Archives of Biochemistry and Biophysics 504 (2010) 132–141

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

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

Review

The regulation of carotenoid pigmentation in flowers

Changfu Zhu [a,b], Chao Bai [a], Georgina Sanahuja [a], Dawei Yuan [a], Gemma Farré [a], Shaista Naqvi [a], Lianxuan Shi [b], Teresa Capell [a], Paul Christou [a,c,*]

[a] Departament de Producció Vegetal i Ciència Forestal, Universitat de Lleida, Av. Alcalde Rovira Roure, 191, Lleida 25198, Spain
[b] School of Life Sciences, Northeast Normal University, Changchun 130024, China
[c] Institucio Catalana de Recerca i Estudis Avancats, Passeig Llúis Companys, 23, Barcelona 08010, Spain

ARTICLE INFO

Article history:
Available online 3 August 2010

Keywords:
Carotenoids
Ketocarotenoids
Regulation
Gene expression
Flower color
Chromoplast
Transgenic plants

ABSTRACT

Carotenoids fulfill many processes that are essential for normal growth and development in plants, but they are also responsible for the breathtaking variety of red-to-yellow colors we see in flowers and fruits. Although such visual diversity helps to attract pollinators and encourages herbivores to distribute seeds, humans also benefit from the aesthetic properties of flowers and an entire floriculture industry has developed on the basis that new and attractive varieties can be produced. Over the last decade, much has been learned about the impact of carotenoid metabolism on flower color development and the molecular basis of flower color. A number of different regulatory mechanisms have been described ranging from the transcriptional regulation of genes involved in carotenoid synthesis to the control of carotenoid storage in sink organs. This means we can now explain many of the natural colorful varieties we see around us and also engineer plants to produce flowers with novel and exciting varieties that are not provided by nature.

Carotenoids are organic molecules comprising a C_{40} polyene backbone that is often cyclized to generate terminal ionone rings. This structure allows carotenoids to absorb short-wave visible light, and the wavelengths that are absorbed depend on the number and nature of the double bonds. Carotenoids are therefore pigments that range in color from yellow through orange to red. They are produced mainly by photosynthetic organisms, and in plants they are synthesized in both chloroplasts and chromoplasts, imparting color to photosynthetic tissues as well as fruits, storage organs and flowers [1].

In mature chloroplasts, carotenoids fulfill a number of functions essential in photosynthesis such as photosystem assembly, light harvesting and protection from photo-oxidation at high light intensities [2,3]. In contrast, carotenoids in chromoplasts appear to be synthesized primarily for the purpose of attracting other organisms, such as pollinating insects and seed-distributing herbivores [4]. Carotenoids are also important substrates for a class of carotenoid cleavage dioxygenases (CCDs)[1] that generate biologically active apocarotenoids such as pro-vitamin A [5], abscisic acid [6] and the recently-discovered hormone strigolactone [7,8]. Other apocarotenoids are well known as food colorings, cosmetics, flavors and fragrances, such as bixin, crocetin and β-ionone [9–12].

There is considerable interest in chromoplast-derived carotenoids because of their two direct benefits to humans – as antioxidants and pigments. When expressed in fruits and storage organs, carotenoids are primarily valued for their antioxidant properties, and we have discussed recent advances in the nutritional improvement of cereals, fruits and root vegetables by carotenoid metabolic engineering in other review articles [13–15]. Other excellent reviews have covered carotenoid metabolism and metabolic engineering [16–18], and the regulation of carotenoid biosynthesis using promoters, transcription factors, chromatin regulation and compartmentalization [19]. Here we focus on carotenoids as pigments in flowers. This has a strong impact in the cut flower industry purely for aesthetic reasons, but also in the areas of traditional medicines and nutrition because certain carotenoids are extracted

[1] *Abbreviations used:* CCDs, carotenoid cleavage dioxygenases; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; MEP, methylerythritol 4-phosphate; GGPP, geranylgeranyl diphosphate; GGPPS, GGPP synthase; PSY, phytoene synthase; PDS, phytoene desaturase; Z-ISO, ζ-carotene isomerase; ZDS, ζ-carotene desaturase; CRTISO, carotenoid isomerase; LYCB, lycopene β-cyclase; LYCE, lycopene ε-cyclase; ZEP, zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase; NXS, neoxanthin synthase; ABA, abscisic acid; CCS, capsanthin–capsorubin synthase; DXS, 1-deoxy-D-xylulose 5-phosphate synthase.

* Corresponding author at: Departament de Producció Vegetal i Ciència Forestal, Universitat de Lleida, Av. Alcalde Rovira Roure, 191, Lleida 25198, Spain. Fax: +34 973 238264.
E-mail address: christou@pvcf.udl.es (P. Christou).

0003-9861/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.abb.2010.07.028

Table 1
Carotenoid composition in petals of fully open flowers.

Species	Cultivars or mutants	Major carotenoids	Minor carotenoids	References
Solanum lycopersicum	Wild type	Violanxanthin and neoxanthin	Lutein	[44]
Solanum lycopersicum	*old-gold* (og)[a]	Violanxanthin and neoxanthin	Lutein and lycopene	[52]
Solanum lycopersicum	*tangerine*^{3183}[b]	Neoxanthin, violaxanthin, phytoene and rubixanthin	Prolycopene, phytofluene, ζ-carotene, neurosporene, lycopene, γ-carotene, β-carotene, β-cryptoxanthin and lutein	[56]
Solanum lycopersicum	*tangerine*^{micc}	Prolycopene, phytoene, neoxanthin, phytofluene and ζ-carotene	*cis*-Neurosporene, neurosporene, di-*cis*-lycopene, β-carotene and rubixanthin	[56]
Solanum lycopersicum	*white-flower* (*wf*)[d]	β-Carotene and neoxanthin,	Violaxanthin, lutein and phytoene	[44]
Solanum lycopersicum	*high-pigment 3* (*hp3*)[e]	Zeaxanthin	Antheraxanthin, neoxanthin, violaxanthin and lutein	[57]
Narcissus pseudonarcissus		Lutein	β-Carotene	[58]
Tagetes erecta	Lady	Lutein		[42]
Gentiana lutea		Lutein, violaxanthin, β-carotene and antheraxanthin	Neoxanthin, zeaxanthin and β-cryptoxanthin	[59]
Ipomoea sp.		β-Cryptoxanthin and zeaxanthin	Lutein, neoxanthin and violaxanthin	[60]
Ipomoea obscura			Zeaxanthin, lutein, neoxanthin and violaxanthin	[60]
Lilium spp.[f]	Connecticut King	Antheraxanthin, (9*Z*)-violaxanthin and *cis*-lutein	Violaxanthin, lutein and β-carotene	[61]
Lilium spp.[f]	Montreux	Violaxanthin, lutein and β-carotene	Antheraxanthin, (9*Z*)-violaxanthin and *cis*-lutein	[61]
Lilium spp.[f]	Saija	Capsanthin	Antheraxanthin	[61]
Chrysanthemum morifolium Ramat.	Yellow Paragon	Lutein		[62]
Cucumis sativus	var. Anguria	Lutein, β-carotene, flavoxanthin, auroxanthin and zeaxanthin	β-Crytoxanthin, α-carotene and violaxanthin	[63]
Nicotiana glauca		Lutein and β-carotene	Violaxanthin, antheraxanthin and β-cryptoxanthin	[64]
Lotus japonicus		Violaxanthin and antheraxanthin	Neoxanthin, lutein, zeaxanthin and β-carotene	[65]
Oncidium Gower Ramsey[g]		All-*trans* violaxanthin and 9-*cis*-violaxanthin	All-*trans* neoxanthin, 9-*cis*-neoxanthin and lutein	[66]
Crocus sativus[h]		Zeaxanthin, ζ-carotene and β-carotene	β-Cryptoxanthin, lycopene and phytofluene	[67]
Adonis aestivalis		Astaxanthin	3-Hydroxyechinenone, adonixanthin and adonirubin	[68,69]

[a] Accumulation of ~20% lycopene in the petals of *og* (null mutant of *CrtL-b2* encoding LYCB) compared with that of the wild type.
[b] Prolycopene (CRTISO substrate), phytofluene, ζ-carotene and neurosporene accumulate because *CRTISO* gene expression is impaired in *tangerine*^{3183}.
[c] Prolycopene, phytoene, phytofluene, ζ-carotene, *cis*-neurosporene and di-*cis*-lycopene accumulate due to loss of CRTISO function in *tangerine*^{mic}.
[d] The elimination of *CrtR-b2* (encoding BCH2) in *wf* petals results in an 80% reduction in total carotenoid concentration, possibly due to the inability of petals to store high concentration of carotenoids other than xanthophylls, and by degradation of β-carotene which accumulates as a result of the *wf* mutation.
[e] Petals of *hp3* accumulate large amounts of zeaxanthin, minute quantities of violaxanthin and neoxanthin compared to that of wild type since a substitution mutation in ZEP, which results in the reduction activity of ZEP in *hp3*.
[f] Fully developed tepals.
[g] Fully developed lips.
[h] Fully developed stigmata.

from dried flowers and used as food colorings and flavors. Although the leaves of most plants have a similar carotenoid composition, flowers offer distinct carotenoid profiles that depend on the species and variety, in the latter case often reflecting the effects of single-gene mutations (Table 1) [20]. In this review, we provide an overview of the molecular basis of carotenoid synthesis in flower chromoplasts and discuss recent advances in metabolic engineering that have been used to alter the carotenoid content of flowers.

