Planta (2005) 221: 305–308
DOI 10.1007/s00425-005-1533-5

PROGRESS REPORT

Susanne Römer · Paul D. Fraser

Recent advances in carotenoid biosynthesis, regulation and manipulation

Received: 21 February 2005 / Accepted: 22 February 2005 / Published online: 15 April 2005

Keywords Carotenoids · Carotenoid biosynthesis · β-carotene · Lycopene · Manipulation · Regulation · Transgenic plants · Xanthophylls

Carotenoids are the most widespread group of pigments found in nature. In plants, carotenoids have functional roles in development, photosynthesis and membrane stability. They act as phytohormone precursors (Schwartz et al. 2003) and are involved in environmental adaptation (Demmig-Adams and Adams 2002). Carotenoids exert protective functions due to their high antioxidative potential and are also important components of the human diet. Strong evidence suggests that diets rich in carotenoids can prevent the onset of some chronic disease states (Mares-Perlman et al. 2002) and certain cancers (Giovannucci 2002).

The elucidation of the carotenoid biosynthetic pathway and the enormous progress in gene cloning provided the tools necessary to embark upon the metabolic engineering (also termed genetic manipulation or engineering) of the pathway (for recent reviews, see Tucker 2003; Fraser and Bramley 2004). This has resulted in stable transgenic plants with improved nutritional quality, increased ability to synthesise high value carotenoids and enhanced tolerance to abiotic stress. The majority of manipulations have targeted single steps in the pathway with the objective to elevate the lycopene and β-carotene content. Several constitutive overexpression approaches (Fray et al. 1995; Busch et al. 2002) focused on phytoene synthase (PSY), and led to various unintended pleiotropic and/or co-suppression effects. These experiments also revealed the delicate precursor balance and intricate interaction between different isoprenoid forming pathways (compare Laule et al. 2003). The use of promoters showing high organ or developmental specificity in combination with low homology transgenes successfully elevated the carotenoid content, avoided co-suppression effects and reduced metabolic perturbances in the transformed plants (Fraser et al. 2002; Shewmaker et al. 1999) in most cases. Due to its pro-vitamin A activity, increasing β-carotene level in crops and vegetables has also been a primary goal of genetic engineering. Over-expression of different lycopene cyclases gave rise to tomato fruits with significantly higher β-carotene content (reviewed in Fraser and Bramley 2004).

Recently, multiple carotenoid genes were transformed into plants in an attempt to further increase the amount of total carotenoids. In canola seeds, Ravanello et al. (2003) have generated a triple construct containing genes encoding PSY and phytoene desaturase from a bacterial source as well as lycopene cyclases of either plant or bacterial origin. Unfortunately, these constructs have made little improvement to that obtained previously with a bacterial PSY solely. The production of the so-called "golden rice" referring to the engineering of β-carotene (pro-vitamin A) synthesis in normally carotenoid-devoid rice endosperm also required the transfer of several genes from different origins to obtain the desired property (Ye et al. 2000). To provide a staple food source high in provitamin A thereby alleviating vitamin A deficiency in developing countries, the provitamin A phenotype was recently transferred into two Indica rice varieties which are widely grown in the target countries (Hoa et al. 2003) taking first steps to commercial exploitation. The authors also modified their construct by replacing the controversially discussed

S. Römer (✉)
School of Biological Sciences,
University of Bristol, Woodland Road,
Bristol, BS8 1UG, UK
E-mail: roemer.susanne@web.de

P. D. Fraser
School of Biological Sciences,
Royal Holloway University London,
Egham, Surrey, TW20 OEX, UK
E-mail: p.fraser@rhul.ac.uk

Present address: S. Römer
Fachbereich Biologie,
Universität Konstanz,
Universitätsstr. 10, 78457 Konstanz,
Germany

