Plant Science 208 (2013) 58–63

Contents lists available at SciVerse ScienceDirect

Plant Science

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

Review

The carotenoid biosynthetic pathway: Thinking in all dimensions

Maria Shumskaya [a], Eleanore T. Wurtzel [a,b,*]

[a] Department of Biological Sciences, Lehman College, The City University of New York (CUNY), Bronx, NY 10468, United States
[b] The Graduate School and University Center-CUNY, 365 Fifth Ave., New York, NY 10016-4309, United States

ARTICLE INFO

Article history:
Received 31 January 2013
Received in revised form 15 March 2013
Accepted 19 March 2013
Available online 27 March 2013

Keywords:
Carotenoid biosynthesis
Plants
Plastids
Plant biochemistry
Metabolic engineering
Biosynthetic pathways

ABSTRACT

The carotenoid biosynthetic pathway serves manifold roles in plants related to photosynthesis, photoprotection, development, stress hormones, and various volatiles and signaling apocarotenoids. The pathway also produces compounds that impact human nutrition and metabolic products that contribute to fragrance and flavor of food and non-food crops. It is no surprise that the pathway has been a target of metabolic engineering, most prominently in the case of Golden Rice. The future success and predictability of metabolic engineering of carotenoids rests in the ability to target carotenoids for specific physiological purposes as well as to simultaneously modify carotenoids along with other desired traits. Here, we ask whether predictive metabolic engineering of the carotenoid pathway is indeed possible. Despite a long history of research on the pathway, at this point in time we can only describe the pathway as a parts list and have almost no knowledge of the location of the complete pathway, how it is assembled, and whether there exists any trafficking of the enzymes or the carotenoids themselves. We discuss the current state of knowledge regarding the "complete" pathway and make the argument that predictive metabolic engineering of the carotenoid pathway (and other pathways) will require investigation of the three dimensional state of the pathway as it may exist in plastids of different ultrastructures. Along with this message we point out the need to develop new types of visualization tools and resources that better reflect the dynamic nature of biosynthetic pathways.

© 2013 Elsevier Ireland Ltd. All rights reserved.

Contents

1. Introduction 58
2. Carotenoid biosynthetic pathway enzymes and model systems 59
3. Phytoene synthase: at the head of an enzyme metabolon 59
4. Localization of the carotenoid biosynthetic pathway – where is it? 59
5. Pathway metabolon: new visualization tools are required 60
6. Conclusion and future prospects 62
Acknowledgments 62
References 62

1. Introduction

What level of understanding is required to predictably manipulate a plant biosynthetic pathway, or controllably breed plant varieties to achieve a desired chemical profile? Recent research in metabolic engineering demonstrates feasibility for altering plant secondary metabolism, but reveals the inadequacies of having an incomplete picture of metabolic regulation. The functional alteration of biosynthetic pathways has the potential to improve the nutritional or survival characteristics of plants, which is of significant importance in addressing food security in the face of climate change. Yet, the more that is explored in control of plant metabolism, the more questions that arise. Metabolon formation may be influenced by unique combinations of enzymes for which interaction may alter activity and/or localization [1,2]. The ability to conduct predictable metabolic engineering is limited by understanding of the dynamic landscape of metabolons, for which research would benefit from improved three-dimensional modeling and visualization of plant metabolism.

* Corresponding author at: Department of Biological Sciences, Lehman College, The City University of New York (CUNY), Bronx, NY 10468, United States. Tel.: +1 718 960 8643; fax: +1 718 960 8236.
E-mail address: wurtzel@lehman.cuny.edu (E.T. Wurtzel).

0168-9452/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.plantsci.2013.03.012

An example of how fundamental research on a pathway improved traditional breeding is that of plant carotenoid biosynthesis. Carotenoids are essential hydrophobic plant compounds usually of yellow, orange or red color, and play important roles in photosynthesis and photoprotection, as well as in production of phytohormones [3]. Certain carotenoids, such as α- and β-carotene, are precursors for vitamin A, good antioxidants and necessary for human health. Carotenoid derivatives participate in signaling in plant developmental programs and responses to abiotic and biotic stress, and mediate response to the presence of beneficial and non-beneficial organisms. Carotenoids and their derivatives impart colors and fragrance to flowers and other plant parts. Volatile apocarotenoids mediate plant–animal interactions (e.g. attracting insect pollinators) and enhance the flavor characteristics of food crops. Nutritional value of certain food crops and stress tolerance of any plant are directly related to carotenoid content.

