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More than meets the eye: from carotenoid biosynthesis, to new insights into apocarotenoid signaling

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Carotenoids are a class of isoprenoids synthesized almost exclusively in plants involved in a myriad of roles including the provision of flower and fruit pigmentation for the attraction of pollinators and seed dispersing organisms. While carotenoids are essential throughout plant development, they are also extremely important in human diets providing necessary nutrition and aiding in the prevention of various cancers, age-related diseases and macular degeneration. Utilization of multiple plant models systems (i.e. *Arabidopsis*; maize; and tomato) has provided a comprehensive framework detailing the regulation of carotenogenesis throughout plant development covering all levels of genetic regulation from epigenetic to post-translational modifications. That said, the understanding of how carotenoids self-regulate remains fragmented. Recent reports demonstrate the potential influence of carotenoid-cleavage products (apocarotenoids) as signaling molecules regulating carotenoid biosynthesis in addition to various aspects of plants development (i.e. leaf and root development). This review highlights recent advances in carotenogenic regulation and insights into potential roles of novel apocarotenoids in plants.

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Introduction

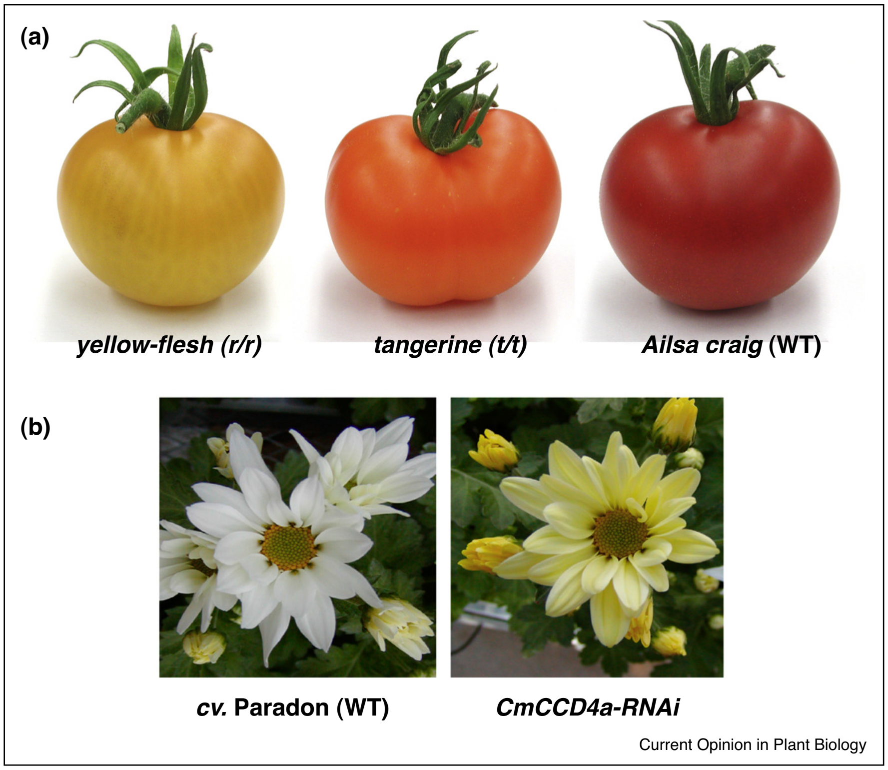
Carotenoids are lipophilic isoprenoids that make up a large number of the most vibrant biological pigments we observe daily. Found in invertebrates, birds, plants, bacteria and fungi, the reflective properties of carotenoids provide the yellow, orange and red pigments in nature. In plants, carotenoids play a myriad of roles including provision of flower and fruit pigmentation for the attraction of pollinators and seed dispersing organisms (Figure 1). In photosynthetic organs, carotenoid accumulation in chloroplasts is crucial for the absorption of excess light energy, scavenge reactive oxygen species, and provide precursors to plant hormones as well as carotenoid-derived signaling molecules [1]. It is thus anticipated that a strict regulatory mechanism tuned to endogenous and exogenous signals must be in place to ensure efficient and precise accumulation of carotenoids and subsequent hormones/signaling molecules as required. Animals, invertebrate and vertebrate, lack the capacity to synthesize carotenoids *de novo* and rely on plants in their diet as carotenoid sources. Moreover, researchers have shown that diets high in carotenoids provide numerous health benefits (e.g. promote healthy ocular development, prevention of various cancers, macular degeneration, cardiac disease and other age-related diseases [2–4]). Thus research efforts are focused on investigating the regulation of carotenoid synthesis, metabolism and accumulation in plants. That said, researchers are finding it increasingly important to understand the mechanisms in which carotenoid cleavage-derived or apocarotenoid signals regulate carotenoid biosynthesis as well as cell and plant development. This review considers recent advances in the regulation of carotenoid biosynthesis and some potential roles for novel apocarotenoids.

