, primarily as the monohydroxy α-carotene derivative zeinoxanthin (133). One likely explanation for this result is the existence of a fourth carotenoid hydroxylase in Arabidopsis that has a major activity toward the β-ring of α-carotene (134). One possible candidate for this activity is CYP97A3, a putative cytochrome P450 with 50% identity to the ε-ring hydroxylase (133, 134).

Carotenoid Cleavage Products

The carotenoid cleavage enzymes are variously referred to as carotenoid cleavage dioxygenases (CCDs), related to carotenoid dioxygenase, or 9-*cis*-epoxycarotenoid dioxygenases (NCEDs), which was the first characterized member of this gene family (83). The CCD gene family is responsible for the formation of abscisic acid (ABA), vitamin A, volatiles used in the perfume industry such as β-ionone, colored food additives such as saffron and bixin, and novel classes of plant hormones (33, 74, 83). The expression and subcellular localization of five of the nine Arabidopsis CCDs were studied (126). All were targeted to the plastid, with AtNCED5 thylakoid bound, AtNCED2, AtNCED3, and AtNCED6 both thylakoid-bound and stromal, and AtNCED9 in the stroma (126). All CCDs tested to date act on carotenoids but do show differences in substrate specificity (113) and tissue distribution (126). The crystal structure of a cyanobacterial retinal-forming carotenoid oxygenase reveals a tunnel for holding the β-ionone ring during processing, and this structure will undoubtedly aid in future functional studies of the entire family of enzymes (61).

Abscisic acid. The pathway for synthesis of the plant hormone ABA involves NCEDs that cleave 9-*cis*-neoxanthin or 9-*cis*-violaxanthin to form xanthoxin. Xanthoxin is further modified to produce ABA (reviewed in 83).

Vitamin A. Vitamin A (retinaldehyde) is a C_{20} carotenoid cleavage product essential for animal survival as both a chromophore in vision (retinals) and a hormone (retinoic acids) that exerts most of the effects of vitamin A (144). Any carotenoid containing an unmodified β-ionone ring, such as β-carotene, has provitamin A activity and can be utilized by an animal carotene dioxygenase to produce retinaldehyde. Despite the importance of vitamin A in mammalian physiology, it was not until 2000 that a β-carotene 15,15′-dioxygenase was cloned from *Drosophila melanogaster* (145) and chicken (148) based on similarity to plant CCDs. Since then, progress in understanding vitamin A synthesis in animals has been rapid (144). These studies have shown isoprenoid processing enzymes are often encoded by related genes in plants and animals. These include CRTISO and RetSat (which saturates the 13–14 double bond of retinol), CYP707A (involved in ABA oxidation) and CYP26 (involved in retinoic acid oxidation), and RALDH1 (oxidizes retinaldehyde to retinoic acid) and its plant homolog that oxidizes ABA aldehyde to ABA (77).

Novel carotenoid cleavage products in plants. Evidence for the requirement of novel carotenoid-derived signaling compounds that regulate aspects of plant development, in particular apical dominance and branching, has been accumulating in recent years (6, 74). The Arabidopsis *more axillary growth* mutants (*max3* and *max4*), pea *ramosus* mutant (*rms1*), and petunia *decreased apical dominance* mutant (*dad1*) all cause increased branching of which *max4*, *rms1*, and *dad1* are disrupted in an orthologous carotenoid cleavage enzyme, CCD8, and *max3* in CCD7 (7, 10, 120, 122). CCD7 and CCD8 can sequentially cleave β-carotene to form the C_{18}

compound 13-apo-carotenone in vitro (112). Further modifications (11) are presumably required in vivo to produce the active, graft-transmissible compound that enables wild-type root stocks to complement *max* and *rms* shoots (7, 122, 138). Thus, there is growing evidence demonstrating a novel, mobile, carotenoid-derived hormone that acts downstream of auxin and inhibits shoot branching. Finally, another signal identifed by the *bypass1* mutation, which affects plant architecture, is apparently carotenoid derived and graft transmissible (142).

Carotenoid cleavage products (enzymatic and photooxidative derivatives) are also important in the food, fragrance, and cosmetic industries. Although other aroma constituents such as esters, terpenes, and pyrazines are usually also present (43), the C_9 to C_{13} carotenoid derivatives are often essential to the odor profile (119). Bixin (annatto) is a red-colored dicarboxylic monomethyl ester apocarotenoid, traditionally derived from the plant *Bixa orellana*. Bouvier et al. (13) identified a lycopene cleavage dioxygenase, bixin aldehyde dehydrogenase, and norbixin carboxyl methyltransferase that are required to produce bixin. Saffron, another commercially important colored compound from *Crocus sativus*, derives most of its characteristic color, flavor, and aroma from the accumulation of carotenoid derivatives. A crocus zeaxanthin 7,8(7′,8′)-cleavage dioxygenase was cloned and found to be targeted to the chromoplast where it initiated the production of the modified cleavage products (15), of which the final step is glucosylation (78).