Overview of carotenoid synthesis and metabolism in plants

In plants, carotenoids are synthesized *de novo* from the isomeric C_5 precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) [21]. The reaction occurs in plastids and both precursors are derived primarily from the plastidial methylerythritol 4-phosphate (MEP) pathway [22,23] as shown in Fig. 1. The condensation of three IPP units and one molecule of DMAPP produces geranylgeranyl diphosphate (GGPP, C_{20}), a reaction catalyzed by the enzyme GGPP synthase (GGPPS). The first committed step in the pathway is the condensation of two molecules of GGPP into the colorless carotenoid precursor 15-*cis*-phytoene (C_{40}) by phytoene synthase (PSY) [24]. This is converted into bright red all-*trans* lycopene, the first pigmented carotenoid, in a series of four desaturation reactions catalyzed by phytoene desaturase (PDS), ζ-carotene isomerase (Z-ISO), ζ-carotene desaturase (ZDS) and carotenoid isomerase (CRTISO). The product of the first desaturation is 9,15,9′-tri-*cis*-ζ-carotene, which is isomerized by the recently characterized Z-ISO [25] and/or light to yield 9,9′-di-*cis*-ζ-carotene, the substrate for ZDS (Fig. 1) [26]. The end product of the desaturation reactions, 7,9,7′,9′-tetra-*cis*-lycopene (prolycopene), is converted to all-*trans* lycopene by CRTISO in non-green tissue, but by light in green tissue [26,27].

Lycopene represents the branch point of the carotenoid pathway because it acts as the substrate for two competing enzymes, lycopene β-cyclase (LYCB) and lycopene ε-cyclase (LYCE). Both enzymes cyclize the linear backbone to generate terminal ionone rings, but the structures of these rings are distinct. The addition of one β-ring to lycopene by LYCB generates γ-carotene, and the addition of a second β-ring to the free end by the same enzyme produces the orange pigment β-carotene, which is further converted to the yellow pigment zeaxanthin by the di-iron non-heme β-carotene hydroxylase BCH and/or the P450-type β-carotene hydroxylase CYP97A and CYP97B, in a two-step reaction via β-cryptoxanthin [28–33]. Alternatively, the addition of one ε-ring to lycopene by LYCE generates δ-carotene. This is a poor substrate for LYCE so it is unusual for the second ε-cyclization to take place, but it is a good substrate for LYCB which adds a β-ring to the free end generating the orange pigment α-carotene. In turn, α-carotene is converted into zeinoxanthin by BCH and/or CYP97A and CYP97B, and then into the yellow pigment lutein by the P450-type ε-hydroxylase, CYP97C [32–34] (Fig. 1). Whereas lutein represents the natural end point of the α-carotene branch, zeaxanthin enters the xanthophyll cycle [35] through the stepwise

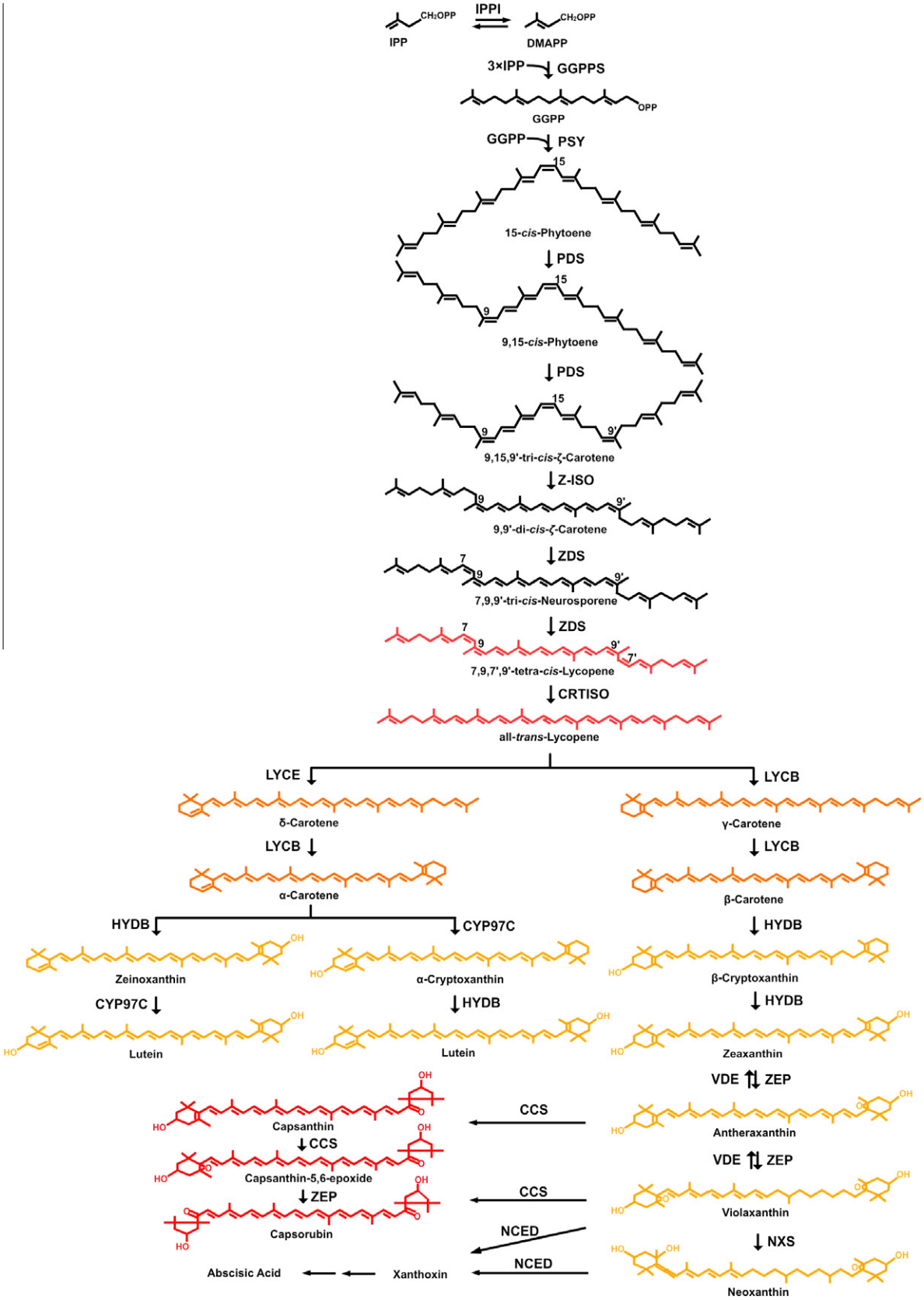

Fig. 1. Carotenoid biosynthetic pathway in higher plants. Abbreviations: IPP, isopentenyl diphosphate; IPPI, isopentenyl diphosphate isomerase; DMAPP, dimethylallyl diphosphate; GGPP, geranylgeranyl diphosphate; GGPPS, GGPP synthase; PSY, phytoene synthase; PDS, phytoene desaturase; Z-ISO, ζ-carotene isomerase; ZDS, ζ-carotene desaturase; CRTISO, carotenoid isomerase; LYCB, lycopene β-cyclase; LYCE, lycopene ε-cyclase; CCS, capsanthin–capsorubin synthase; CYP97C, carotene ε-ring hydroxylase; HYDB, β-carotene hydroxylase (BCH, CYP97A or CYP97B); ZEP, zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase; NXS, neoxanthin synthase; NCED, 9-*cis*-epoxycarotenoid dioxygenase.

activities of zeaxanthin epoxidase (ZEP) and violaxanthin de-epoxidase (VDE). The resulting yellow pigments antheraxanthin and violaxanthin are then converted to yellow neoxanthin by neoxanthin synthase (NXS) [36,37]. Finally, the enzyme 9-*cis*-epoxycarotenoid dioxygenase (NCED, VP14) cleaves the 11,12(11′,12′) double bonds of the 9-*cis* isomers violaxanthin and neoxanthin to produce xanthoxin, the precursor of abscisic acid (ABA) (Fig. 1) [38,39].

In some plants, the biosynthesis pathway extends further reflecting an ability to synthesize specialized ketocarotenoids. One such example is the red fruits of chili peppers, in which the red ketocarotenoids capsanthin and capsorubin are synthesized from antheraxanthin and violaxanthin by capsanthin–capsorubin synthase (CCS) (Fig. 1) [40]. Another example, which we consider in more detail later, is the ornamental plant Summer pheasant's-eye (*Adonis aestivalis*) whose petals synthesize the red ketocarotenoid astaxanthin (Fig. 2), which is usually found only in microbes [41].

The quantity and diversity of carotenoids varies widely in the chromoplasts of different plants even within a single species (Table 1), and this reflects the different regulatory mechanisms affecting the carotenoid biosynthesis pathway. Many flowers with white petals contain few carotenoid molecules, whereas the dark orange petals of some marigold (*Tagetes erecta*) flowers contain up to 20-fold the carotenoid content of leaves [42]. Three major mechanisms are known to affect carotenoid accumulation in chromoplasts: (1) the transcriptional or post-transcriptional regulation of genes controlling carotenoid biosynthesis; (2) the transcriptional or post-transcriptional regulation of genes controlling carotenoid degradation; and (3) the regulation of lipoprotein-sequestering structures that act as carotenoid sinks. These mechanisms are discussed in more detail below using specific case studies.