2. Carotenoid biosynthetic pathway enzymes and model systems

In plants, enzymatic formation of carotenoids occurs on plastid membranes and is mediated by nuclear-encoded enzymes [3]. The pathway per se starts with condensation of geranylgeranylpyrophosphate (GGPP), a precursor from the upstream methylerythritol (MEP) pathway, to produce the C_{40} 15-cis-phytoene. This step is catalyzed by phytoene synthase (PSY), a rate-limiting enzyme for the pathway. Phytoene undergoes consecutive modifications such as desaturation and isomerization to form lycopene, which is later cyclized to carotenes. Carotenes are oxygenated to xanthophylls. A generalized pathway of plant carotenoid biosynthesis can be found through online web portals such as PlantCyc (http://pmn.plantcyc.org/PLANT/NEW-IMAGE?type=PATHWAY&object=CAROTENOID-PWY).

Extensive study of carotenoid biosynthesis in *Zea mays* (maize), a major food crop, has benefitted from the diverse germplasm collection, phenotypic mutants, genetic and physical map, and quantitative trait loci (QTL) affecting carotenoid biosynthesis (as reviewed in [4]). Other important models for understanding carotenogenesis include Arabidopsis (as reviewed in [5]), tomato, pepper, and daffodil (as examples: [6–8]). However, there is much that we do not understand about the regulation of carotenoid biosynthesis in the context of the manifold roles of carotenoids in plants. Most importantly, the location of the “fully assembled” biosynthetic pathway is unknown. Therefore, how can we really achieve predictable metabolic engineering of this important pathway?

3. Phytoene synthase: at the head of an enzyme metabolon

PSY is considered a key enzyme in carotenoid biosynthesis. It has been extensively studied and engineered in numerous plants where modifying carotenoid content is desired [9,10]. In maize, nutritionally important carotenoids are accumulated in edible seed endosperm. Maize PSY is encoded by three paralogous genes. In certain genotypes containing an insertion in the *PSY1* gene promoter [11,12], *PSY1* expression is anomalously induced in endosperm and thus conditions the characteristic yellow endosperm color. *PSY1* is also expressed in leaves along with *PSY2*. The expression of *PSY3* is limited to roots and induced by stress conditions [13–15]. Discovery of multiple *PSY* genes has been extended to many plant families. Based on studies in maize, it appears that *PSY* gene paralogs show tissue-specificity of expression that might impart the ability to control carotenogenesis independently of photosynthesis or in response to certain stresses.

Seeds of another grass staple, rice (*Oryza sativa*), lack expression of endosperm-specific *PSY*. In an attempt to add bioavailable carotenoids into the rice seed endosperm, a functional carotenoid biosynthetic pathway was introduced into rice by transforming plants with endosperm-expressed maize *PSY1* and *CrtI* (a multifunctional bacterial enzyme able to convert phytoene to lycopene) [16]. The result of such modification is Golden Rice 2 accumulating high amounts of β-carotene [17]. It took many years to successfully create Golden Rice 2. The first attempt utilized PSY from daffodil, which did not give a sufficient amount of carotenoids in rice seeds [16]. Through extensive trials with phytoene synthases from different plant species, it was discovered that in plant calli, PSY1 from maize was most effective to increase carotenoid production [17], and maize and rice PSY1 enzymes were most successful in promoting endosperm carotenoid accumulation in rice plant transformants. The reason of such a preference for PSYs is not clear.

What was so special about the maize PSY1 enzyme that made it optimal for carotenoid production in certain tissues? As recently discovered [18], the maize PSY1 isozyme differs from others in localization to a distinct plastid compartment. While the majority of phytoene synthases, such as maize PSY2 and PSY3, rice and *Arabidopsis* PSYs, are found to localize to plastoglobuli, maize PSY1 localization depends on its allelic variant. Three maize PSY1 variants differ from each other by only 1–2 amino acids at positions 168 and 257. Amino acid #257 may be any of three different residues: proline, serine or threonine. Threonine_{257} in combination with asparagine_{168} is the variant encoded by *PSY1* in all maize varieties with yellow endosperm. This was likely the allele that gained the promoter insertion mutation that activated endosperm expression and therefore became a selected trait for maize breeding. The threonine_{257} version of PSY appears as a soluble protein in plastids [18], and interestingly, this was the particular variant effectively expressed in Golden Rice 2 endosperm (while other PSY1 allelic variants were not tested). The localization of the proline_{257} variant of PSY1 in maize plastids is different from the threonine_{257} version. When expressed in protoplasts, the proline_{257} variant of PSY is accompanied with formation of fibrils, a sign of high concentration of carotenoids [18]. Similar fibrils are observed inside chromoplasts of fruits such as tomato, loquat and papaya [19,20], or carrot roots [21,22], where carotenoids are naturally accumulated and stored. Would a proline_{257} variant increase carotenoid production in rice, if used instead of maize PSY1 with threonine_{257}?