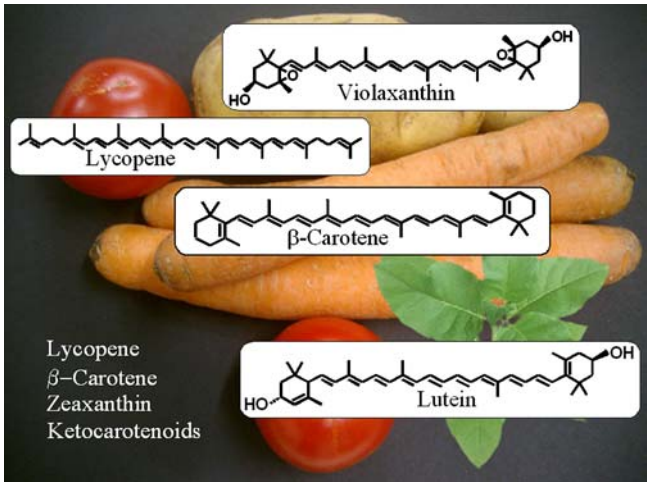

Fig. 1 Carotenoids in green leaves, fruits and vegetables. Boxed are the chemical structures of some carotenoids being widely distributed in higher plants; listed are some examples of carotenoids that are targets for genetic engineering and conventional breeding

antibiotic resistance marker with the phosphomannose isomerase selection system.

The emerging health benefits of xanthophylls have attracted increasing attention. The xanthophylls lutein and zeaxanthin are located in the yellow spot of the retina and the decrease of these pigments with age is thought to result in macular degeneration (AMD) (Mares-Perlman et al. 2002), a major cause of blindness in the elderly. Evidence is accumulating that the dietary intake of these xanthophylls can ameliorate or prevent the onset of such chronic degenerative diseases. Since very few vegetables and fruits contain significant amounts of zeaxanthin, genetic engineering was applied to alter xanthophyll composition. In tomato fruits, higher amounts of zeaxanthin and *β*-cryptoxanthin, as well as enhanced levels of *β*-carotene were achieved (Dharmapuri et al. 2002). In potato tubers, zeaxanthin concentration could be increased up to 130-fold using antisense inhibition of zeaxanthin epoxidase and co-suppression by the corresponding sense construct (Römer et al. 2002). Manipulation of xanthophyll biosynthesis by overexpression of various *β*-carotene hydroxylase genes also improved abiotic stress resistance of *Arabidopsis thaliana* and *Nicotiana tabacum* (Davison et al. 2002; Götz et al. 2002). Moreover, the cloning and characterisation of various carotene hydroxylases revealed the complexity of carotenoid biosynthesis and indicated overlapping functions within the pathway (reviewed in Tian and DellaPenna 2004). Following the identification of several *β*-carotene hydroxylases, the ε-hydroxylase gene was finally isolated by positional cloning. The use of *Arabidopsis* T-DNA knockout mutants for both *β*-hydroxylases and ε-hydroxylase in single, double and triple combinations has elegantly shown that the two *β*-hydroxylases can compensate for each other and the *β*-hydroxylase also possesses ε-hydroxylation activity.

The carotenoid pathway in higher plants can be used as a source of precursors for the synthesis of high-value