Carotenoid biosynthesis in plants

Diverse germplasm resources including Arabidopsis, tomato and maize have provided the tools necessary to piece together a comprehensive framework of carotenoid biosynthesis in both photosynthetic and non-photosynthetic tissues of plants (Figure 2). In plastids, carotenoid biosynthesis is one of many isoprenoid and isoprenoid-related pathways relying on the methylerythritol 4-phosphate (MEP) pathway for the production of the 5-carbon isoprenoid building blocks isopentenyl diphosphate and dimethylallyl diphosphate (IPP and DMAPP, respectively) [5]. The MEP pathway end product, IPP, is isomerized by isopentenyl diphosphate isomerase (IDI) generating DMAPP and the subsequent condensation of one IPP and three DMAPP in a series of prenyltransferase reactions supply the immediate precursor for carotenoid biosynthesis, geranylgeranyl diphosphate (GGPP) [6,7].

The first committed step in carotenogenesis requires the condensation of 2 GGPPs by phytoene synthase (PSY) producing 15-*cis*-phytoene [8,9]. At the start of the poly-*cis*-transformation from 15-*cis*-phytoene to *all-trans*-lycopene,

Figure 1



Current Opinion in Plant Biology

Visual phenotypes associated with perturbations in carotenoid metabolism. **(a)** Fruit-specific carotenoid mutants in *PSY1* (*yellow-flesh*) and *CRTISO* (*tangerine*) compared to the lycopene-rich wild-type tomato (*Ailsa Craig*). **(b)** Altered carotenoid content in flowers with reduced *CCD4a* expression (*CmCCD4a-RNAi*) compared to wild-type (*cv.* Paradon) [57••].

phytoene desaturase catalyzes two desaturation reactions converting 15-*cis*-phytoene to 9,15,9′-*tri-cis*-ζ-carotene via a 15,9-*di-cis*-phytofluene intermediate [10–12]. Subsequently, 9,15,9′-*tri-cis*-ζ-carotene can either be photoisomerized or enzymatically isomerized by ζ-carotene isomerase (ZISO) to create 9,9′-*di-cis*-ζ-carotene, the substrate for ZDS [13]. The two subsequent desaturation reactions by ZDS produces 7,9,7′,9′-*tetra-cis*-lycopene (prolycopene) [14,15] which is subsequently isomerized by CRTISO creating *all trans*-lycopene, the red color characteristic of tomato [16–18]. Next, *all trans*-lycopene undergoes cyclization steps by lycopene ε-cyclase (CRTL-E) and or lycopene β-cyclase (CRTL-B1(2)) making α-carotene (ε-ring and β-ring) and/or β-carotene (β-rings) [19,20]. Subsequently, lutein is synthesized through the ε-hydroxylation and β-hydroxylation of α-carotene two ferredoxin-dependent non-heme P450-type carotenoid hydroxylases, CYP97A3 and CYP97C1, respectively [21,22]. β-Carotene undergoes β-hydroxylation on both rings almost exclusively by two non-heme di-iron monooxygenases, CRTR-B1 and CRTR-B2 generating zeaxanthin [23–25]. Further epoxidation of both β-rings on zeaxanthin produces violaxanthin through the antheraxanthin intermediate [26,27]. Violaxanthin can then be converted to neoxanthin by neoxanthin synthase [28]. Both violaxanthin and neoxanthin can be isomerized and cleaved by 9-*cis*-epoxycarotenoid dioxygenases, NCEDs providing xanthoxin, the apocarotenoid precursor to abscisic acid, ABA, and signifying the final step of carotenoid biosynthesis (Figure 3a) [29].

Upon completion of the tomato genome sequence steps catalyzed by PSY, and CRTL-B were confirmed to be performed by gene families each containing three genes (PSY1, PSY2, and PSY3; CRTL-B1, CRTL-B2, and CRTL-B3) (Figure 2) [30]. This phenomenon is hypothesized to have occurred through a triplication event during the evolution of tomato and appears to be consistent across other higher plants [30,31]. Furthermore, from the tomato genome sequence carotenoid isomerase-like genes, CRTIL1 and CRTIL2, were identified and via virus-induced gene silencing were proposed to catalyze the isomerization of 9,9′-*di-cis*-z-carotene to form *all-trans*-z-carotene (Figure 2) [30,32•]. As researchers discover and characterize new members of the various carotenogenic gene families, unique enzymatic, potentially catabolic reactions within the pathway may be exposed, thus allowing an opportunity to better understand the regulation of