Carotenoids in Nongreen Plastids

A substantial body of work exists on the biosynthesis and manipulation of carotenoids in chromoplasts (see 33, 49, 100, 128). During the chloroplast-to-chromoplast transformation process, carotenoids become localized in plastoglobuli before incorporation into the chromoplast (130). Carotenoids within plastoglobuli exhibit much higher light stability than carotenoids in chloroplast membranes, indicating pigments are better protected from light-mediated destruction in these structures (75). Carotenoids accumulated in fruits are important for protection of triacylglycerols, unsaturated lipids, proteins, membranes, and phenol quinones from photooxidation (75). Chromoplasts accumulate carotenoids in lipoprotein structures (39, 143). For example, in a novel cauliflower mutant with orange curd, β-carotene accumulates in the plastids of the pith and curd as sheets, ribbons, and crystals (66). Additionally, catabolism may also be an important component regulating carotenoid content in some tissues and developmental stages; for example, knockouts of *CCD1* increased total carotenoids in seeds by 40%, with some individual carotenoids increasing by three- to fivefold (3). Thus, carotenoid accumulation in nongreen plastids relies not only on the balance between carotenoid biosynthesis and degradation, but also on the development of structures capable of storing and retaining carotenoids.

Elaioplasts, which are specialized lipid-storing plastids in oil seeds, provide an ideal hydrophobic sink for accumulation of carotenoids. Seed-specific overexpression of PSY in canola resulted in an impressive 50-fold increase in total carotenoid content, in particular the provitamin A α- and β-carotenes (114). A similar approach in Arabidopsis seed resulted in a 43-fold increase in β-carotene and concomitant increases in other carotenoids and chlorophyll, but germination was delayed, reflecting higher levels of the carotenoid-derived hormone, ABA (68). Amyloplasts are "colorless" plastids specialized for storage of starch granules. Lutein is the predominant carotenoid present in many seed amyloplasts, including maize (56) and wheat (48). The antioxidant properties of carotenoids help to combat seed aging, with the loss of lutein subsequently accompanied by an increase in free radicals and reactive oxygen species and a loss of seed viability (20, 91).

The dark-grown etioplast is distinguished by the prolamellar body (PLB), a uniformly curved lattice of tubular membranes, which contains several of the biochemical building blocks required for the chloroplast including the xanthophylls, lutein, and violaxanthin. The Arabidopsis *ccr2* mutant accumulates tetra-*cis*-lycopene and lacks a PLB (89). The absence of this structure suggests that different carotenoids either directly or indirectly impede PLB formation, which results in a delay in photomorphogenesis (greening).

Engineering the Carotenoid Pathway to Benefit Human Health and Agriculture

An increasing body of work on the transgenic manipulation of carotenoids in food plants is emerging (for recent reviews see 100, 128). Because of the prevalence and dire consequences of vitamin A deficiency, provitamin A–carotenoid levels have been targeted for increase by plant breeding and genetic modification. One such example is the breeding of orange-fleshed sweet potato for local conditions in Kenya to provide a new source of β-carotene (46). The best-known example of carotenoid enhancement by molecular manipulation is Golden Rice. *PSY* from daffodil and the bacterial phytoene desaturase (*crtI*) from *Erwinia uredorva* were targeted for expression in rice endosperm (149). Both β-carotene and xanthophylls were produced indicating expression of later pathway enzymes normally occurs in rice endosperm (108). A second generation of Golden Rice has been produced using the maize PSY, which in conjunction with a larger population of transgenics enabled the elevation of carotenoids by up to 23-fold (87). Elite lines will be bred into local cultivars and subject to nutritional and risk assessment (26). In tomato, overexpressing the *Erwinia uredovora* PSY gene (*crtB*) resulted in a two- to fourfold increase in fruit carotenoids (42). Fruit-specific suppression of a photomorphogenic gene, *DET1*, increased the carotenoid and flavonoid content in tomato fruit (36).

Lutein and zeaxanthin, which are important for photoprotection in plants, have been implicated in protecting against the leading cause of age-related blindness in the developed world, macular degeneration (33). Nontransgenic strains of a green alga, *Dunaliella salina*, were developed that accumulate zeaxanthin as the primary xanthophyll (57). *Dunaliella* is already cultivated as a source of β-carotene by the natural products industry. Potato, which usually accumulates lutein and violaxanthin, was genetically modified to accumulate zeaxanthin (101). Serendipitously, this resulted in elevated transcript levels of PSY and a concomitant two- to threefold increase in α-tocopherol (vitamin E) (101). Additional research has shown a correlation with transcript levels and genetic variability in carotenoid content across 20 potato cultivars (79). Intriguingly, overexpression of *PSY* in transgenic potatoes resulted in an increase in transcript for a protein known to function in carotenoid storage, fibrillin, in concert with an increase in carotenoid content (40).