PSY1 variants, forming different types of plastoglobuli – globular or fibrillar – might direct carotenoid biosynthesis to different suborganellar locations. As a consequence, perhaps PSY isozyme choice might affect carotenoid storage and bioavailability which is related to storage location [21,22].

4. Localization of the carotenoid biosynthetic pathway – where is it?

Early biochemical studies made clear that carotenoid enzyme location is critical for activity [23,24]. Unexpected results in metabolic engineering indicate the need for better understanding of carotenoid pathway regulation at many levels [25]. Carotenoids are localized on membranes of plastids, and plastids are known to be the site of carotenoid biosynthesis. However, depending on the tissue and plastid type, plastids are architecturally unique, being dynamic organelles that may go through a developmental program altering the ultrastructure, chemistry, and structural aspects of the carotenoid biosynthesis machinery. Carotenoids on the envelope may have a different destiny (e.g. conversion to apocarotenoids involved in mediating signaling) as compared to carotenoids that function as structural components for photosynthesis or

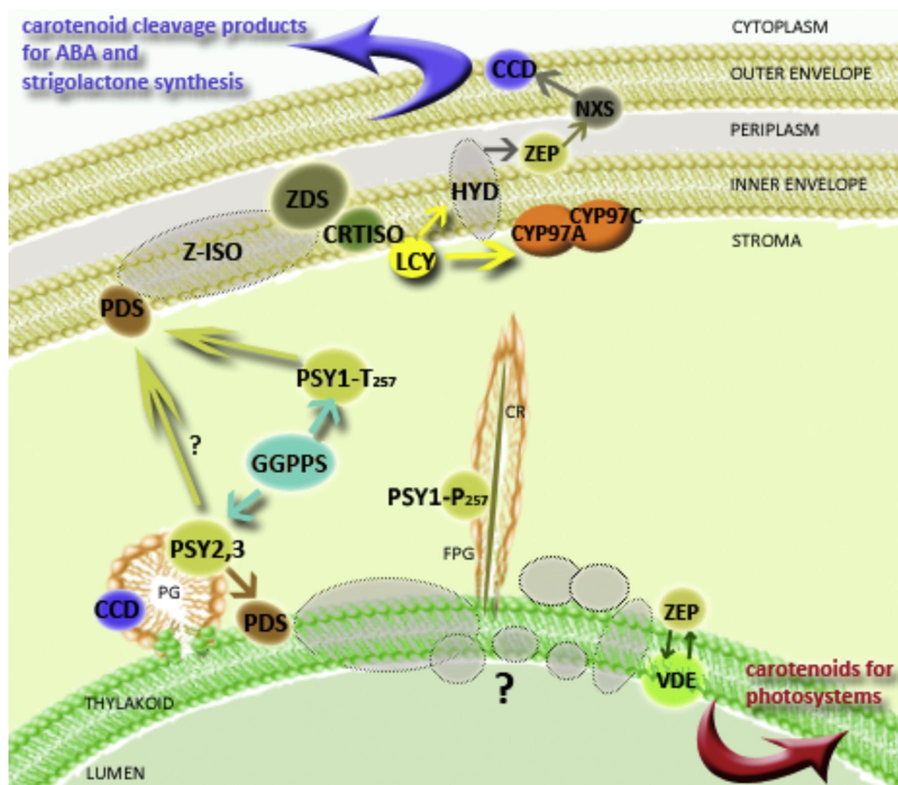

Fig. 1. Hypothetical topology of carotenoid biosynthetic pathway in maize chloroplasts. Gray shadows – proteins were not localized experimentally. PG globular plastoglobuli, FPG – fibrillar plastoglobuli, CR – hypothetical carotenoid crystals. For the legend, see Table 1.

photoprotection on thylakoid membranes. Enhancement of carotenoids in edible tissues for human benefit, for example, will require understanding of carotenogenesis in the context of diverse biological roles and locations of carotenoids.