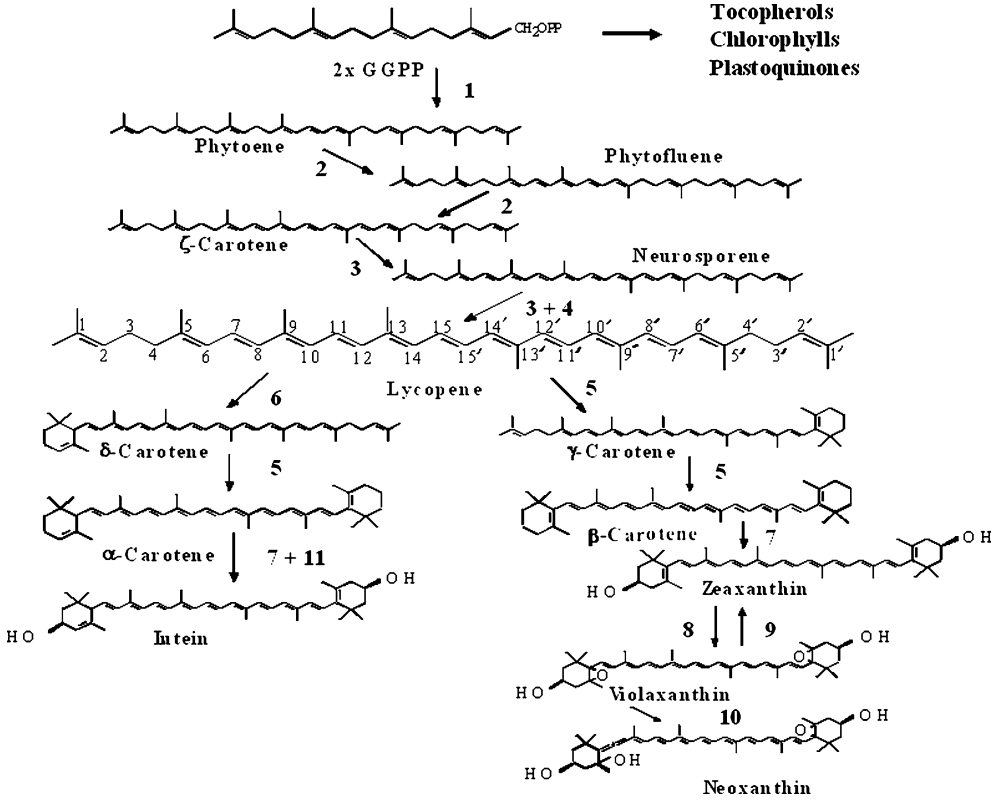

Fig. 2 Schematic representation of carotenoid synthesis in higher plants. GGPP geranylgeranyl diphosphate. Enzymes: 1 PSY phytoene synthase, 2 PDS phytoene desaturase, 3 ZDS ζ-carotene desaturase, 4 CRTISO carotene isomerase, 5 LCY-b /CYC-B lycopene *β*-cyclase, 6 LCY-e lycopene ε-cyclase, 7 CRTL-b *β*-ring hydroxylase, 11 CRTL-e ε-ring hydroxylase, 8 ZEP zeaxanthin epoxidase, 9 VDE violaxanthin de-epoxidase, 10 NXS neoxanthin synthase

ketocarotenoids (e.g. canthaxanthin, 4-ketozeaxanthin and astaxanthin), too. A variety of ketolated carotenoids were formed by the expression of the *Haematococcus* carotene oxygenase (*crt*O) in either tobacco (Hirschberg 2001) or *Arabidopsis* (Stalberg et al. 2003). In addition, the utility of the astaxanthin biosynthetic genes from marine bacteria to synthesise ketocarotenoids in higher plants was demonstrated (Ralley et al. 2004). Naturally produced astaxanthin is expected to become more important due to its evolving health benefits (Guerin et al. 2003). The isolation of carotenoid cleavage enzymes (reviewed in Giuliano et al. 2003) is also likely to facilitate the production of high-value apocarotenoids in higher plants and unravel their physiological roles.

Although the carotenoid biosynthetic pathway was already successfully targeted by genetic engineering, the complex regulation of carotenogenesis still remains poorly understood. Regulatory studies have primarily concentrated on three model systems: (1) fruit ripening, (2) flower colour development, and (3) light-dependent etioplast to chloroplast transition (de-etiolation). Induction of (coordinated) gene expression seems to be an important feature of carotenoid accumulation during flower (Zhu et al. 2003) and plastid development (von Lintig et al. 1997; Woitsch and Römer 2003). PSY, the first committed step of carotenoid biosynthesis, was transcriptionally up-regulated during tomato fruit ripening (Bramley 2002) and de-etiolation of mustard and tobacco seedlings (von Lintig et al. 1997). Its gene expression was shown to be under phytochrome control and the location of the PSY protein influenced prolamellar body formation (Welsch et al. 2000). Recently, the analysis of the PSY promoter of *A. thaliana* allowed the identification of promoter regions responsible for the modulation to different light qualities (Welsch et al. 2003). In accordance, phytochrome is also thought to be involved in the regulation of the expression of various xanthophyll biosynthetic genes in tobacco (Woitsch and Römer 2003). The transcript level of β-carotene hydroxylase was affected by different light qualities, whereas post-transcriptional or translational control might be a more decisive factor in the regulation of violaxanthin de-epoxidase (Rossel et al. 2002). Evidence for a redox control of some of the xanthophyll biosynthetic genes by the plastoquinone pool was derived from studies using site-specific photosynthetic electron transport inhibitors (Woitsch and Römer 2003). In tomato, components of the light signal transduction pathways were shown to have positive and negative regulatory effects on carotenoid formation, principally by increasing plastid numbers in the cell (Cookson et al. 2003; Liu et al. 2004). Light and developmental programs are not the only clues to induce expression of carotenoid biosynthetic genes, since also intermediates of the mevalonic acid (MVA)-independent pathway were capable to up-regulate expression of downstream carotenoid biosynthetic genes during tomato fruit ripening (Lois et al. 2000). Furthermore, genetic manipulation has sometimes led to alterations of endogenous gene expression in the transgenic plants (Römer et al. 2000; Römer et al. 2002).

