# Engineering novel carotenoids in microorganisms

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A considerable number of microbial and plant carotenoid biosynthesis genes have been cloned over the past few years. Functional heterologous expression of most of these genes has made it possible to engineer carotenoid biosynthesis in non-carotenogenic *E. coli* and yeasts. Recently, gene combination and molecular breeding of pathways have been used to produce novel and rare high-value carotenoids.

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**Abbreviations**

| | |
|---|---|
| **IDP** | isopentyl diphosphate |
| **DMADP** | dimethylallyl diphosphate |
| **GGDP** | geranylgeranyl diphosphate |

## Introduction

Carotenoids are currently produced for use as food colorants in animal feeds, nutritional supplements and for cosmetic and pharmaceutical purposes. Most of the commercially used carotenoids (e.g. β-carotene, astaxanthin and canthaxanthin) are produced by chemical synthesis [1]. The demand and market for carotenoids, however, is anticipated to change with the discovery that carotenoids exhibit tumor suppressing activity and play an important role in the prevention of chronic diseases. In addition to their antioxidant properties, a number of unexpected biological effects of carotenoids, for example, in junctional communication and gene regulation, have been recently discovered and attributed to their tumor suppressing activity [2••,3–5], boosting the interest in evaluating the pharmaceutical potential of carotenoids. Supply of structurally diverse carotenoids for medical studies is limited though, as only a restricted number of carotenoids can be synthesized chemically, isolated from natural sources or produced by microbial fermentation. Elucidation of a number of carotenoid biosynthetic pathways at a molecular level has provided a toolbox of carotenogenic (crt) genes that can be used to engineer microorganisms for carotenoid production. This review describes the progress made in engineering structurally diverse carotenoids in non-carotenogenic microorganisms.

## Biosynthesis of carotenoids

Thorough biochemical analysis of carotenoid biosynthesis, classical genetics and more recently molecular genetics resulted in the elucidation of the main routes for the synthesis of acyclic and cyclic carotenoids at a molecular level (reviewed in [6••]; Figure 1). Little is known, however, about the biosynthesis of carotenoids containing additional modifications of the end groups, the polyene chain, the methyl groups or molecular rearrangements that contribute to the tremendous structural diversity of carotenoids. At present, hundreds of individual carotenoids have been characterized [7] and novel carotenoids continue to be isolated.