The control of location-specific carotenogenesis is poorly understood. Though all of the pathway enzymes are known, there is neither insight into the three-dimensional structural features of the "pathway" nor whether such putative metabolon organization consists of one or more sets of complexes with additional yet to be described transporters. The carotenoid biosynthetic enzymes are likely to be organized in protein complexes in plants as proposed by Cunningham and Gantt [26]. This hypothesis is supported by limited detection of pathway intermediates [2,27], evidence of in vivo channeling [28,29], and observation of high molecular weight complexes containing biosynthetic enzymes [23,30–34]. Recently, it was also shown that hydroxylases CYP97A and CYP97C form a complex for synergistic conversion of α-carotene to lutein [2].

The question remains, what is the site of carotenoid biosynthesis? Most studies to date feature proteomic approaches that inform location of individual enzymes but lack information on context within multi-enzyme complexes. Mass spectrometry studies of the chromoplast proteome found individual carotenoid enzymes mostly in plastoglobuli [35] or in membrane fractions [36]. In Arabidopsis photosynthetic chloroplasts, many, but not all, carotenoid biosynthetic enzymes are found in envelope membranes, while only a few carotenoid enzymes are identified in thylakoids [5,37]. The plastoglobuli attached to thylakoids of chloroplasts contain PSY (with the exception of the "yellow endosperm" allele for maize PSY1), which would suggest that PSY is physically separated from the remainder of the pathway [18]. So, where is the pathway localized and how are components recruited to assemble a functionally complete metabolon, or does such a structure actually exist (Fig. 1)? What about chromoplasts – carotenoid-accumulating plastids, where carotenoid enzymes are distributed differently: for example, enzymes localized to the envelope in chloroplasts are found in plastoglobuli of chromoplasts (Table 1)? The envelope localization of carotenoid enzymes can be explained by the need to supply carotenoids for abscisic acid (ABA) synthesis, for which later steps occur outside of the plastid [38]. How do carotenoids get to thylakoids, where they serve photosynthetic and photoprotection needs, if only a few carotenoid biosynthetic enzymes are found there? Are these missing enzymes actually there but undetectable in proteomic screens due to their low abundance, or are carotenoids transferred from envelope membranes via vesicular transport (or other yet to be discovered transport system)? What happens to carotenoid enzymes when chloroplasts change into chromoplasts? Are chromoplast carotenoids synthesized *de novo* and then somehow sequestered in plastoglobuli? What directs enzymes to plastoglobuli rather than to envelopes or thylakoids? After years of thorough study of the carotenoid pathway genes and enzymes, the enigma of pathway assembly and the question of whether trafficking of carotenoids or enzymes exists, await further exploration.

5. Pathway metabolon: new visualization tools are required

To enable metabolic engineering strategies to predictably enhance carotenogenesis, we will also need to evolve the supporting pathway databases and network tools that exist today to better represent the complexity of the carotenoid pathway, as well as all other biosynthetic pathways.

Genome scale reconstruction of metabolism is available for Arabidopsis and includes transcript and other data to facilitate predictive modeling of metabolism in specific tissues [39]. Such a network adds to the limited number of plant genome scale models of metabolism [40]. Even though cell-type specific data may be included, these models do not fully integrate, within a single framework, repositories of protein interactions, suborganellar

Table 1
The localization of carotenoid biosynthetic enzymes in plastids, based on experimental evidence.