Carotenoid biosynthesis and accumulation controlled by transcription of carotenogenic genes

The transcriptional regulation of carotenogenic genes is an important mechanism that contributes to the accumulation of specific carotenoids during flower development. The next section looks at specific case studies to illustrate how transcriptional regulation affects carotenoid accumulation in the flowers of different species.

Tomato

Tomato (*Solanum lycopersicum* L.) is widely regarded as the most important model plant species for studying carotenoid accumulation because of its diverse germplasm and the different carotenoid profiles in leaves, flowers and fruits [43]. The red color of ripe fruits reflects the accumulation of lycopene, whereas the intense yellow color of the flowers reflects accumulation of the xanthophylls violaxanthin and neoxanthin [44]. As in other species, the color of tomato flowers is thought to attract pollinating insects and that of the fruit is thought to attract herbivores for seed dispersal [45].

Carotenoid biosynthesis is controlled by different regulatory mechanisms in tomato tissues containing chloroplasts and chromoplasts [46]. Underlying this fact, at least four of the carotenogenic genes in tomato come in pairs, one set expressed preferentially in leaves (containing chloroplasts) and the other in flowers and fruit (containing chromoplasts) [44]. The enzymes GGPPS, PSY and BCH are thus represented by *GGPPS1*, *PSY2* and *CRTR-b1*, which are expressed preferentially in photosynthetic tissue, and by *GGPPS2*, *PSY1* and *CRTR-b2*, which are expressed more strongly in flowers and fruits [44,47–50]. In the case of LYCB, *CRTL-b1* is expressed in leaves and at very low levels in flowers whereas *CRTL-b2* (CYC-B) is expressed strongly in flowers and at very low levels in fruits [51,52]. The carotenoid content of tomato flowers increases approximately 10-fold during development and this is coincident with strong increases in the steady state levels of *PSY1* and *PDS* mRNAs [53]. This evidence indicates that carotenoid accumulation in tomato flowers is predominantly controlled at the level of transcription. Galpaz et al. [44] concluded that there has been strong selection pressure to maintain separate chromoplast isoforms of the carotenogenic enzymes to regulate the pigmentation of flowers and fruit. The regulation of carotenoid biosynthesis

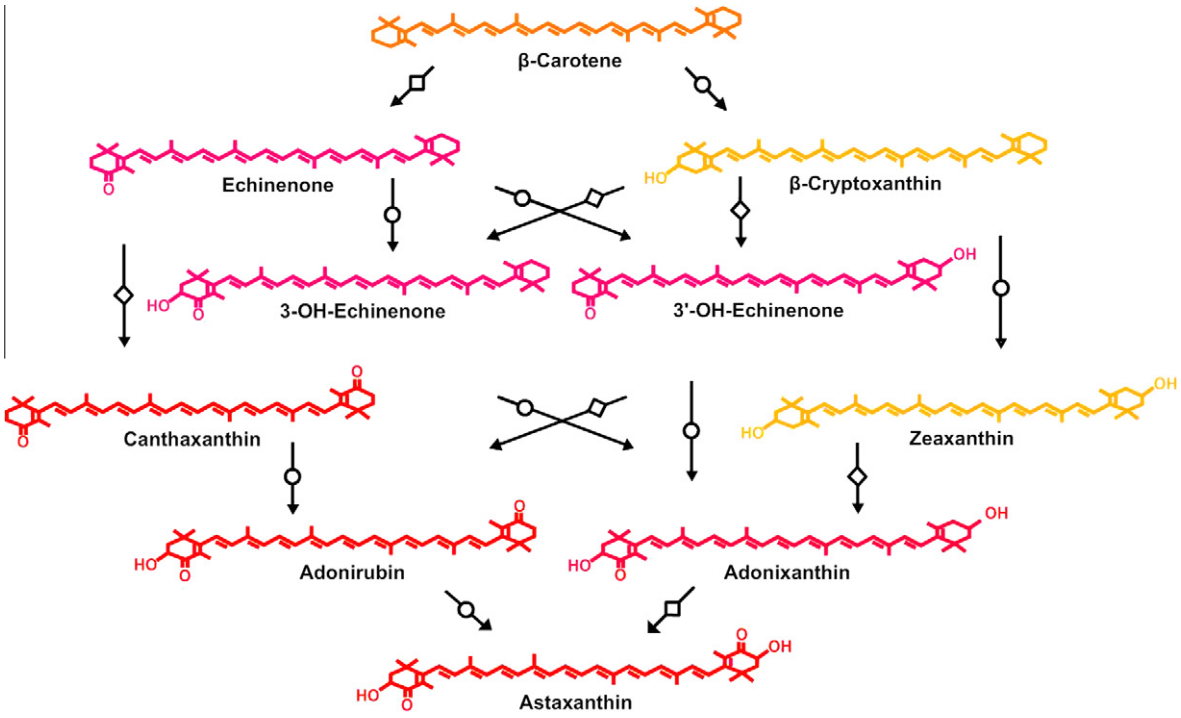

Fig. 2. Astaxanthin biosynthesis pathway from β-carotene. Arrow with inset square represents β-carotene ketolase – BKT, CRTW or CRTO. Arrow with inset circle represents β-carotene hydroxylase (HYDB) – BCH, CYP97A, CYP97B or CRTZ.

tomato flowers and fruits has been elucidated by functional analysis of the *PDS* and *CYC-B* promoters, both of which are preferentially expressed in chromoplast-containing tissues [54,55]. *PDS* is upregulated in the chromoplast-rich anthers of flowers, and in fruits at different stages of ripening [54]. The *CYC-B* promoter contains conserved *cis*-acting elements such as RAP2.2 and ERE (ethylene responsive element), which are responsible for a common regulation of carotenoid accumulation in flowers and fruits [55]. It will be interesting to characterize the signal transduction components and transcription factors that control *CYC-B* expression in order to provide further insight into carotenoid metabolism in tomato.

Marigold

Marigold flowers are a major commercial source of lutein, which is responsible for the orange to yellow hues of marigold petals. Carotenoid yields vary approximately 100-fold between white flowers and the most pigmented varieties [42], the latter of which are ground up and added to poultry feed [70] in order to improve the quality of egg yolks [71]. As in tomato, carotenoid accumulation in marigold appears to be regulated primarily at the level of transcription, but there is conflicting evidence as to which genes represent the primary bottlenecks in carotenoid biosynthesis.

Moehs et al. [42] cloned many of the relevant carotenogenic genes, i.e. those encoding PSY, PDS, LYCB, LYCE, BCH, and also the genes coding for 1-deoxy-D-xylulose 5-phosphate synthase (DXS) responsible for the first and rate-limiting step of the mevalonate-independent MEP pathway, isopentenyl diphosphate isomerase (IPP) and geranylgeranyl diphosphate (GGPP) synthase. They also cloned two genes involved in plastid division and replication (*MinD* and *FtsZ*). They surveyed mRNA levels in four marigold cultivars ranging in color from white to dark orange, with approximately equal carotenoid levels in green tissue. The clear finding was that the abundance of *PSY* mRNA correlated precisely with the carotenoid levels in flowers [42]. They also found that the mRNA for LYCE was expressed strongly whereas that for LYCB was not induced in the more pigmented flowers, indicating that the amount of lutein was determined predominantly by LYCE activity. In contrast to the above, Del Villar-Martinez et al. [72] found that the amount of *PSY* mRNA was not related to carotenoid content, e.g. there was more *PSY* mRNA in the yellow Alcosa variety than the deep orange Crackerjack variety. They also found that the mRNA for LYCB was barely detectable in the white variety Snowdrift, but was strongly expressed in Alcosa and Crackerjack [72]. It is possible that these differences are attributable to distinct regulatory mechanisms in different varieties with similar phenotypes, e.g. Moehs et al. [42] used the non-pigmented variety French Vanilla rather than the Snowdrift variety used by Del Villar-Martinez et al. [72].

Gentian

Gentian (*Gentiana lutea*) is a perennial plant with medicinal properties that has been adopted as model for carotenogenesis because of its bright yellow flowers, which are rich in β-carotene and xanthophylls [59]. Genes encoding all the major carotenogenic enzymes have been cloned from a yellow petal cDNA library, and the corresponding enzyme activities (i.e. GGPPS, PSY, PDS, ZDS, LYCB, LYCE, BCH and ZEP) have been verified by complementation in *Escherichia coli* [59,73].

Flower development involves the upregulation of carotenoid synthesis and a switch in emphasis from the ε-branch to the β-branch, with a corresponding shift in carotenoid profile from lutein to zeaxanthin, antheraxanthin and neoxanthin [59,73]. Underlying this, the *PSY* and *ZDS* mRNAs are strongly upregulated (6- to 7-fold) during development, coincident with a moderate upregulation of *LYCB*, *BCH* and *ZEP* (2-fold) and a fall in the level of *LYCE* mRNA to approximately 50% of its original level [59]. Interestingly, the level of *GGPPS* mRNA decreases over the same period, which suggests carotenoid synthesis utilizes an exclusive pool of GGPP that accumulates in the early stages of development [73].