Astaxanthin is a powerful antioxidant and hence a beneficial human dietary component. However, its main value is as a feed additive in aquaculture of salmon, which bioaccumulate astaxanthin in their flesh resulting in its characteristic pink color (71). Astaxanthin is expensive to synthesize chemically and is produced by a limited number of organisms (58); hence it is a target for biotechnological production. In plants, this includes expression of the ketocarotenoid biosynthetic enzyme(s), such as the *Haematococcus pluvialis* β-carotene ketolase (*CrtO*) gene in tobacco (73) and Arabidopsis seeds (123). Although only trace amounts of astaxanthin were produced in chloroplast-containing green tissue, astaxanthin esters were >20% of total carotenoids in chromoplasts of floral nectaries (73). A fusion protein produced from two astaxanthin biosynthetic genes from the bacterium *Paracoccus* expressed in tobacco nectaries resulted in a 10-fold increase in total carotenoid

content, of which a small fraction was ketocarotenoids indicating the efficiency is affected by the high degree of esterification of xanthophyll precursors in this tissue (97). Astaxanthin biosynthesis is found in a limited number of plants, one being the flowers of *Adonis aestivalis*, which have an unexpected biosynthetic route to produce it (32). Two novel *Adonis aestivalis* enzymes with 60% sequence similarity to nonheme βOHases were identified, but neither enzyme displayed typical C3 β-hydroxylase activity and instead preferred to desaturate the 3,4-bond of the β-ring and hydroxylate the fourth carbon, resulting in the 4-keto-β-ring characteristic of astaxanthin and other ketocarotenoids (32).

The production of novel carotenoids has been made possible by innovative molecular shuffling of carotenoid biosynthetic genes. Random shuffling of bacterial phytoene desaturase (*crtI*) and β-cyclase (*crtY*) genes allowed the production of a variety of colored compounds, including the highly desaturated compound 3,4, 3′, 4′-tetra-dehydro-lycopene, despite limited knowledge of the enzymes' catalytic mechanisms (110). They also produced the monocyclic carotenoid, torulene, for the first time in *E. coli* by an entirely new metabolic route, different from any mechanism found in nature (110). A similar strategy enabled the creation of new bioactive compounds and the production of carotenoids otherwise inefficient to synthesize or extract (2, 64, 146).

TOCOCHROMANOL BIOSYNTHESIS IN CYANOBACTERIA AND PLANTS

General Considerations: Structures, Chemistry, and Vitamin E Activities

Tocochromanols are a group of four tocopherols and four tocotrienols produced at various levels and in different combinations by all plant tissues and some cyanobacteria. Tocochromanols are amphipathic molecules with the general structures shown in **Figure 3**. The polar head group is derived from aromatic amino-acid metabolism whereas the saturated tail is derived from phytyl-diphosphate (phytyl-DP) or (GGDP) for tocopherols and tocotrienols, respectively. α-, β-, γ- and δ-tocochromanols differ only in the number and position of methyl substituents on the aromatic ring. Plant tissues vary enormously in their tocochromanol content and composition (45) with photosynthetic tissues generally containing low levels of total tocochromanols (<50 μ/gfw) and a high percentage of α-tocopherol whereas seeds contain 10–20 times this level of total tocochromanols, but α-tocopherol is most often a minor component. α-tocopherol content is especially important from a nutritional perspective as it has the highest vitamin E activity of all tocochromanols (**Figure 3**). This difference is a result of the preferential retention and distribution of α-tocopherol in animals, rather than differential absorption of tocochromanol species during digestion (136). Retention is mediated by a hepatic α-tocopherol transfer protein (α-TTP) with binding kinetics that correlate well with the relative vitamin E activity of each tocochromanol species (53). The importance of α-TTP binding for determining vitamin E activity is clear from the severe phenotypes of α-TTP knockout mice (129, 150). Although tocotrienol vitamin E activity is much lower than the corresponding tocopherols, dietary tocotrienols have been associated with other health benefits (131).

Although tocochromanols are only synthesized by photosynthetic organisms, most of our understanding of their chemistry and function comes from studies in artificial membranes and animal systems because of the vitamin E activity of tocochromanols in animal diets. From a chemical perspective, tocochromanols interact with polyunsaturated acyl groups and protect membrane lipids (especially polyunsaturated fatty acids) from oxidative damage by scavenging lipid peroxy radicals and quenching or chemically reacting with $^1O_2{}^*$ and other reactive oxygen species (ROS) (**Figure 4**) (reviewed in 111). Singlet

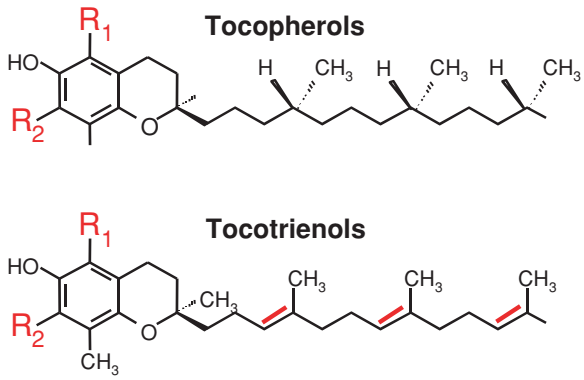

Figure 3

Tocochromanol structures and activities. Key differences in molecules are indicated in red. The table indicates the number and position of ring methyls in α-, β-, γ-, and δ-tocopherol and tocotrienols. The binding of each tocochromanol to an α-tocopherol transfer protein (α-TTP) (53, 88) and the vitamin E activity in the rat resorption-gestation assay (65) are expressed as a percent relative to α-tocopherol.