	Protein	Localization in chloroplasts	Localization in chromoplasts	Localization in amyloplasts
Carotenoid precursors	GGPPS (all isozymes)	*A. thaliana* – stroma[a]	*Capsicum annuum*, *Citrullus lanatus* subsp. *vulgaris*, *Daucus carota* subsp. *sativus*, *Brassica oleracea* L. var. *botrytis*, *Carica papaya* – stroma[i]	n/d
	PSY (isozyme not specified)	*A. thaliana* – PG[b]	*C. lanatus*, *D. carota* – membrane[i]	–
	PSY1-T_{257}	*Z. mays* – stroma, peripheral thylakoid membrane bound[b]	n/d	*Z. mays* – envelope[c]
	PSY1-P_{257}	*Z. mays* – FPG[b]; *O. sativa* – PG[b]	*Solanum lycopersicum* – membrane[i]	n/d
	PSY2	*Z. mays*, *O. sativa* – PG[b]	*C. annuum* – stroma/membrane[i]; *S. lycopersicum* – membrane[i]	n/d
	PSY3	*Z. mays*, *O. sativa* – PG[b]	n/d	n/d
	PDS	*A. thaliana* – 84% envelope, 16% thylakoid[a,d]	*C. annuum, D. carota, B. oleracea*–membrane[i]; *C. lanatus, S. lycopersicum* – stroma/membrane[i]	*Z. mays* – stroma[e]
	Z-ISO	n/d	n/d	n/d
Carotenoid biosynthesis	ZDS	*A. thaliana* – 90% envelope, 10% stroma[a]	*C. annuum*, – PG,[f] membrane[i]; *C. lanatus, S. lycopersicum, D. carota, B. oleracea, C. papaya* – membrane[i]	n/d
	CRTISO	*A. thaliana* – envelope[a]	*C. annuum* – stroma/membrane[i]; *S. lycopersicum*, *C. papaya* – membrane[i]	n/d
	LCYE	n/d	n/d	n/d
	LCYB	*A. thaliana* – envelope[a]	*C. annuum* – PG,[f] membrane[i]; *C. lanatus* – membrane[i]	n/d
	HYD	*Z. mays* - membrane[j]	*C. annuum* – PG,[f] membrane[i]	n/d
	CYP97A	*A. thaliana* – envelope[a]; *O. sativa* - membrane[j]	n/d	n/d
	CYP97C	*A. thaliana* – envelope[a]; *O. sativa* - membrane[j]	n/d	n/d
	ZEP	*A. thaliana* – 75% envelope, 14% stroma, 11% thylakoid[a]	*C. annuum* – membrane[i]	n/d
	VDE	*A. thaliana* – thylakoid,[a] thylakoid lumen[g]	n/d	n/d
	NXS	*A. thaliana* – envelope[a]	n/d	n/d
Carotenoid degradation	CCD/NCED (all isozymes)	*A. thaliana* – envelope,[a] PG[f,h]	*C. annuum* – stroma/membrane[i]; *C. lanatus, S. lycopersicum, D. carota, B. oleracea* – membrane[i]	n/d

PG, plastoglobuli; FPG, fibrillar plastoglobuli; n/d, not detected; GGPPS, geranylgeranyl pyrophosphate synthase; PSY, phytoene synthase; PDS, phytoene desaturase; Z-ISO, 15-*cis*-ζ-carotene isomerase; ZDS, ζ-carotene desaturase; CRTISO, carotenoids isomerase; LCYE, lycopene ε-cyclase; LCYB, lycopene β-cyclase; HYD, nonheme diiron carotene hydroxylase; CYP97A, P450 carotene hydroxylase 97A; CYP97C, P450 carotene hydroxylase 97C; ZEP, zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase; NXS, neoxanthin synthase; CCD/NCED, carotenoid cleavage enzymes.

[a] Ref. [37].
[b] Ref. [18].
[c] Ref. [15].
[d] Ref. [49].
[e] Ref. [50].
[f] Ref. [35].
[g] Ref. [51].
[h] Ref. [52].
[i] Ref. [36].
[j] Ref. [2].

localization, and diurnal variation, all of which may influence pathway flux [41]. Future tools will need to integrate pathway flux data and genetic variation. Such variation can affect enzyme location (for example: [18]), enzyme interactions with other components, and enzyme activity, including both primary and side reactions associated with metabolite repair (reviewed in [42]). Current network models generally do not capture the structural intricacies (such as involvement of transporters) needed to connect biosynthetic pathways that operate across different cell types (e.g. indole alkaloid biosynthesis [43]) or are distributed among several subcellular (e.g. fatty acid biosynthesis [44]) or suborganellar locations (e.g. chlorophyll [45] and carotenoid biosynthesis [18]). The challenge to link multi-dimension data related to metabolism across multiple cell types has been addressed in modeling neurotransmission in the human brain [46]. Additional details on the structural aspects of multi-component assembled pathways

in plants will require greater input from the structural biology community to provide three-dimensional visualization of assembled pathways. Future enhancement of multi-dimension plant metabolism models would be accelerated by building interfaces to facilitate community input of new information.

So where should we look to design such supporting tools to drive the development of metabolic engineering? Perhaps, it is time for plant biochemistry to move from 2D to 3D by enlisting the collaboration of 3D graphic modelers. Experimentation with 3D visualization of plant metabolism is in its infancy as seen in the use of virtual reality simulations that allow a user to "virtually" explore a metabolism network [47] or interactive visualization tools that aim to provide insight into connections across different layers of data [48]. As cinema evolved to 3D graphics, predictive metabolic engineering will require improved tools that reflect the three-dimensional structures that likely exist, taking into account the location, interplay between pathway enzymes, and dynamic changes over time and space, instead of the current network tools and databases that are static and incomplete. Development and use of such 3D models would advance future efforts in plant metabolic engineering.