Morning glory

The genus *Ipomoea* (morning glory) is renowned for its diverse flower colors including many species with carotenoid-rich yellow and orange petals. But the Japanese morning glory (*Ipomoea nil*) lacks a yellow cultivar and does not accumulate carotenoids. A recent study by Yamamizo et al. [60] compared Japanese morning glory with two yellow-flowered species in an attempt to determine the molecular basis of this phenomenon. During early flower development, all the species accumulated lutein, violaxanthin and β-carotene in the petals, the same carotenoids that accumulate in leaves. However, the yellow flowers switched to chromoplast-type carotenoid accumulation later in development (β-cryptoxanthin, zeaxanthin and β-carotene) and also contained esterified β-cryptoxanthin and zeaxanthin, as also seen in marigold and *Eustoma* [42,74]. The white flowers, however, did not accumulate any chromoplast-type carotenoids [60].

Genes encoding the enzymes involved in isoprenoid and carotenoid biosynthesis (*IPPI*, *GGPPS*, *PSY*, *PDS*, *ZDS*, *CRTISO*, *LYCB*, *LYCE* and *BCH*) and carotenoid cleavage (*ccd1* and *ccd4*) were isolated from immature petals [60] and it was noted that many of the genes were expressed at lower levels in the white petals compared to the yellow ones, particularly *BCH*. However, the expression of *CCD1* and *CCD4* did not correlate with the carotenoid level in petals [60]. These results suggest that the white color in petals is due to the lack of the ability to synthesize chromoplast-type carotenoids because of the transcriptional down-regulation of carotenogenic genes [60].

Lilies

The Asiatic hybrid lily (*Lilium* spp.) is another commercially valuable ornamental plant with flower colors ranging from red, orange and yellow (carotenoids), through pink (anthocyanins) to white. Carotenoid profiling has shown that most of the carotenoids in yellow petals are antheraxanthin, (9*Z*)-violaxanthin, *cis*-lutein and violaxanthin, whereas red petals are unusual in that they accumulate capsanthin (Table 1) [61,75]. The increase in carotenoid content in yellow cultivars was mirrored by the induction of *PSY*, *PDS*, *ZDS*, *CRTISO* and *BCH* mRNA expression, while *LYCB* expression remained constant and *LYCE* expression diminished, suggesting that transcriptional regulation is the predominant control mechanism [61]. However, the steady state levels of *PSY*, *PDS*, *ZDS*, *CRTISO* and *BCH* were similar in red and white petals, indicating another mechanism was probably responsible for the lack of carotenoids in white petals, perhaps the expression of CCDs although this remains to be demonstrated [61].

Carotenoid biosynthesis and accumulation controlled by carotenogenic enzyme levels

Post-transcriptional regulation of carotenogenic enzyme activity also plays a role in controlling carotenoid biosynthesis and accumulation in flowers. The yellow flowers of the wild daffodil *Narcissus pseudonarcissus* reflect the accumulation of large amounts of lutein and lower amounts of β-carotene and its derivatives [58]. Isolated chromoplasts can be used to synthesize β-carotene when fed with IPP, but upon disintegration they also yield significant amounts of α-carotene and ζ-carotene [76]. As is the case in tomato, there is evidence that carotenogenic genes in daffodil are transcriptionally upregulated during flower development [77] but several studies

have also shown that the corresponding enzymes are regulated post-transcriptionally by compartmentalization.

Daffodil flower development is characterized by the production of chromoplasts from chloroplast-like precursors, a process that involves the disassembly of thylakoids and the photosynthetic apparatus, the enhanced synthesis of membrane lipids and enhanced carotenoid biosynthesis [78]. Carotenoid accumulation is strongly induced during daffodil flower development and steady-state *PSY* transcript levels remain constant or even decrease from stage 1 to stage 4 [81]. In contrast to *PSY* expression there is an increase in PSY protein levels during flower stages 1 and 2 [81]. The level of PDS protein also increases to some extent during flower development [80]. However, the increases in PSY and PDS expression do not appear to be related to functionality, as the proteins detected include one soluble, complexed and inactive form, and a second, membrane-bound and active form [80,81]. The carotenogenic enzymes, which are all encoded by nuclear genes, are imported into the chromoplast and assembled into oligomeric complexes. The plastid molecular chaperones Cpn60 and Hsp70 are strongly upregulated during this process to facilitate the correct folding of the imported enzymes and the assembly of the multi-protein complex [79]. The carotenogenic complex is functional only when it is associated with the chromoplast membrane, but Al-Babili et al. [80] reported that the enzymes PSY and PDS could either be found associated with the membrane or as free, soluble proteins in the chromoplast milieu. The soluble forms of both PSY and PDS were non-functional, but could become activated by membrane association [80,81]. The PDS enzyme was localized correctly with respect to the membrane-bound lipophilic substrate and lipophilic co-factors such as quinones [80]. It has been speculated that the posttranslational mechanisms, a redox mechanism, in which PDS in conjunction with a membrane-localized redox chain employing quinones as intermediate electron acceptors [82] and oxygen as the final electron acceptor [76], may contribute to color formation, given the very low expression of PDS in carotenoid-overaccumulating chromoplasts [80].

Regulating carotenoid degradation

The abundance of CCDs

The enzyme 9-*cis*-epoxycarotenoid dioxygenase (NCED) has been isolated from many different plants, the first being the corn *viviparous14* (*vp14*) gene product [6,83]. It is not yet clear whether this enzyme significantly affects carotenoid metabolism in flowers. For example, Zhu et al. [84] recently cloned two NCED cDNAs from gentian, one of which (*GlNCED1*) was expressed in flowers (strongly in the stamens, less strongly in the petals) but its impact on carotenoid accumulation is unknown. There are nine NCED homologs in Arabidopsis, with complex spatiotemporal expression patterns [85], and *AtNCED6* is the most closely related to *GlNCED1*. The first Arabidopsis NCED to be discovered (AtNCED1 or AtCCD1) has many orthologs including genes in petunia [11], tomato [12], crocus [9,86], grape [87] and rose [88]. The expression of *CCD1* is temporally associated with the emission of apocarotenoid volatiles in petunia corolla [11], grape berries [89] and tomato fruits [12]. The highest *CCD1* mRNA levels were detected in flower tissue, specifically in corollas, and *CCD1* regulation appears to fit with similar oscillations in the expression of *PDS* and *ZDS* indicating a circadian rhythm [11]. The examples below discuss specific case studies where CCDs are known to have a significant impact on carotenoid levels in flowers.

Regulating carotenoid degradation in crocus

Crocus (*Crocus sativus)* is a triploid sterile plant characterized by its long red stigmata, which when desiccated give rise to the expensive spice saffron [89]. The intense color of saffron is caused by apocarotenoids such as *cis*- and *trans*-crocins (crocetin digentiobiose ester), picrocrocin and its degradation product safranal, which represents 70% of total volatiles and gives saffron its distinct aroma [90]. These saffron apocarotenoids are generated by the cleavage and glucosylation of zeaxanthin [91]. The ability to synthesize these compounds is not common in plants. Crocin and picrocrocin are only found in crocus stigmata, in fruits of the genus *Gardenia* [92], in flowers of the genus *Buddleja* [93], and in two further species, *Jacquinia angustifolia* [94] and *Coleus forskolii* [95].

As is the case for other flowers, the accumulation of carotenoids in crocus appears to be controlled by the transcriptional regulation of corresponding carotenogenic genes during flower development, in this case the genes encoding PSY, LYCB and BCH [67]. Two different lycopene cyclase genes have been identified (*LYCB1* and *LYCB2a*), and the expression of *LYCB2a* in different crocus species indicates that transcriptional regulation of this gene affects both the carotenoid and apocarotenoid content in the stigmata [96]. The absence of *LYCB2a* orthologs in plants that do not accumulate high levels of carotenoids suggests that *LYCB2a* is a *LYCB1* paralog that has acquired a new expression pattern by sub-functionalization, allowing the tight control of carotenoids in very specific tissues. A positive correlation between zeaxanthin accumulation and *BCH* expression in fully developed stigmata suggests that zeaxanthin production is regulated at the transcriptional level, and that BCH enzyme activity could be the rate-limiting step in the formation of saffron apocarotenoids in the stigma [67].

The predominant apocarotenoid product in developing crocus stigmata is crocetin, although this is converted into its glucoside derivatives and picrocrocin as each stigma matures [97]. The volatiles profile undergoes significant changes during development, and in red stigmata the predominance of β-cyclocitral suggests that both β-carotene and zeaxanthin are involved in the synthesis of crocetin. As the stigmata mature, hydroxy-β-ionone and β-ionone are produced along with very low quantities of safranal [97]. These important volatiles are derived from carotenoids by the action of CCDs [98].

The crocus zeaxanthin 7,8(7′,8′) cleavage dioxygenase gene (*ZCD*) encodes a chromoplast enzyme that catalyses the synthesis of crocetin dialdehyde (C_{20}) and hydroxy-β-cyclocitral (C_{10}) from zeaxanthin [9]. More recently, Rubio et al. [86] identified four stigma-specific CCD genes (*CsCCD1a*, *CsCCD1b*, *CsCCD4a* and *CsCCD4b*), which belong to two different families, *CCD1* and *CCD4*. They have the same enzymatic activity even though they are localized in different compartments. It has been suggested that each enzyme class is responsible for carotenoid metabolism in a different subcellular compartment, during both normal development and in response to stress [86].

The subsequent step in apocarotenoid synthesis involves glucosylation of the crocetin and picrocrocetin cleavage products. Two crocetin GTase activities (UDP-glucose:crocetin 8,8′-O-glucosyltransferase and UDP-glucose:crocetinglucosylester 6′-O-glucosyltransferase) are thought to be present in saffron [99]. A glycosyltransferase gene (*UGTCs2*) encoding an enzyme that adds glucose to crocetin has also been isolated [100].