Tocochromanol type	R_1	R_2	Activity versus α-tocopherol: α-TPP binding	Activity versus α-tocopherol: Vitamin E activity
α-tocopherol	CH_3	CH_3	100	100
α-tocotrienol	CH_3	CH_3	12.5	21-50
β-tocopherol	CH_3	H	38	25-50
β-tocotrienol	CH_3	H	nd	nm
γ-tocopherol	H	CH_3	9	8-19
γ-tocotrienol	H	CH_3	nd	nm
δ-tocopherol	H	H	1.5	<3
δ-tocotrienol	H	H	nd	nm

oxygen quenching occurs by a highly efficient charge-transfer mechanism. Termination of polyunsaturated fatty acid free radical chain reactions by tocochromanols occurs by donation of a hydrogen atom from the tocochromanol ring hydroxyl resulting in a "tocopherol radical." In mammals, the tocopherol radical is rapidly recycled back to the corresponding tocopherol allowing each tocopherol to participate in many lipid peroxidation chain-breaking events before being degraded. Whether a similar regeneration cycle occurs in plastids has not been demonstrated. Finally, tocochromanols can chemically scavenge various ROS and become converted to the corresponding quinone (and other derivatives), some of which have been shown to also participate in electron-transfer reactions (63, 118). Though it seems logical these quinones might be converted back to the corresponding tocopherols for additional rounds of scavenging, such reactions have not been reported in plants or animals. Biological functions of tocochromanols in mammalian systems and plants are discussed below.

The Tocochromanol Pathway Succumbs to Biochemical Genomics

The tocochromanol biosynthetic pathway (**Figure 5)** was elucidated from radiotracer studies in isolated chloroplasts and cyanobacteria in the mid-1980s (reviewed in 45). The tocochromanol pathway utilizes cytosolic aromatic amino-acid metabolism for head group synthesis and the plastidic deoxyxylulose 5-phosphate pathway for tail synthesis (phytyl-PP for tocopherols and GGDP for tocotrienols). The committed step in headgroup synthesis is the conversion of p-hydroxyphenylpyruvate (HPP) to homogentisic acid (HGA) by the enzyme HPP

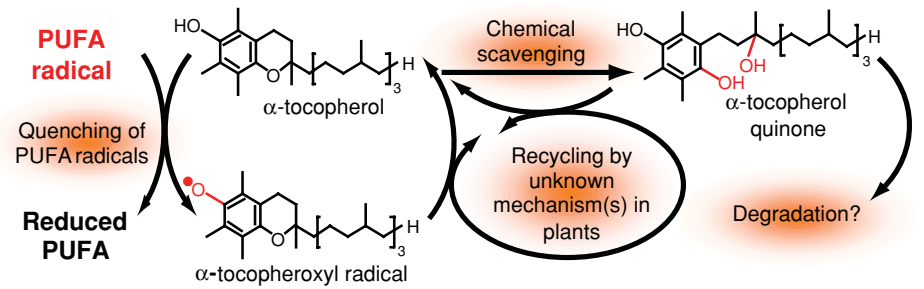

Figure 4

Polyunsaturated fatty acid (PUFA) quenching and reactive oxygen species (ROS) scavenging by tocopherols. Not all possible intermediates, reactions, or products are shown. Key differences in molecules are indicated in red.

dioxygenase (HPPD). Phytyl-PP or GGDP are condensed with HGA by a class of homogentisate prenyl transferases to yield 2-methyl-6-phytylplastoquinol (MPBQ) and 2-methyl-6-geranylgeranylplastoquinol (MGGBQ), respectively, the first committed intermediates in tocopherol and tocotrienol synthesis. The substrate specificity of this reaction is key for determining whether one or both tocochromanol classes are produced in a tissue. MPBQ and MGGBQ are methylated to form 2,3-dimethyl-5-phytyl-1, 4-benzoquinone (DMPBQ) or 2,3-dimethyl-5-geranylgeranyl-1, 4-benzoquinone (DMGGBQ), respectively. MPBQ and DMPBQ (or MGGBQ and DMGGBQ) are substrates for tocopherol cyclase to yield δ- and γ-tocopherols (and δ- and γ-tocotrienols), respectively. Finally, reaction with γ-tocopherol methyltransferase converts δ- and γ-tocopherols (and tocotrienols) to β- and α-tocopherols (and tocotrienols), respectively.

During the past decade our understanding of the molecular genetics of tocochromanol synthesis has become increasingly sophisticated. This is due to the directed application of "omics" and associated technologies to understand the synthesis of plant compounds important to human health and agriculture, an approach termed nutritional genomics (37). Several groups have targeted the tocochromanol pathway with this approach, such that all the core pathway enzymes have been isolated and studied in detail from *Synechocystis* sp. PCC6803 and *Arabidopsis thaliana* (19, 22, 23, 85, 86, 95, 105, 107, 109, 115, 116, 141). With the exception of MPBQ MT, the enzymes share significant sequence similarity between plants and cyanobacteria, which has facilitated genomics-driven ortholog isolation between the two organism groups. The sections below focus primarily on tocochromanol research in model photosynthetic organisms and highlight efforts, where appropriate, to use the knowledge obtained to manipulate tocochromanol levels in food crops.