6. Conclusion and future prospects

Predictable control of the carotenoid biosynthetic pathway will require more than just introducing genes encoding the biosynthetic enzymes. Carotenoids in different plastid suborganellar locations have different functions. Do we want to affect ABA and strigolactone production from envelope carotenoids, and/or regulate stress response or plant development? Do we want to improve regeneration of carotenoids in thylakoids lost due to photooxidation in photosynthetic tissues? Or, do we want to modify the amount of nutritionally important carotenoids in endosperm?

In the future, we will develop better insight into the assembly of the "parts" of the carotenoid biosynthetic pathway that will create the "whole" pathway in a known suborganellar location. We will be able to describe how carotenoids are produced to address unique physiological needs that arise on short and long time scales within specific suborganellar regions as can be found in the range of plastid ultrastructures.

Acknowledgments

The carotenoid research in the Wurtzel lab has been funded by the US National Institutes of Health, Rockefeller Foundation International Rice Biotechnology Program, McKnight Foundation, American Cancer Society, US National Science Foundation, United States Department of Agriculture, PSC-CUNY, and New York State. We thank Jesús Beltrán for insightful discussions.

References

[1] J.-E. Bassard, et al., Protein–protein and protein–membrane associations in the lignin pathway, Plant Cell 24 (2012) 4465–4482.
[2] R.F. Quinlan, et al., Synergistic interactions between carotene ring hydroxylases drive lutein formation in plant carotenoid biosynthesis, Plant Physiol. 160 (2012) 204–214.
[3] A.J. Cuttriss, C.I. Cazzonelli, E.T. Wurtzel, B.J. Pogson, Carotenoids, in: F. Rébeillé, R. Douce (Eds.), Biosynthesis of Vitamins in Plants, Elsevier, Amsterdam, The Netherlands, 2011, pp. 1–36.
[4] E.T. Wurtzel, A. Cuttriss, R. Vallabhaneni, Maize provitamin A carotenoids, current resources and future metabolic engineering challenges, Front. Plant Sci. 3 (2012) 29.
[5] M. Águila Ruiz-Sola, M. Rodríguez-Concepción, Carotenoid biosynthesis in Arabidopsis: a colorful pathway, Arabidop. Book 10 (2012) e0158.
[6] D.E. Kachanovsky, S. Filler, T. Isaacson, J. Hirschberg, Epistasis in tomato color mutations involves regulation of phytoene synthase 1 expression by cis-carotenoids, Proc. Natl. Acad. Sci. U.S.A. 109 (2012) 19021–19026.
[7] P. Beyer, M. Mayer, K. Kleinig, Molecular oxygen and the state of geometric isomerism of intermediates are essential in the carotene desaturation and cyclization reactions in daffodil chromoplasts, Eur. J. Biochem. 184 (1989) 141–150.
[8] O. Dogbo, A. Laferrière, A. D'Harlingue, B. Camara, Carotenoid biosynthesis: isolation and characterization of a bifunctional enzyme catalyzing the synthesis of phytoene, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 7054–7058.
[9] G. Farré, C. Bai, R.M. Twyman, T. Capell, P. Christou, C. Zhu, Nutritious crops producing multiple carotenoids – a metabolic balancing act, Trends Plant Sci. 16 (2011) 532–540.
[10] G. Giuliano, R. Tavazza, G. Diretto, P. Beyer, M.A. Taylor, Metabolic engineering of carotenoid biosynthesis in plants, Trends Biotechnol. 26 (2008) 139–145.
[11] K.A. Palaisa, M. Morgante, M. Williams, A. Rafalski, Contrasting effects of selection on sequence diversity and linkage disequilibrium at two phytoene synthase loci, Plant Cell 15 (2003) 1795–1806.
[12] F. Li, O. Tzfadia, E.T. Wurtzel, The *Phytoene Synthase* gene family in the grasses: subfunctionalization provides tissue-specific control of carotenogenesis, Plant Signal. Behav. 4 (2009) 208–211.
[13] C.E. Gallagher, P.D. Matthews, F. Li, E.T. Wurtzel, Gene duplication in the carotenoid biosynthetic pathway preceded evolution of the grasses (Poaceae), Plant Physiol. 135 (2004) 1776–1783.
[14] F. Li, R. Vallabhaneni, E.T. Wurtzel, *PSY3*, a new member of the phytoene synthase gene family conserved in the Poaceae and regulator of abiotic-stress-induced root carotenogenesis, Plant Physiol. 146 (2008) 1333–1345.
[15] F. Li, R. Vallabhaneni, J. Yu, T. Rocheford, E.T. Wurtzel, The maize phytoene synthase gene family: overlapping roles for carotenogenesis in endosperm, photomorphogenesis, and thermal stress-tolerance, Plant Physiol. 147 (2008) 1334–1346.