Regulating carotenoid degradation in chrysanthemum

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is a commercially valuable ornamental plant with bright yellow petals, mainly reflecting the accumulation of lutein [101]. Flower development involves a switch from lutein, β-carotene and violaxanthin synthesis to the predominant synthesis of lutein, which appears to involve the transcriptional upregulation of *LYCE* [102]. In contrast to the flowers, *LYCB* expression is higher in leaves, favoring the accumulation of β-carotene and its derivatives.

Interestingly, the yellow color of chrysanthemum is a recessive trait, with the dominant white trait apparently resulting from an allele that blocks carotenoid biosynthesis [103,104]. In this dominant white variety, the early accumulation of lutein, β-carotene and violaxanthin proceeds as normal, but the later accumulation of lutein is inhibited, resulting in the loss of pigmentation. In order to identify genes responsible for the loss of carotenoid accumulation in white flowers, Ohmiya et al. [62] cloned cDNAs that were differentially expressed in the yellow and white varieties, leading to the isolation of *CmCCD4a*, encoding a CCD homolog. The gene was petal-specific and was strongly expressed in white flowers but weakly expressed in yellow ones. RNAi constructs targeting the endogenous *CmCCD4a* mRNA were expressed in the white variety, resulting in a strong yellow color (Fig. 3a). This experiment confirmed that white chrysanthemums do synthesize carotenoids, but they are immediately degraded into colorless apocarotenoids.

'Jimba' is the most popular white-flowered chrysanthemum cultivar in Japan. A yellow-flowered cultivar with the same growth properties as 'Jimba' will benefit growers because both forms could be produced under the same conditions. Ohmiya et al. [105] recently succeeded in altering the petal color of 'Jimba' from white to yellow by introducing two separate *CmCCD4a* RNAi constructs. The transgenic 'Jimba' line with the deepest yellow flowers contained 102 μg/g FW carotenoids in the petals, and the expression level of *CmCCD4a* was 0.4% of the wild type level. Although the transformed plants were significantly smaller than the wild type, flower size was unchanged [105].

Extended ketocarotenoid synthesis in Adonis

Adonis (*A. aestivalis*), also known as Summer pheasant's-eye, has long petals which are orange for most of their length but have a deep red basal patch which gives the flower a striking, ocellar appearance. The color is caused by carotenoid accumulation, but unlike all other known flowering plants the pigments that accumulate are ketocarotenoids (Fig. 2) [68,69], predominantly astaxanthin (3,3′-dihydroxy-4,4′-diketo-β,β-carotene) with lesser amounts of 3-hydroxyechinenone (3-hydroxy-4-keto-β,β-carotene), adonirubin (3-hydroxy-4,4′-diketo-β,β-carotene) and adonixanthin (3,3′-dihydroxy-4-keto-β,β-carotene) [41]. The ability of *Adonis* flowers to synthesize and accumulate astaxanthin in such large quantities is therefore unique in the plant kingdom [106,107].

The ketocarotenoids are derived from zeaxanthin (3,3′-dihydroxy-β,β-carotene) which is present in the green tissues of most higher plants. Yu et al. [108] isolated the *CRTH1* (*BCH1*) gene, whose product was able to catalyze the formation of zeaxanthin and its intermediate precursor β-cryptoxanthin from β-carotene in functional assays carried out in *E. coli*. This gene is highly expressed in petals, roots and stems, with relatively low expression in leaves and developing seeds. The formation of astaxanthin from zeaxanthin requires only that a carbonyl group be introduced at the number 4 carbon of each β-ring, but it is thought this addition must occur prior to hydroxylation, i.e. β-carotene must be the substrate and echinenone (4-keto-β,β-carotene) and canthaxanthin (4,4′-diketo-β,β-carotene) would be the immediate products [109,110]. The genes for two β-carotene 4-ketolases required for this enzymatic step have been identified in *Adonis* and are named *Adketo1* and *Adketo2*. Sequence analysis suggested they were homologous to plant-type β-carotene 3-hydroxylases, but despite this similarity the enzymes demonstrated neither 4-ketolase nor 3-hydroxylase activity when presented with β-carotene as the substrate in *E. coli* [41]. Further investigation showed that they add carbonyl groups at the appropriate site via an indirect route that involves keto-enol tautomerization, i.e. desaturation at the 3,4 position and hydroxylation of the number 4 carbon.

Coupling carotenogenesis to chromoplast development and carotenoid storage

As stated above, Moehs et al. [42] cloned many of the carotenogenic genes from marigold to investigate their impact on flower color, and also found that the *MinD* and *FtsZ* genes were upregu-

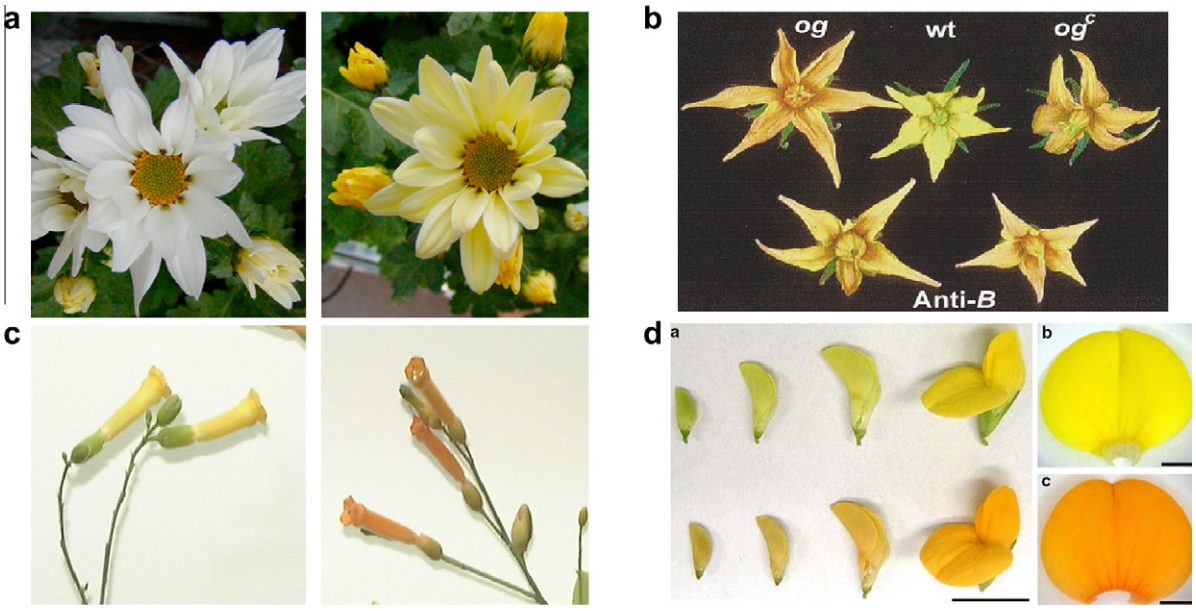

Fig. 3. (a) Suppression of endogenous carotenoid cleavage dioxygenase 4a (*CmCCD4a*) by RNAi in chrysanthemum, showing fully open wild type white flowers (left) and yellow flowers resulting from the inhibition of *CmCCD4a* (right) (from [62] with permission from Plant Physiology). (b) Wild type (wt) tomato flowers compared with mutants *old-gold* (*og*) and *old-gold crimson* (og^c) and with transgenic plant expressing antisense *CRTL-b2* (LYCB2) mRNA (from [52], Copyright 2000 National Academy of Sciences, USA, used with permission). (c) *Nicotiana glauca* flowers, wild type on left, transgenic plant expressing *Synechocystis* sp. PCC 6803 β-carotene ketolase (*CRTO*) on right (photo provided by C. Zhu and G. Sandmann). (d) The upper row shows a wild type *Lotus japonicus* flower, four developmental stages with sepals removed (bar = 2 mm) and a fully opened petal (bar = 10 mm). The bottom row shows the same stages for a transgenic flower expressing the *Agrobacterium aurantiacum* β-carotene ketolase gene (*CRTW*) (from [65] with permission from Plant Cell Reports).

lated in the most pigmented varieties during development while remaining at basal levels in paler varieties, suggesting a correlation between carotenogenesis and plastid replication. A full-length *FtsZ* cDNA has also been isolated from gentian petals but in this case the opposite expression profile was observed, with expression gradually declining during development [111]. There is only limited plastid replication during petal development in marigold [72] and gentian [111] because most chromoplasts arise from proplastids or pre-existing fully developed chloroplasts [112], so a link between plastid replication and carotenogenesis remains unlikely.

Although there is no definitive link between plastid replication and carotenoid accumulation, it is very likely that accumulation goes hand in hand with chromoplast differentiation. In support of this, a novel dominant mutation (*Orange*, *Or*) has been identified recently in cauliflower which causes the curd (edible inflorescence) to develop with a deep orange color [113]. The corresponding gene encodes a DnaJ homolog, not a carotenogenic enzyme, so it appears to be involved in protein folding and assembly. Further investigation has shown that the cellular phenotype of the mutation involves the arrest of plastid division and the differentiation of plastids into carotenoid-accumulating chromoplasts that act as a sink for carotenoid molecules [113]. Expression of an *Or* transgene in potato tubers did not affect the expression of endogenous carotenogenic genes, but nevertheless resulted in a 6-fold increase in carotenoid levels by increasing the capacity for carotenoid storage [114].