Tocochromanol Aromatic Headgroup Synthesis

p-hydroxyphenylpyruvic acid dioxygenase (HPPD) was the first tocochromanol pathway enzyme to be cloned from Arabidopsis (86). HPPD catalyzes HGA synthesis, and mutation of the Arabidopsis locus (the *PDS1* locus, At1g06590) conclusively demonstrated HPPD activity was essential for both tocopherol and plastoquinone biosynthesis in plants (85). Interestingly, disruption of the single HPPD in *Synechocystis* sp. PCC6803 (slr0090) only impacted tocopherol synthesis, indicating plastoquinone synthesis in *Synechocystis* sp. PCC6803 is HGA independent

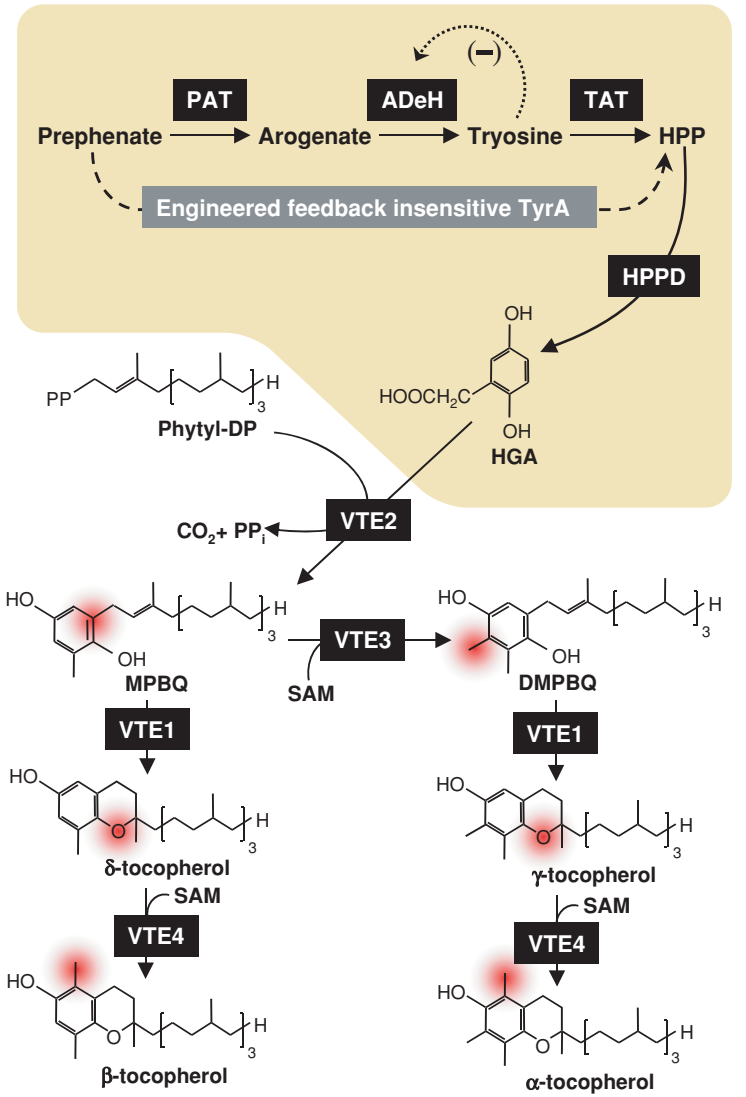

Figure 5

The tocopherol biosynthetic pathway in plants. The pathway and enzyme nomenclature, loci, and genes are in reference to studies in Arabidopsis, as described in detail in the text. Organisms that produce tocotrienols utilize the same pathway except the prenyltransferase reaction (VTE2) in these organisms can also utilize geranylgeranyl diphosphate (GGDP) in addition to or in place of phytyl-diphosphate (phytyl-DP). (*Yellow highlight*) Pathway leading from prephenate to homogentisic acid (HGA). Feedback inhibition of arogenate dehydrogenase (AdeH) by tyrosine is indicated by a dotted line. The activity of the feedback insensitive TyrA activity is indicated by a gray box and dashed line. α-tocopherol is the most abundant tocopherol produced in wild-type Arabidopsis leaves and in *Synechocystis* sp. PCC6803. γ-tocopherol is the most abundant tocopherol in Arabidopsis seed. The activity of VTE1, -2, -3, and -4 in generating the product for each step is highlighted in red on the relevant structure. DMPBQ, 2,3-dimethyl-5-phytyl-1, 4-benzoquinone; HPP, p-hydroxyphenylpyruvate; HPPD, HPP dioxygenase; PAT, prephenate amino transferase; SAM, S-adenosyl methionine; TAT, tyrosine amino transferase; VTE1, tocopherol cyclase; VTE2, homogentisate phytyltransferase; VTE3, MPBQ methyltransferase; VTE4, γγ-tocopherol methyltransferase.

(34). Given the key location of HPPD in the tocochromanol pathway, it seemed a likely candidate regulating pathway flux. To test this hypothesis HPPD was overexpressed in Arabidopsis seed and leaves (137) resulting in more than a 20-fold increase in activity but only a 15% and 30% increase in seed and leaf tocopherols, respectively. Targeting overexpressed HPPD protein to either the cytosol or plastid of tobacco yielded similarly modest increases in seed tocochromanols (41). These data indicated that although HPPD is required, this activity alone is not a significant limitation to tocochromanol flux.