[16] X. Ye, et al., Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm, Science 287 (2000) 303–305.
[17] J.A. Paine, et al., Improving the nutritional value of Golden Rice through increased pro-vitamin A content, Nat. Biotechnol. 23 (2005) 482–487.
[18] M. Shumskaya, L.M.T. Bradbury, R.R. Monaco, E.T. Wurtzel, Plastid localization of the key carotenoid enzyme phytoene synthase is altered by isozyme, allelic variation, and activity, Plant Cell 24 (2012) 3725–3741.
[19] R.M. Schweiggert, C.B. Steingass, A. Heller, P. Esquivel, R. Carle, Characterization of chromoplasts and carotenoids of red- and yellow-fleshed papaya (*Carica papaya L.*), Planta 234 (2011) 1031–1044.
[20] X. Fu, et al., Plastid structure and carotenogenic gene expression in red- and white-fleshed loquat (*Eriobotrya japonica*) fruits, J. Exp. Bot. 63 (2012) 341–354.
[21] J. Jeffery, A. Holzenburg, S. King, Physical barriers to carotenoid bioaccessibility. Ultrastructure survey of chromoplast and cell wall morphology in nine carotenoid-containing fruits and vegetables, J. Sci. Food Agric. 92 (2012) 2594–2602.
[22] R.M. Schweiggert, D. Mezger, F. Schimpf, C.B. Steingass, R. Carle, Influence of chromoplast morphology on carotenoid bioaccessibility of carrot, mango, papaya, and tomato, Food Chem. 135 (2012) 2736–2742.
[23] S. Al-Babili, J. von Lintig, H. Haubruck, P. Beyer, A novel, soluble form of phytoene desaturase from *Narcissus pseudonarcissus* chromoplasts is Hsp70-complexed and competent for flavinylation, membrane association and enzymatic activation, Plant J. 9 (1996) 601–612.
[24] M. Schledz, et al., Phytoene synthase from *Narcissus pseudonarcissus*: functional expression, galactolipid requirement, topological distribution in chromoplasts and induction during flowering, Plant J. 10 (1996) 781–792.
[25] P. Schaub, S. Al-Babili, R. Drake, P. Beyer, Why is golden rice golden (yellow) instead of red? Plant Physiol. 138 (2005) 441–450.
[26] F.X. Cunningham, E. Gantt, Genes and enzymes of carotenoid biosynthesis in plants, Annu. Rev. Plant Physiol. Plant Mol. Biol. 49 (1998) 557–583.
[27] E.T. Wurtzel, Genomics, genetics, and biochemistry of maize carotenoid biosynthesis, in: J. Romeo (Ed.), Recent Advances in Phytochemistry, Elsevier Ltd., Amsterdam, Netherlands, 2004, pp. 85–110.
[28] R. Candau, E.R. Bejarano, E. Cerda-Olmedo, In vivo channeling of substrates in an enzyme aggregate for beta-carotene biosynthesis, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 4936–4940.
[29] M.D. De la Guardia, C.M. Aragon, F.J. Murillo, E. Cerda-Olmedo, A carotenogenic enzyme aggregate in Phycomyces: evidence from quantitive complementation, Proc. Natl. Acad. Sci. U.S.A. 68 (1971) 2012–2015.
[30] M. Bonk, et al., Chloroplast import of four carotenoid biosynthetic enzymes *in vitro* reveals differential fates prior to membrane binding and oligomeric assembly, Eur. J. Biochem. 247 (1997) 942–950.
[31] B. Camara, F. Bardat, R. Moneger, Sites of biosynthesis of carotenoids in *Capsicum* chromoplasts, Eur. J. Biochem. 127 (1982) 255–258.
[32] K. Kreuz, P. Beyer, H. Kleinig, The site of carotenogenic enzymes in chromoplasts from *Narcissus pseudonarcissus* L., Planta 154 (1982) 66–69.
[33] A.B. Lopez, Y. Yang, T.W. Thannhauser, L. Li, Phytoene desaturase is present in a large protein complex in the plastid membrane, Physiol. Plant. 133 (2008) 190–198.
[34] B. Maudinas, M.L. Bucholtz, C. Papastephanou, S.S. Katiyar, A.V. Briedis, J.W. Porter, The partial purification and properties of a phytoene synthesizing enzyme system, Arch. Biochem. Biophys. 180 (1977) 354–362.
[35] A.J. Ytterberg, J.-B. Peltier, K.J. van Wijk, Protein profiling of plastoglobules in chloroplasts and chromoplasts. A surprising site for differential accumulation of metabolic enzymes, Plant Physiol. 140 (2006) 984–997.
[36] Y.-Q. Wang, et al., Proteomic analysis of chromoplasts from six crop species reveals insights into chromoplast function and development, J. Exp. Bot. 64 (2013) 949–961.
[37] J. Joyard, et al., Chloroplast proteomics and the compartmentation of plastidial isoprenoid biosynthetic pathways, Mol. Plant 2 (2009) 1154–1180.