In chloroplasts, almost all carotenoid molecules are associated with functional light-harvesting complexes [115]. In contrast, chromoplasts have evolved as specialized structures whose major function is the storage and accumulation of carotenoids and other pigments [116,117]. Storage in certain chromoplast-types is achieved by carotenoid esterification which allows their association with specialized proteins known as fibrillins or plastid lipid associated proteins. Hydroxylation is an important process for the esterification of carotenoids, and the upregulation of BCH activity has been shown to accelerate carotenoid accumulation in many flowers, including morning glory [60], tomato [44] and lily [61].

The fibrillin-carotenoid conjugates are assembled into crystalline, membranous, fibrillar or tubular structures [116], or they may be packaged into plastoglobuli (lipid bodies), which consist of a layer of polar lipids and proteins covering the surface, with non-polar components (15–25% carotenoids) in the center. The fibrillar and tubular chromoplasts consist of equal parts proteins and lipids, and the non-polar compounds are mainly esterified xanthophylls [116]. The membranous chromoplasts are less well characterized. The crystalline chromoplasts contain pure carotenoid crystals [116].

Recent research has provided new information about specific fibrillins, which are also known as chromoplast proteins. CHRC (chromoplast protein C) is an abundant 35-kDa carotenoid-associated protein found in the corolla of cucumber flowers; it is not present in chloroplasts, and is therefore undetectable in cucumber leaves and fruits. Its abundance increases during flower development, peaking in mature flowers before rapidly disappearing [118]. Using an *in vitro* flower bud culture system that mimics flower development, Vainstein et al. [119] and Vishnevetsky et al. [120] showed that the accumulation of carotenoids, *CHRC* mRNA and CHRC protein was markedly enhanced when gibberellin (GA_3) was added to the medium, but was inhibited by ABA and ethylene. A similar developmental profile and hormone response was demonstrated for the 14-kDa CHRD (chromoplast protein D) [121]. *CHRC* promoter analysis revealed a GA response element whose deletion prevented GA_3 induction, and also led to the identification of MYBYS, a flower-specific MYB-like transcriptional activator with a similar expression profile to *CHRC* and *CHRD*. MYBYS activates the *CHRC* gene in flowers accumulating carotenoids and flavonoids [122,123]. Inhibiting carotenoid biosynthesis affects *CHRC* and *CHRD* expression at the post-transcriptional and translational levels, indicating some form of feedback circuit dependent on carotenoid abundance [117]. The inhibition of *CHRC* by RNAi in transgenic tomato plants reduced total carotenoid levels by 30%, indicating that carotenoid accumulation also depends on the presence of this protein [122,123]. A flower-specific *CHRC* ortholog was recently isolated from the hybrid orchid, *Oncidium* Gower Ramsey [124].

Carotenoid metabolic engineering to introduce new flower colors

Modulating the existing carotenoid pathway

Flowers evolved to attract pollinators but they are also aesthetically pleasing to humans, and breeding flowers to generate visually appealing color varieties has been an important aspect of floriculture for hundreds of years [20]. One of the obstacles faced by flower breeders is that, for many species, there are colors that cannot be accessed by conventional breeding because the pigments cannot be synthesized using available genotypes, e.g. vivid red petunias and delphiniums, and the elusive blue rose.

Metabolic engineering can overcome these limitations by allowing genes for the appropriate metabolic enzymes to be transferred to flowering plants under the control of a flower-specific promoter, thus altering the color phenotype of the engineered flower without affecting other traits. Until recently, research has focused on flavonoids rather than carotenoids, but the information now available about carotenogenesis in flowers means that carotenoid engineering is becoming a realistic prospect.

In the first report of carotenoid engineering in flowers, Bird et al. [125] generated transgenic tomato plants with an antisense TOM5 (*PSY1*) gene under the control of the constitutive CaMV 35S promoter. As anticipated, this reduced the total carotenoid content by 80%, resulting in yellow ripe fruits and pale yellow flowers. However, leaf carotenoid levels were not affected because (as stated earlier) *PSY1* is not involved in carotenoid synthesis in green tissues, a function fulfilled by *PSY2* [48,126]. Since LYCB enzyme activity in tomato is also represented by different genes for photosynthetic and non-photosynthetic tissues, an antisense fragment of the *CRTL-b2* gene expressed under the control of the CaMV 35S promoter had a similarly negligible impact on vegetative growth while causing marked changes in the flower phenotype [52]. Transgenic flowers displayed a tawny-orange color (Fig. 3b), typical of the *old-gold* mutant (a natural loss-of-function mutation affecting *CRTL-b2*; see Table 1) resulting from the accumulation of up to 12% lycopene in flowers. The fruit color was not affected significantly because the fruits normally accumulate large amounts of lycopene.

Extending the pathway to include ketocarotenoids

Most plants do not produce ketocarotenoids such as astaxanthin and canthaxanthin, so metabolic engineering that aims to achieve ketocarotenoid synthesis in flowers can introduce vivid red and scarlet colors that would otherwise be unachievable. As stated earlier, astaxanthin is derived from β-carotene by 3-hydroxylation and 4-ketolation at both ionone end groups, reactions catalyzed by β-carotene hydroxylase (HYDB) and β-carotene ketolase, respectively (Fig. 2). The hydroxylation reaction is widespread in higher plants but ketolation only occurs naturally in *Adonis* petals. Even so, the substrates for ketolation are abundant in both green and non-green tissue, such that ketocarotenoid production can be achieved simply by the introduction of a single heterologous enzyme.

When tobacco was transformed with the β-carotene ketolase (*BKT*) gene from the alga *Haematococcus pluvilis* under the control of the tomato *PDS* promoter [54], high levels of astaxanthin and other ketocarotenoids were produced in the nectary, the only tobacco tissue that contains chromoplasts [127]. These results were improved still further when Ralley et al. [128] simultaneously expressed *Paracoccus* sp. β-carotene ketolase (*CRTW*) and β-carotene hydroxylase (*CRTZ*) genes, increasing the ketocarotenoid content of the nectary tissue 9-fold. Constitutive expression of a *Synechocystis* sp. PCC 6803 ketolase gene (*CRTO*) shifted the pathway towards the formation of 3′-hydroxyechinenone, 3-hydroxyechinenone, 4-ketozeaxanthin (adonixanthin) esters, 4-ketolutein and 4′-ketolutein esters, representing more than 50% of the total carotenoid content [129].

Unlike cultivated tobacco, the wild tobacco species *Nicotiana glauca* has carotenoid-pigmented petals, sepals, pistils, ovaries and nectary tissues. The constitutive expression of a *Nostoc* sp. 73102 ketolase gene (*CRTW*) resulted in the accumulation of 4-ketozeaxanthin (adonixanthin) as the only detectable ketocarotenoid [129], whereas the expression of a *Synechocystis* sp. PCC 6803 *CRTO* gene resulted in a wider spectrum of ketocarotenoids comprising 4′-ketolutein, echinenone, 3′-hydroxyechinenone and 4-ketozeaxanthin [64]. The total ketocarotenoid content in petals was more than three times greater in the latter plants, due in part to the unexpected upregulation of the entire carotenoid pathway in leaves and even more so in flowers – consequently the color of flower petals changed from the original light yellow to deep orange (Fig. 3c). Likewise, the petals of *Lotus japonicus* plants overexpressing an *Agrobacterium aurantiacum CRTW* gene changed from yellow to deep yellow or orange (Fig. 3d) [65].

Outlook and conclusions

Over the last decade we have learned a great deal about the accumulation of carotenoid pigments in flowers, the genes and enzymes responsible for carotenoid synthesis and degradation, the cellular mechanisms responsible for carotenoid storage, and how all these processes are regulated during development and in response to external stimuli. The availability of efficient genetic transformation methods for commercially important cut flower varieties and flower-specific promoters [54,55,124] means that a full armory of molecular biology techniques can now be brought to bear, including the overexpression of heterologous enzymes and the abolition of endogenous enzyme activity using antisense suppression and RNAi, to modulate carotenoid metabolism in flowers.

The future therefore looks colorful, as we embark on an era in which it will become possible not only to dissect carotenoid metabolism using molecular biology techniques but also to engineer flowers expressing pigments ranging from pale yellow through to deep scarlet by modulating carotenoid metabolism. It may also be possible to use flowers as a source of nutritionally important carotenoid molecules, including those rarely produced in significant quantities by flowering plants.

Acknowledgments

We would like to thank Prof. G. Sandmann for permission to use an unpublished photograph in Fig. 3. This research was supported by the Ministry of Science and Innovation, Spain (BFU2007-61413 and BIO2007-30738-E) and European Research Council Advanced Grant (BIOFORCE) to P.C., C.Z. and L.S. are supported in part by grants from the National Natural Science Foundation of China (Nos. 30870222 and 30370123). S.N. and G.F. are supported by Ministry of Science and Innovation (Spain) Ph.D. fellowships. C.B. is supported by a fellowship from the University of Lleida, Spain.