An alternative approach to engineering headgroup flux yielded unexpected results: greatly increased levels of tocotrienols in seed and leaves of various plants. The endogenous regulation of HPP production in plants, feedback inhibition of arogenate dehydrogenase by its product tyrosine, was bypassed by engineering a feedback insensitive, bifunctional prephenate dehydratase (TyrA) for overexpression in plants (60, 98). TyrA catalyzes HPP synthesis directly from prephenate but had little impact on tocochromanols when overexpressed alone. However, coexpression of TyrA with Arabidopsis HPPD in tobacco leaf resulted in an eightfold increase in total leaf tocochromanol levels, which occurred almost entirely because of increases in various tocotrienols normally only produced in tobacco seed (98). Similar results were obtained by seed-specific co-overexpression of TyrA and HPPD in Arabidopsis, canola, and soybean: two- to three fold increases in total seed tocochromanols, almost entirely because of increased tocotrienols (60). These results suggest flux to HGA is indeed limiting but requires both increased HPPD activity and increased flux to HPP. However, why co-overexpression of TyrA and HPPD specifically increases tocotrienols but not tocopherols remains unclear; perhaps the high HGA levels in the transgenics induce a GGDP-utilizing homogentisate prenyl transferase. It should be noted that seed of TyrA/HPPD overexpressing Arabidopsis and soybean were black as a result of oxidative polymerization of HGA (present at 60- and 800-fold higher levels, respectively, than wild types), and germination was negatively impaired, an undesirable agronomic trait (60).

Prenylation of Homogentisic Acid

Homogentisate prenyl transferases catalyze the committed step in tocochromanol synthesis: condensation of phytyl-DP and HGA for tocopherols, and GGDP and HGA for tocotrienols. This class of hydrophobic, integral membrane proteins was cloned by approaches utilizing whole genome information from *Synechocystis* sp. PCC6803 and Arabidopsis (23, 107, 109). It was hypothesized that homogentisate prenyltransferases would show some level of similarity to related cyanobacterial and plant prenyltransferases that utilize similar prenyl-DPs as substrates. Chlorophyll synthesis involves one such enzyme: chlorophyll synthase (ChlG), which attaches PDP or GGDP to chlorophyllide (70). Query of the *Synechocystis* sp. PCC6803 genome database with the ChlG sequence identified several candidate genes including slr1736, which had ~20% protein identity with ChlG. Disruption of the slr1736 locus eliminated production of all tocopherols and pathway intermediates in *Synechocystis* sp. PCC6803. An Arabidopsis ortholog (At2g18950, the *VTE2* locus) was isolated, and both the *Synechocystis* sp. PCC6803 and Arabidopsis enzymes were expressed in *E. coli* and assayed. Both enzymes utilized phytyl-DP as a cosubstrate, but only the *Synechocystis* sp. PCC6803 enzyme could also utilize GGDP, which is intriguing, as *Synechocystis* sp. PCC6803 does not accumulate tocotrienols.

Mutation of the *VTE2* locus resulted in complete tocopherol deficiency in all tissues demonstrating it is the only activity for the synthesis of tocopherols in wild-type Arabidopsis (106). VTE2 was shown to be a limiting activity in unstressed

Arabidopsis as VTE2 overexpression increased total tocopherol levels up to fivefold and twofold in leaves and seeds, respectively (23, 107). VTE2 activity was also limiting for tocopherol synthesis during combined high light and nutrient stress (25). Isolation of paralogs (40%–50% protein identity to Arabidopsis VTE2) from various tocotrienol-producing monocots demonstrated the key role of the enzyme in determining the tocochromanol composition of a tissue (19). Active enzyme could not be produced in *E. coli* for direct analysis of substrate specificity. However, overexpression of a barley enzyme in Arabidopsis leaves and maize embryos increased tocotrienols up to 15-fold and sixfold of the total tocochromanol content of the respective wild-type tissues without impacting tocopherols. Because the bulk of the increase was γ-tocotrienol, which has low vitamin E activity (**Figure 3**), the vitamin E content of transgenics was increased less than 50% relative to wild-type tissues.

An Alternate Route for Phytyl-Tail Synthesis

It has long been assumed the phytyl tail of tocopherols is primarily derived from reduction of GGDP, and the phenotypes of tobacco and *Synechocystis* sp. PCC6803 lines with decreased GGDP reductase activity are consistent with this thinking (117, 127). However, the identification of a novel Arabidopsis mutant that reduces leaf and seed tocopherols 80% and 65%, respectively, relative to wild type indicates a second pathway provides an important source of phytyl-DP for tocopherol synthesis (139). The locus (VTE5, At5g04490) encodes a gene with similarity to yeast and Arabidopsis dolichol kinase, a polyisoprenoid substrate similar to phytol. VTE5 protein has phytol kinase activity when expressed in *E. coli*, and a second kinase in plants presumably acts on the phytyl monophosphate produced by VTE5 to yield phytyl-DP. The identification of VTE5 helps explain the inverse correlations between tocopherol levels and chlorophyll degradation during natural and induced leaf senescence (99) and canola-seed development (44). Although the relative contributions of phytyl-DP from GGDP and the VTE5-based recycling pathway to tocopherol synthesis have not been directly evaluated, this alternative source of phytyl-DP for tocopherol synthesis may help explain some surprising pathway-engineering results. Recall that expression of barley homogentisate geranylgeranyldiphosphate transferase (19) caused large increases in tocotrienols in Arabidopsis without negatively impacting tocopherol levels. This result could be readily explained if phytyl and geranylgeranyl tails were derived from separate precursor pools, with the majority of tocopherol phytyl tails coming from activation of free phytol rather than reduction of GGDP.