[38] S.R. Cutler, P.L. Rodriguez, R.R. Finkelstein, S.R. Abrams, Abscisic acid: emergence of a core signaling network, Annu. Rev. Plant Biol. 61 (2010) 651–679.
[39] S. Mintz-Oron, S. Meir, S. Malitsky, E. Ruppin, A. Aharoni, T. Shlomi, Reconstruction of Arabidopsis metabolic network models accounting for subcellular compartmentalization and tissue-specificity, Proc. Natl. Acad. Sci. U.S.A. 109 (2012) 339–344.
[40] S.M.D. Seaver, C.S. Henry, A.D. Hanson, Frontiers in metabolic reconstruction and modeling of plant genomes, J. Exp. Bot. 63 (2012) 2247–2258.
[41] G.W. Bassel, A. Gaudinier, S.M. Brady, L. Hennig, S.Y. Rhee, I. De Smet, Systems analysis of plant functional, transcriptional, physical interaction, and metabolic networks, Plant Cell 24 (2012) 3859–3875.
[42] C.L. Linster, E. Van Schaftingen, A.D. Hanson, Metabolite damage and its repair or pre-emption, Nat. Chem. Biol. 9 (2013) 72–80.
[43] A. Oudin, et al., Spatial distribution and hormonal regulation of gene products from methyl erythritol phosphate and monoterpene-secoiridoid pathways in *Catharanthus roseus*, Plant Mol. Biol. 65 (2007) 13–30.
[44] C. Benning, A role for lipid trafficking in chloroplast biogenesis, Prog. Lipid Res. 47 (2008) 381–389.
[45] O. Czarnecki, B. Grimm, Post-translational control of tetrapyrrole biosynthesis in plants, algae, and cyanobacteria, J. Exp. Bot. 63 (2012) 1675–1687.
[46] N.E. Lewis, et al., Large-scale in silico modeling of metabolic interactions between cell types in the human brain, Nat. Biotechnol. 28 (2010) 1279–1285.
[47] Y. Yang, L. Engin, E.S. Wurtele, C. Cruz-Neira, J.A. Dickerson, Integration of metabolic networks and gene expression in virtual reality, Bioinformatics 21 (2005) 3645–3650.
[48] M. Jia, S.Y. Choi, D. Reiners, E.S. Wurtele, J.A. Dickerson, MetNetGE: interactive views of biological networks and ontologies, BMC Bioinform. 11 (2010) 469.
[49] M. Ferro, et al., AT_CHLORO, a comprehensive chloroplast proteome database with subplastidial localization and curated information on envelope proteins, Mol. Cell. Proteomics 9 (2010) 1063–1084.
[50] Y. Balmer, W.H. Vensel, F.M. DuPont, B.B. Buchanan, W.J. Hurkman, Proteome of amyloplasts isolated from developing wheat endosperm presents evidence of broad metabolic capability, J. Exp. Bot. 57 (2006) 1591–1602.
[51] A. Hager, K. Holocher, Localization of the xanthophyll-cycle enzyme violaxanthin de-epoxidase within the thylakoid lumen and abolition of its mobility by a (light-dependent) pH decrease, Planta 192 (1994) 581–589.
[52] P.-A. Vidi, et al., Tocopherol cyclase (VTE1) localization and vitamin E accumulation in chloroplast plastoglobule lipoprotein particles, J. Biol. Chem. 281 (2006) 11225–11234.