As yet, no regulatory genes involved in carotenoid formation have been isolated. However, the *Or* gene defines a mutation resulting in an unusual accumulation of β-carotene in cauliflower (Li et al. 2003). Crosses of different carrot varieties and the creation of recombinant inbred lines of tomato enabled the identification of quantitative trait loci (QTLs) involved in the production of lycopene and β-carotene (Santos and Simon 2002). These findings will facilitate the breeding for these quality traits in the future by more consumer acceptable non-GM approaches.

Future prospects

Over the last decade, carotenoid research was predominantly focused on the biosynthetic pathway. The successful isolation of biosynthetic genes, identification of pathway products/intermediates and the manipulation of key steps in the pathway are major achievements. However, the generation of transgenic plants and mutants has frequently resulted in unexplainable physiological, molecular and biochemical effects, which presumably reflect our poor understanding of the regulatory aspects associated with carotenoid synthesis. The exploitation of the genetic resources generated and application of modern genomic and post-genomic techniques could considerably improve our knowledge about gene products and physiological processes that indirectly regulate and affect carotenoid formation enabling both non-GM and GM approaches to enhance food crops and improve the environmental adaptation of plants.

References

Bramley PM (2002) Regulation of carotenoid formation during tomato fruit ripening and development. J Exp Bot 53:2107–2113

Busch M, Seuter A, Hain R (2002) Functional analysis of the early steps of carotenoid biosynthesis in tobacco. Plant Physiol 128:439–453

Cookson PJ, Kiano JW, Shipton CA, Fraser PD, Römer S, Schuch W, Bramley PM, Pyke KA (2003) Increases in cell elongation, plastid compartment size and phytoene synthase activity underlie the phenotype of the high pigment 1 mutant of tomato. Planta 217:896–903

Davison PA, Hunter CN, Horton P (2002) Overexpression of β-carotene hydroxylase enhances stress tolerance in *Arabidopsis*. Nature 418:203–206

Demmig-Adams B, Adams III WW (2002) Antioxidants in photosynthesis and human nutrition. Science 298:2149–2153

Dharmapuri S, Rosati C, Pallara P, Aquilani R, Bouvier F, Camara B, Giuliano G (2002) Metabolic engineering of xanthophyll content in tomato fruits. FEBS Lett 519:30–34

Fraser PD, Bramley PM (2004) The biosynthesis and nutritional uses of carotenoids. Prog Lipid Res 43:228–265

Fraser PD, Romer S, Shipton CA, Mills PB, Kiano JW, Misawa N, Drake RG, Schuch W, Bramley PM (2002) Evaluation of transgenic tomato plants expressing an additional phytoene synthase in a fruit-specific manner. Proc Natl Acad Sci USA 99:1092–1097

Fray RG, Wallace A, Fraser PD, Valero D, Hedden P, Bramley PM, Grierson D (1995) Constitutive expression of a fruit phytoene synthase gene in transgenic tomatoes causes dwarfism by redirecting metabolites from the gibberellin pathway. Plant J 8:693–701

Giovannucci E (2002) Lycopene and prostate cancer risk. Methodological considerations in the epidemiologic literature. Pure Appl Chem 74:1427–1434

Giuliano G, Al-Babili S, von Lintig J (2003) Carotenoid oxygenases: cleave it or leave it. Trends Plant Sci 8:145–149

Götz T, Sandmann G, Römer S (2002) Expression of a bacterial carotene hydroxylase gene (*crtZ*) enhances UV tolerance in tobacco. Plant Mol Biol 50:129–142

Guerin M, Huntley ME, Olaizola M (2003) *Haematococcus* astaxanthin: applications for human health and nutrition. Trends Biotechnol 21:210–216

Hirschberg J (2001) Carotenoid biosynthesis in flowering plants. Curr Opin Plant Biol 4:210– 216

Hoa TTC, Al-Babili S, Schaub P, Potrykus I, Beyer P (2003) Golden indica and japonica rice lines amenable to deregulation. Plant Physiol 133:1612–169

Laule O, Furholz A, Chang HS, Zhu T, Wang X, Heifetz PB, Gruissem W, Lange M (2003) Crosstalk between cytosolic and plastidial pathways of isoprenoid biosynthesis in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 100:6866–6871