The Methyltransferases of Tocochromanol Synthesis

MPBQ MT and γ-TMT are key activities in determining the types of tocochromanols that accumulate in a tissue (**Figures 3** and **5**). *Synechocystis* sp. PCC6803 γ-TMT (slr0089) was the first pathway methyltransferase to be identified (reviewed in 45), in part because of its physical proximity to HPPD (slr0090) in the *Synechocystis* sp. PCC6803 genome (115). Briefly, disruption of slr0089 resulted in α-tocopherol deficiency and γ-tocopherol accumulation, consistent with loss of γ-TMT activity. Both slr0089 and the Arabidopsis γ-TMT ortholog (*VTE4*, At1g64970) were found to use δ- and γ-tocopherols as substrates to produce β- and α-tocopherols, respectively. Seed-specific overexpression of VTE4 in Arabidopsis resulted in the near-complete conversion of γ-tocopherol to α-tocopherol and a ninefold increase in vitamin E activity (115). Interestingly, VTE2 and VTE4 overexpression were additive in Arabidopsis leaves and seeds increasing total tocochromanols while simultaneously

converting virtually all of the γ-tocopherol to α-tocopherol. In seed, this resulted in a nearly 12-fold increase in vitamin E activity (24).

Synechocystis sp. PCC6803 MPBQ MT (sll0418) was identified based on sequence similarity to γ-TMT and shown to use MPBQ but not δ- or β-tocopherols as substrates (22, 116). Disruption of the sll0418 gene only partially eliminated α-tocopherol accumulation suggesting an additional, partially redundant activity is present in *Synechocystis* sp. PCC6803 for this reaction. Surprisingly, unlike all other tocochromanol pathway steps, the SLL0418 protein sequence was not useful for identifying a plant ortholog. Instead, two groups used map-based cloning approaches to isolate ethyl methane sulfonate mutant alleles for Arabidopsis MPBQ MT (the *VTE3* locus, At3g63410) (22, 141). Unlike the sll0418 mutant, the phenotypes of *VTE3* mutants make it clear there are no redundant activities for the reaction in plants. VTE3 and SLL0418 have identical activities and substrate specificities in vitro but less than 20% amino-acid identity and represent a clear case of convergent evolution (22). Interestingly, the proteins with the highest identity to VTE3 are only present in Archaea (and other plants). The Chlamydomonas genome was unique in containing orthologs for both SLL0418 and VTE3.

VTE3 and VTE4 are key enzymes for metabolic engineering the tocochromanol content of crop plants because most crop plants accumulate δ-, β- and γ-tocochromanols (45). An outstanding example of the relevance of basic studies of metabolism in model systems to agricultural crops was reported by Van Eenennaam et al. (141) where coexpression of Arabidopsis VTE4 and VTE3 in soybean seed resulted in near-complete conversion of β-, γ-, and δ-tocopherols to α-tocopherol. The resulting fivefold increase in vitamin E activity of the transgenic soybean oil represents one of the clearest examples of nutritional genomics applied from a model plant to an agricultural crop.

The Tocopherol Cyclase Enzyme

The tocopherol cyclase gene (*VTE1*, At4g32770) was isolated by chromosome walking to mutations in Arabidopsis that eliminated the synthesis of tocopherols and caused accumulation of the pathway intermediate, DMPBQ (95, 105). The homologous *Synechocystis* sp. PCC6803 gene, slr1737, was present in a two open reading frame operon with slr1736, the *Synechocystis* sp. PCC6803 VTE2 ortholog. A maize tocopherol cyclase ortholog, whose activity was unknown at the time, had been previously cloned and studied based on the negative impact of mutating the locus on carbon translocation in germinating maize seedlings (96). The gene was originally designated *SXD1* for *sucrose export defective 1* and suggests tocopherols have impacts beyond acting as lipid-soluble antioxidants in plants, in this instance by somehow regulating carbon translocation from source tissues to sink tissues. The phenotype resulting from RNAi inhibition of tocopherol cyclase expression in potato was similar to maize *sxd1* suggesting such functions for tocopherols may be conserved in plants (50).

Overexpression of *VTE1* in Arabidopsis leaves produced surprising results (59). Total leaf tocochromanols increased several fold solely as a result of increased γ-tocopherol rather than α-tocopherol, the major tocopherol normally found in wild-type Arabidopsis leaves. This was interpreted as a limitation in VTE4 activity (59), which is puzzling as overexpression of VTE2 and VTE4, singly and in combination, clearly demonstrated VTE4 activity only becomes limiting when plants are stressed (24, 25, 115). Further, this conclusion does not address why ascorbate and glutathione levels were reduced 60% and 40%, respectively, relative to wild type in VTE1 overexpressing plants (59). Such decreases are especially significant as ascorbate and glutathione are present in 10^2 and 10^3 molar excesses, respectively, relative to tocopherols in wild type. A mechanistic

explanation for the surprising and interesting consequences of VTE1 overexpression is still lacking.