References

[1] G. Britton, S. Liaaen-Jensen, H. Pfander, Carotenoids Handbook, Birkhäuser, Basel, Switzerland, 2004.
[2] H.A. Frank, R.J. Cogdell, Photochem. Photobiol. 63 (1996) 257–264.
[3] B. Demmig-Adams, W.W. Adams III, Science 298 (2002) 2149–2153.
[4] G.E. Bartley, P.A. Scolnik, Plant Cell 7 (1995) 1027–1038.
[5] J. Von Lintig, K. Vogt, J. Nutr. 134 (2004) 251S–256S.
[6] S.H. Schwartz, B.C. Tan, D.A. Gage, J.A. Zeevaart, D.R. McCarty, Science 276 (1997) 1872–1874.
[7] V. Gomez-Roldan, S. Fermas, P.B. Brewer, V. Puech-Pagès, E.A. Dun, J.P. Pillot, F. Letisse, R. Matusova, S. Danoun, J.C. Portais, H. Bouwmeester, G. Becard, C.A. Beveridge, C. Rameau, S.F. Rochange, Nature 455 (2008) 189–194.
[8] M. Umehara, A. Hanada, S. Yoshida, K. Akiyama, T. Arite, N. Takeda-Kamiya, H. Magome, Y. Kamiya, K. Shirasu, K. Yoneyama, J. Kyozuka, S. Yamaguchi, Nature 455 (2008) 195–200.
[9] F. Bouvier, C. Suire, J. Mutterer, B. Camara, Plant Cell 15 (2003) 47–62.
[10] F. Bouvier, O. Dogbo, B. Camara, Science 300 (2003) 2089–2091.
[11] A.J. Simkin, B.A. Underwood, M. Auldridge, H.M. Loucas, K. Shibuya, E. Schmelz, D.G. Clark, H.J. Klee, Plant Physiol. 136 (2004) 3504–3514.
[12] A.J. Simkin, S.H. Schwartz, M. Auldridge, M.G. Taylor, H.J. Klee, Plant J. 40 (2004) 882–892.
[13] G. Farré, G. Sanahuja, S. Naqvi, C. Bai, T. Capell, C. Zhu, P. Christou, Plant Sci. 179 (2010) 28–48.
[14] C. Zhu, S. Naqvi, S. Gomez-Galera, A.M. Pelacho, T. Capell, P. Christou, Trends Plant Sci. 12 (2007) 548–555.
[15] C. Zhu, S. Naqvi, T. Capell, P. Christou, Arch. Biochem. Biophys. 483 (2009) 182–190.
[16] G. Sandmann, S. Römer, P.D. Fraser, Metab. Eng. 8 (2006) 291–302.
[17] S. Lu, L. Li, J. Integr. Plant Biol. 50 (2008) 778–785.
[18] P.D. Fraser, E.M. Enfissi, P.M. Bramley, Arch. Biochem. Biophys. 483 (2009) 196–204.
[19] C.I. Cazzonelli, B.J. Pogson, Trends Plant Sci. 15 (2010) 266–274.
[20] Y. Tanaka, N. Sasaki, A. Ohmiya, Plant J. 54 (2008) 733–749.
[21] J. Chappell, Annu. Rev. Plant Physiol. Plant Mol. Biol. 46 (1995) 521–547.
[22] H.K. Lichtenthaler, Annu. Rev. Plant Physiol. Plant Mol. Biol. 50 (1999) 47–65.
[23] W. Eisenreich, F. Rohdich, A. Bacher, Trends Plant Sci. 6 (2001) 78–84.
[24] N. Misawa, M.R. Truesdale, G. Sandmann, P.D. Fraser, C. Bird, W. Schuch, P.M. Bramley, J. Biochem. 116 (1994) 980–985.
[25] Y. Chen, F. Li, E.T. Wurtzel, Plant Physiol. 153 (2010) 66–79.
[26] J. Breitenbach, G. Sandmann, Planta 220 (2005) 785–793.
[27] T. Isaacson, I. Ohad, P. Beyer, J. Hirschberg, Plant Physiol. 136 (2004) 4246–4255.
[28] Z. Sun, E. Gantt, F.X. Cunningham Jr., J. Biol. Chem. 271 (1996) 24349–24352.
[29] F. Bouvier, Y. Keller, A. d'Harlingue, B. Camara, Biochim. Biophys. Acta 1391 (1998) 320–328.
[30] L. Tian, V. Musetti, J. Kim, M. Magallanes-Lundback, D. DellaPenna, Plant Cell 15 (2003) 1320–1332.
[31] R.F. Quinlan, T.T. Jaradat, E.T. Wurtzel, Arch. Biochem. Biophys. 458 (2007) 146–157.
[32] J. Kim, D. DellaPenna, Proc. Natl Acad. Sci. USA 103 (2006) 3474–3479.
[33] J.E. Kim, K.M. Cheng, N.E. Craft, B. Hamberger, C.J. Douglas, Phytochemistry 71 (2010) 168–178.
[34] L. Tian, D. DellaPenna, Plant Mol. Biol. 47 (2001) 379–388.
[35] B. Demmig-Adams, A.M. Gilmore, W.W. Adams III, FASEB J. 10 (1996) 403–412.
[36] F. Bouvier, A. d'harlingue, R.A. Backhaus, M.H. Kumagai, B. Camara, Eur. J. Biochem. 267 (2000) 6346–6352.
[37] S. Al-Babili, P. Hugueney, M. Schledz, R. Welsch, H. Frohnmeyer, O. Laule, P. Beyer, FEBS Lett. 485 (2000) 168–172.
[38] M. Seo, T. Koshiba, Trends Plant Sci. 7 (2002) 41–48.
[39] S.H. Schwartz, X. Qin, J.A. Zeevaart, Plant Physiol. 131 (2003) 1591–1601.
[40] F. Bouvier, P. Hugueney, A. d'Harlingue, M. Kuntz, B. Camara, Plant J. 6 (1994) 45–54.
[41] F.X. Cunningham Jr., E. Gantt, Plant J. 41 (2005) 478–492.
[42] C.P. Moehs, L. Tian, K.W. Osteryoung, D. DellaPenna, Plant Mol. Biol. 45 (2001) 281–293.
[43] P.M. Bramley, J. Exp. Bot. 53 (2002) 2107–2113.
[44] N. Galpaz, G. Ronen, Z. Khalfa, D. Zamir, J. Hirschberg, Plant Cell 18 (2006) 1947–1960.
[45] P.G. Kevan, in: C.E. Jones, R.J. Little (Eds.), Handbook of Experimental Pollination Biology, Van Nostrand Reinhold, New York, 1983, pp. 3–30.
[46] M. Thelander, J.O. Narita, W. Gruissem, Curr. Top. Plant Biochem. Physiol. 5 (1986) 128–141.
[47] G.E. Bartley, P.V. Viitanen, K.O. Bacot, P.A. Scolnik, J. Biol. Chem. 267 (1992) 5036–5039.
[48] G.E. Bartley, P.A. Scolnik, J. Biol. Chem. 268 (1993) 25718–25721.
[49] P.D. Fraser, M.R. Truesdale, C.R. Bird, W. Schuch, P.M. Bramley, Plant Physiol. 105 (1994) 405–413.
[50] P.D. Fraser, J.W. Kiano, M.R. Truesdale, W. Schuch, P.M. Bramley, Plant Mol. Biol. 40 (1999) 687–698.