Li L, Garvin DF (2003) Molecular mapping of *Or*, a gene inducing beta-carotene accumulation in cauliflower (*Brassica oleracea* L. var. botrytis). Genome 46:588–594

von Lintig J, Welsch R, Bonk M, Giuliano G, Batschauer A, Kleinig H (1997) Light- stimulated carotenoid biosynthesis occurs at the level of phytoene synthase expression and is mediated by phytochrome in *Sinapis alba* and *Arabidopsis thaliana* seedlings. Plant J 12:625–634

Liu Y, Roof S, Ye Z, Barry C, van Tuinen A, Vrebalov J, Bowler C, Giovannoni J (2004) Manipulation of light signal transduction as a means of modifying fruit nutritional quality in tomato. Proc Natl Acad Sci 101:9897–9902

Lois LM, Rodríguez-Concepción M, Gallego F, Campos N, Boronat A (2000) Carotenoid biosynthesis during tomato fruit development: regulatory role of 1-deoxy-D-xylulose 5-phosphate synthase. Plant J 22:503–513

Mares-Perlman JA, Millen AE, Ficek TL, Hankinson SE (2002) The body of evidence to support a protective role for lutein and zeaxanthin in delaying chronic disease. J Nutr 132:518S–524S

Ralley L, Enfissi EMA, Misawa N, Schuch W, Bramley PM, Fraser PD (2004) Metabolic engineering of ketocarotenoids in higher plants. Plant J 39:477–485

Ravanello MP, Ke D, Alvarez J, Huang B, Shewmaker CK (2003) Coordinate expression of multiple bacterial carotenoid genes in canola leading to altered carotenoid production. Metab Eng 5:255–263

Römer S, Fraser PD, Kiano JW, Shipton CA, Misawa N, Schuch W, Bramley PM (2000) Elevation of the provitamin A content of transgenic tomato plants. Nat Biotechnol 18:666–669

Römer S, Lübeck J, Kauder F, Steiger S, Adomat C, Sandmann G (2002) Genetic engineering of a zeaxanthin-rich potato by antisense inactivation and co-suppression of carotenoid epoxidation. Metab Eng 4:263–272

Rossel JB, Wilson IW, Pogson B (2002) Global changes in gene expression in response to high light in *Arabidopsis*. Plant Physiol 130:1109–1120

Santos CAF, Simon PW (2002) QTL analysis reveal clustered loci for accumulation of major provitamin A carotenes and lycopene in carrot roots. Mol Gen Genomics 268:122–129

Schwartz SH, Qin X, Zeevaart JAD (2003) Elucidation of the indirect pathway of abscisic acid biosynthesis by mutants, genes and enzymes. Plant Physiol 131:1591–1601

Shewmaker CK, Sheehy JA, Daley M, Colburn S, Ke DY (1999) Seed-specific over- expression of phytoene synthase: increase in carotenoids and other metabolic effects. Plant J 20:401–412

Stalberg K, Lindgren O, Ek B, Hogland AS (2003) Synthesis of ketocarotenoids in the seed of *Arabidopsis thaliana*. Plant J 36:771–779

Tian L, DellaPenna D (2004) Progress in understanding the origin and functions of carotenoid hydroxylases in plants. Arch Biochem Biophys 430:22–29

Tucker G (2003) Nutritional enhancement of plants. Curr Opin Biotechnol 14:221–225

Welsch R, Beyer P, Hugueney P, Kleinig H, v. Lintig J (2000) Regulation and activation of phytoene synthase in carotenoid biosynthesis during photomorphogenesis. Planta 211:846–854

Welsch R, Medina J, Giuliano G, Beyer P, v. Lintig J (2003) Structural and functional characterization of the phytoene synthase promoter from *Arabidopsis thaliana*. Planta 216:523–534

Woitsch S, Römer S (2003) Expression of xanthophylls biosynthetic genes during light- dependent chloroplast differentiation. Plant Physiol 132:1508–1517

Ye X, Al-Babili S, Kloti A Zhang J, Lucca P, Beyer P, Potrykus I (2000) Engineering provitamin A (b-carotene) biosynthesis pathway into (carotenoid-free) rice endosperm. Science 287:303–305

Zhu C, Yamamura S, Nishihara M, Koiwa H, Sandmann G (2003) cDNAs for the synthesis of cyclic carotenoids in petals of *Gentiana lutea* and their regulation during flower development. Biochim Biophys Acta 1625:305–308