Tocopherol Functions

As described above, because of their roles as vitamin E, tocochromanol functions have been studied most extensively in animal systems, and our understanding of plant tocochromanol functions pales by comparison. The antioxidant and radical scavenging roles of tocochromanols in animals are well-defined (111), and during the past decade additional nonantioxidant functions for specific tocochromanols have been described in a variety of animal systems (4, 151). Most often nonantioxidant functions are mediated by tocopherol-dependent alterations in the synthesis of lipid-derived signaling molecules, membrane-associated signaling pathways, and gene expression. Analogous nonantioxidant functions for tocochromanols have been proposed for plants (80–82), but this seems premature, as supporting evidence is nonexistent and tocochromanol biological functions may differ between the two kingdoms as a result of fundamental physiological differences.

The plant and cyanobacterial tocochromanol biosynthetic mutants described throughout this review will be essential tools for furthering understanding of tocochromanol functions in photosynthetic organisms. These mutants are already providing unanticipated results, the most obvious that, unlike tocopherol deficiency in animals, tocopherol deficiency in photosynthetic organisms is not lethal. Tocopherol-deficient *Synechocystis* sp. PCC6803 mutants and mature *vte4*, *vte2*, and *vte1* Arabidopsis plants show surprisingly robust phenotypes and are often virtually indistinguishable from wild-type counterparts in permissive conditions and several abiotic stresses, including high light (22, 23, 72, 109, 116). Indeed, the only well-defined tocochromanol function in plants to date is the protection of seed-storage lipids from oxidation during dormancy and germination in Arabidopsis (106). *vte2* but not *vte1* mutants exhibited massive oxidation of storage lipids during germination and severely reduced fitness, suggesting the DMPBQ accumulated by *vte1* can compensate for the lack of tocopherols in this regard. Once past germination, *vte1* and *vte2* are visually indistinguishable from wild type. This lack of readily observable phenotypes in tocopherol-deficient plants should not be taken as conclusive evidence against a vital role for tocochromanols in photoautotrophic plant tissues. The fact that the synthesis and presence of tocochromanols in photoautotrophic tissues are absolutely conserved throughout the plant kingdom argues for essential and conserved functions. Rather, the surprisingly subtle phenotypes of tocopherol-deficient mutants in plants and cyanobacteria suggest some of our long-held assumptions about tocochromanol functions in photosynthetic organisms need to be reassessed in an unbiased fashion, and this is certain to yield some surprises.

SUMMARY POINTS

1. Integrated molecular, genetic, and genomic approaches during the past decade have allowed isolation of the full set of core pathway genes for synthesis of tocochromanols and carotenoids in plants. This approach allows similar progress in many other previously recalcitrant areas of plant metabolism.
2. Metabolic engineering has demonstrated the feasibility of altering the levels of total and specific carotenoids and tocochromanols in agricultural crops to positively impact nutrition and human health.

3. The identification of novel functions for carotenoid derivatives from the Arabidopsis CCD gene family highlights the expanding and diverse roles of carotenoids beyond photosynthesis.
4. The unanticipated effects of some carotenoid and tocochromanol pathway mutants and transgenic experiments serve to highlight the complex regulation of these pathways at the molecular and biochemical levels.
5. Evolution of the plant carotenoid and tocochromanol pathways provides insight into the complex origins of plant metabolic pathways. The two pathways are a complex mix of genes with orthologs in photosynthetic and nonphotosynthetic bacteria and mammals combined with gene duplication and divergence within the plant kingdom.

FUTURE DIRECTIONS/ UNRESOLVED ISSUES

1. Although some carotenoid and tocochromanol enzymes have been cloned and studied for nearly a decade, our understanding of the overall biochemical and molecular regulation of these pathways in plants remains limited. Research to explain unanticipated results from biosynthetic mutants and metabolic engineering of the pathways promises novel insights into the regulation and integration of these pathways in plants.
2. Although intensive functional studies of carotenoids in photosynthetic tissues have highlighted important structure/function relationships, there is still considerable debate about the role(s) of individual carotenoids. In nonphotosynthetic tissues, carotenoid functions remain much less defined. The emerging roles of novel carotenoid derivatives in plant development and regulation of the pathway suggest many important functions beyond photosynthesis remain to be uncovered.
3. With the exception of germinating/dormant seedlings, tocochromanol functions in photosynthetic organisms remain an open question. The phenotypes of tocochromanol biosynthetic mutants are surprisingly similar to wild type and suggest long-held assumptions of tocochromanol functions in photosynthetic organisms need to be critically reassessed. Mutant and transgenic lines with altered tocochromanol levels and types will be important tools for these studies.

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Excellent, well-referenced short historical overview of nonantioxidant tocochromanol functions studies in animal systems since 1991.

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APPENDIX

Compound Abbreviations

DMPBQ: 2,3-dimethyl-5-phytyl-1,4-benzoquinone
HGA: homogentisic acid
HPP: *p*-hydroxyphenylpyruvate
MPBQ: 2-methyl-6-phytyl-1,4-benzoquinone
phytyl-DP: phytyl-diphosphate
SAM: S-adenosyl methionine

Enzyme Abbreviations

AdeH: arogenate dehydrogenase
HPPD: HPP dioxygenase
PAT: prephenate amino transferase
TAT: tyrosine amino transferase
VTE1: tocopherol cyclase
VTE2: homogentisate phytyltransferase
VTE3: MPBQ methyltransferase
VTE4: γ-tocopherol methyltransferase

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