[51] I. Pecker, R. Gabbay, F.X. Cunningham Jr., J. Hirschberg, Plant Mol. Biol. 30 (1996) 807–819.
[52] G. Ronen, L. Carmel-Goren, D. Zamir, J. Hirschberg, Proc. Natl Acad. Sci. USA 97 (2000) 11102–11107.
[53] G. Giuliano, G.E. Bartley, P.A. Scolnik, Plant Cell 5 (1993) 379–387.
[54] V. Corona, B. Aracri, G. Kosturkova, G.E. Bartley, L. Pitto, L. Giorgetti, P.A. Scolnik, G. Giuliano, Plant J. 9 (1996) 505–512.
[55] M. Dalal, V. Chinnusamy, K.C. Bansal, BMC Plant Biol. 10 (2010) 61.
[56] T. Isaacson, G. Ronen, D. Zamir, J. Hirschberg, Plant Cell 14 (2002) 333–342.
[57] N. Galpaz, Q. Wang, N. Menda, D. Zamir, J. Hirschberg, Plant J. 53 (2008) 717–730.
[58] L.R.G. Valadon, R.S. Mummery, Biochem. J. 106 (1968) 479.
[59] C. Zhu, S. Yamamura, M. Nishihara, H. Koiwa, G. Sandmann, Biochem. Biophys. Acta 1625 (2003) 305–308.
[60] C. Yamamizo, S. Kishimoto, A. Ohmiya, J. Exp. Bot. 61 (2010) 709–719.
[61] M. Yamagishi, S. Kishimoto, M. Nakayama, Plant Breed. 128 (2009) 172–177.
[62] A. Ohmiya, S. Kishimoto, R. Aida, S. Yoshioka, K. Sumitomo, Plant Physiol. 142 (2006) 1193–1201.
[63] G. Neamtu, Z. Nagy, C. Bilaus, T. László, in: Institutum Agronomicum "Dr. Petru Groza" Cluj-Napoca (Romania) Notulae Botanicle Horti Agrobotanici Cluj-Napoca, VIII, 1975/76, pp. 53–58.
[64] C. Zhu, T. Gerjets, G. Sandmann, Transgenic Res. 16 (2007) 813–821.
[65] S. Suzuki, M. Nishihara, T. Nakatsuka, N. Misawa, I. Ogiwara, S. Yamamura, Plant Cell Rep. 26 (2007) 951–959.
[66] A.D. Hieber, R.G. Mudalige-Jayawickrama, A.R. Kuehnle, Planta 223 (2006) 521–531.
[67] R. Castillo, J.A. Fernandez, L. Gomez-Gomez, Plant Physiol. 139 (2005) 674–689.
[68] K. Egger, Phytochemistry 4 (1965) 609–618.
[69] G. Neamtu, V. Tamas, C. Bodea, Rev. Roum. Biochem. 3 (1966) 305–310.
[70] F. Delgado-Vargas, O. Paredes-Lopez, J. Agri. Food Chem. 45 (1997) 1097–1102.
[71] M.I. Scott, I. Ascarelli, G. Olson, Poult. Sci. 47 (1968) 863.
[72] A.A. Del Villar-Martinez, P.A. Garcia-Saucedo, A. Carabez-Trejo, A. Cruz-Hernandez, O. Paredes-Lopez, J. Plant Physiol. 162 (2005) 1046–1056.
[73] C. Zhu, S. Yamamura, H. Koiwa, M. Nishihara, G. Sandmann, Plant Mol. Biol. 48 (2002) 277–285.
[74] M. Nakayama, M. Miyasaka, T. Maoka, M. Yagi, N. Fukuta, J. Jpn. Soc. Hort. Sci. 75 (2006) 161–165.
[75] J. Deli, P. Molnár, Z. Matus, G. Tóth, A. Steck, H. Pfander, Chromatographia 48 (1998) 27–31.
[76] P. Beyer, M. Mayer, H. Kleinig, Eur. J. Biochem. 184 (1989) 141–150.
[77] V. Nievelstein, J. Vandekerchove, M.H. Tadros, J.V. Lintig, W. Nitschke, P. Beyer, Eur. J. Biochem. 233 (1995) 864–872.
[78] B. Liedvogel, H. Kleinig, Planta 133 (1997) 249–253.
[79] M. Bonk, M. Tadros, J. Vandekerckhove, S. Al-Babili, P. Beyer, Plant Physiol. 111 (1996) 931–939.
[80] S. Al-Babili, J. von Lintig, H. Haubruck, P. Beyer, Plant J. 9 (1996) 601–612.
[81] M. Schledz, S. Al-Babili, J. Von Lintig, H. Haubruck, S. Rabbani, H. Kleinig, P. Beyer, Plant J. 10 (1996) 781–792.
[82] M.P. Mayer, P. Beyer, H. Kleinig, Eur. J. Biochem. 191 (1990) 359–363.
[83] B.C. Tan, S.H. Schwartz, J.A. Zeevaart, D.R. McCarty, Proc. Natl Acad. Sci. USA 94 (1997) 12235–12240.
[84] C. Zhu, K. Kauder, S. Römer, G. Sandmann, J. Plant Physiol. 164 (2007) 195–204.
[85] B.C. Tan, L.M. Joseph, W.T. Deng, L.L. Liu, Q.B. Li, K. Cline, D.R. McCarty, Plant J. 35 (2003) 44–56.
[86] A. Rubio, J.L. Rambla, M. Santaella, M.D. Gómez, D. Orzaez, A. Granell, L. Gómez-Gómez, J. Biol. Chem. 283 (2008) 24816–24825.
[87] S. Mathieu, N. Terrier, J. Procureur, F. Bigey, Z. Gunata, J. Exp. Bot. 56 (2005) 2721–2731.
[88] F.C. Huang, G. Horváth, P. Molnár, E. Turcsi, J. Deli, J. Schrader, G. Sandmann, H. Schmidt, W. Schwab, Phytochemistry 70 (2009) 457–464.
[89] G.M. Holloway, J.L. Gainer, J. Appl. Physiol. 65 (1988) 683–686.
[90] P.A. Tarantilis, M. Polissiou, M. Manfait, J. Chromatogr. 664 (1994) 55–61.
[91] H. Pfander, H. Schurtenberger, Phytochemistry 21 (1982) 1039–1042.
[92] S. Pfiter, P. Meyer, A. Steck, H. Pfander, J. Agric. Food Chem. 44 (1996) 2612–2615.
[93] Y.H. Liao, P.J. Houghton, J.R. Hoult, J. Nat. Prod. 62 (1999) 1241–1245.
[94] C.H. Eugster, H. Hurlimann, H.J. Leuenberger, Helv. Chim. Acta 52 (1969) 89–90.
[95] J.S. Tandon, S.B. Katti, P. Rüedi, C.H. Eugster, Helv. Chim. Acta 274 (1979) 2706–2707.
[96] O. Ahrazem, A. Rubio-Moraga, R.C. López, L. Gómez-Gómez, J. Exp. Bot. 61 (2010) 105–119.
[97] A. Rubio, J.L. Rambla, O. Ahrazem, A. Granell, L. Gómez-Gómez, Phytochemistry 70 (2009) 1009–1016.
[98] P. Winterhalter, R.L. Rouseff, Carotenoid-derived Aroma Compounds, American Chemical Society, Washington, DC, 2002.
[99] C. Dufresne, F. Cormier, S. Dorion, Planta Med. 63 (1997) 150–153.
[100] A. Rubio, P.F. Nohales, J.A. Pérez, L. Gómez-Gómez, Planta 219 (2004) 955–966.
[101] S. Kishimoto, T. Maoka, M. Nakayama, A. Ohmiya, Phytochemistry 65 (2004) 2781–2787.
[102] S. Kishimoto, A. Ohmiya, Physiol. Plant 128 (2006) 436–447.
[103] F.A. Langton, Euphytica 29 (1980) 807–812.
[104] K. Hattori, Jpn. J. Breed. 41 (1991) 1–9.
[105] A. Ohmiya, K. Sumitomo, R. Aida, J. Jpn. Soc. Hort. Sci. 78 (2009) 450–455.
[106] B. Czeczuga, Syst. Ecol. 15 (1987) 303–306.
[107] T.W. Goodwin, second ed., The Biochemistry of the Carotenoids, vol. 1, Chapman & Hall, London, 1980. pp. 377.
[108] B. Yu, D.J. Lydiate, U.A. Schäfer, A. Hannoufa, Planta 226 (2007) 181–192.
[109] P.D. Fraser, H. Shimada, N. Misawa, Eur. J. Biochem. 252 (1998) 229–236.
[110] T. Lotan, J. Hirschberg, FEBS Lett. 364 (1995) 125–128.
[111] Y. He, C. Zhu, D. Wang, D. Kong, J. Sun, Prog. Nat. Sci. 12 (2002) 592–597.
[112] M.R. Marano, E.C. Serra, E.G. Orellano, N. Carrillo, Plant Sci. 94 (1993) 1–17.
[113] S. Lu, J. Van Eck, X. Zhou, A.B. Lopez, D.M. O'Halloran, K.M. Cosman, B.J. Conlin, D.J. Paolillo, D.F. Garvin, J. Vrebalov, L.V. Kochian, H. Küpper, E.D. Earle, J. Cao, L. Li, Plant Cell 18 (2006) 3594–3605.
[114] A.B. Lopez, J. Van Eck, B.J. Conlin, D.J. Paolillo, J. O'Neill, L. Li, J. Exp. Bot. 59 (2008) 213–223.
[115] W. Kühlbrandt, D.N. Wang, Y. Fujiyoshi, Nature 367 (1994) 614–621.
[116] M. Vishnevetsky, M. Ovadis, A. Vainstein, Trends Plant Sci. 4 (1999) 232–235.
[117] M. Vishnevetsky, M. Ovadis, A. Zuker, A. Vainstein, Plant J. 20 (1999) 423–431.
[118] I. Smirra, A.H. Halevy, A. Vainstein, Plant Physiol. 102 (1993) 491–496.
[119] A. Vainstein, A.H. Halevy, I. Smirra, M. Vishnevetsky, Plant Physiol. 104 (1994) 321–326.
[120] M. Vishnevetsky, M. Ovadis, H. Itzhaki, A. Vainstein, J. Biol. Chem. 272 (1997) 24747–24750.
[121] Y. Libal-Weksler, M. Vishnevetsky, M. Ovadis, A. Vainstein, Plant Physiol. 113 (1997) 59–63.
[122] Y. Leitner-Dagan, M. Ovadis, E. Shklarman, Y. Elad, D. Rav David, A. Vainstein, Plant Physiol. 142 (2006) 233–244.
[123] Y. Leitner-Dagan, M. Ovadis, A. Zuker, E. Shklarman, I. Ohad, T. Tzfira, A. Vainstein, Planta 225 (2006) 89–102.
[124] C.Y. Chiou, K. Wu, W. Yeh, Biotechnol. Lett. 30 (2008) 1861–1866.
[125] C.R. Bird, J.A. Ray, J.D. Fletcher, J.M. Boniwell, A.S. Bird, C. Teulieres, I. Blain, P.M. Bramley, W. Schuch, Bio/Technology 9 (1991) 635–639.
[126] P. Bramley, C. Teulieres, I. Blain, C. Bird, W. Schuch, Plant J. 2 (1992) 343–349.
[127] V. Mann, M. Harker, I. Pecker, J. Hirschberg, Nat. Biotechnol. 18 (2000) 888–892.
[128] L. Ralley, E.M. Enfissi, N. Misawa, W. Schuch, P.M. Bramley, P.D. Fraser, Plant J. 39 (2004) 477–486.
[129] T. Gerjets, M. Sandmann, C. Zhu, G. Sandmann, Biotechnol. J. 2 (2007) 1263–1269.