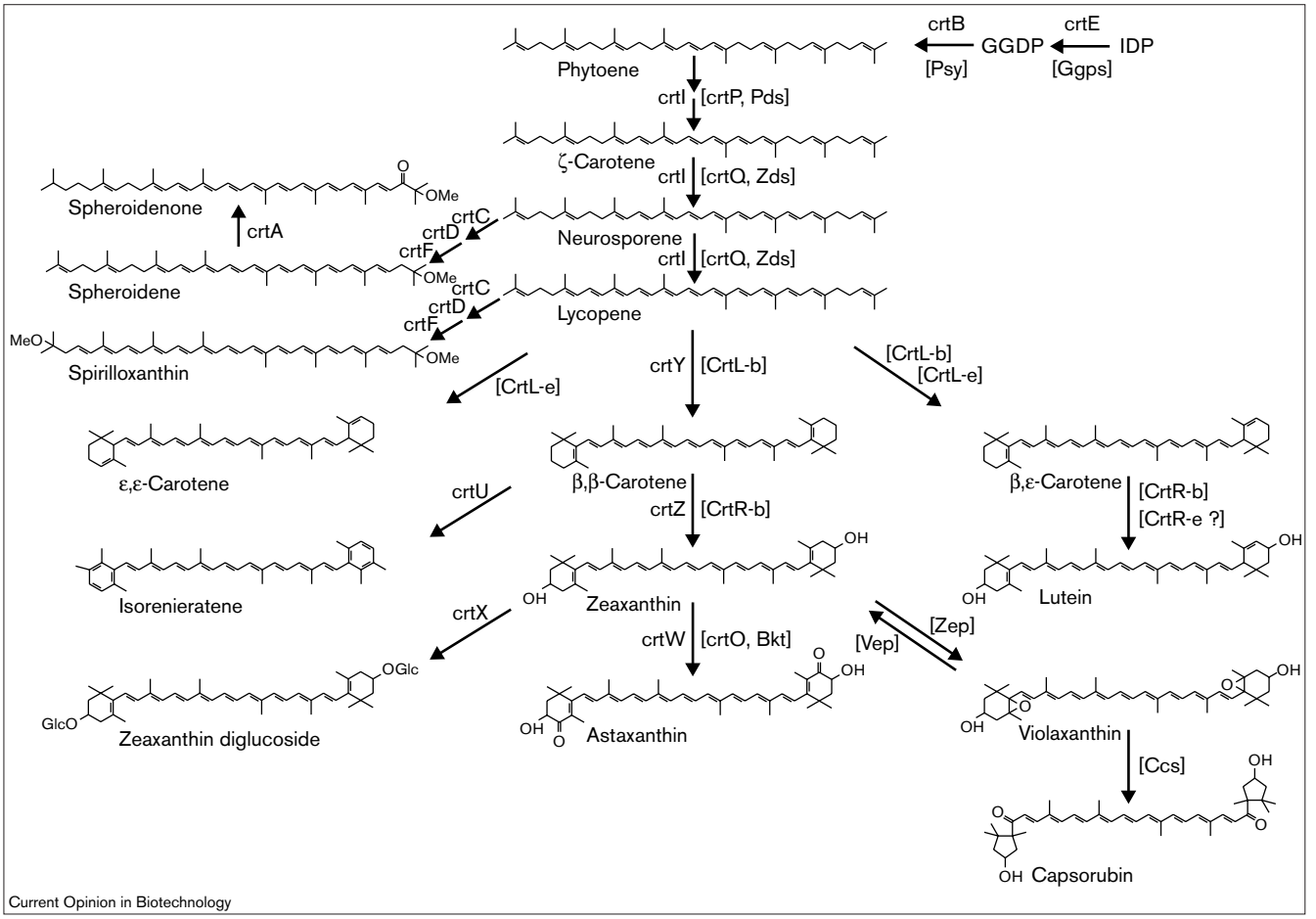
All carotenoids are derived from the isoprenoid or terpenoid pathway. Condensation of one molecule of dimethylallyl diphosphate (DMADP) and three molecules of isopentyl diphosphate (IDP) produces the diterpene geranylgeranyl diphosphate (GGDP) that forms one half of all C40 carotenoids. The head to head condensation of two GGDP molecules results in the first colorless carotenoid, phytoene. Subsequent desaturation reactions lengthen the conjugated double bond system to produce neurosporene or lycopene. Following desaturation, carotenoid biosynthesis branches into routes for acyclic and cyclic carotenoids.

In phototrophic bacteria, additional desaturation at C3,4, introduction of hydroxy and methoxy groups at C1(C1′) of neurosporene and lycopene and, under aerobic conditions, a keto-group at C2 of neurosporene leads to the synthesis of the acyclic xanthophylls spheroidene or spheroidenone and spirilloxanthin, respectively. Synthesis of cyclic carotenoids involves cyclization of one or both end groups of lycopene or neurosporene. Typically, β-rings are introduced, but formation of ε-rings is common in higher plants and carotenoids with γ-rings are found, for example, in certain fungi. Most cyclic carotenoids contain at least one oxygen function at one of the ring carbon atoms. Cyclic carotenoids with keto-groups at C4(C4′) and/or hydroxy groups at C3(C3′) (e.g. zeaxanthin, astaxanthin, echinenone and lutein) are widespread in microorganisms and plants. The leaf xanthophylls violaxanthin and neoxanthin, and algae carotenoids such as fucoxanthin contain β-rings with a C(5,6)-epoxy group. The C(5,6)-epoxy β-end group serves as a key intermediate in the formation of a variety of other unusual end groups, for example, the cyclopentyl end group of capsanthin and capsorubin synthesized in *Capsicum annuum* (red pepper).

## Toolbox of carotenogenic genes

At present more than 150 genes, encoding 24 different crt enzymes, have been isolated from bacteria, plants, algae and fungi (Table 1; for a comprehensive list see [8]). These genes can be used to engineer a variety of diverse carotenoids in recombinant microorganisms. Complete carotenoid biosynthesis pathways have been cloned from a number of bacteria where the crt genes are clustered. The synthetic pathways for the production of zeaxanthin diglucoside and acyclic xanthophylls from *Erwinia* and *Rhodobacter*, respectively, were the first for which all involved enzymes have been identified (reviewed in [9,10]).

**Figure 1**




Carotenoid biosynthesis pathways in microorganisms and plants from which the enzymes have been cloned. Terminal products of biosynthesis routes are shown. Enzymes from plants, algae and cyanobacteria are given in parentheses. Ccs, capsanthin/capsorubin synthase; crtA, spheroidene monooxygenase; crtB, phytoene synthase; crtC, hydroxyneurosporene synthase; crtD, methoxyneurosporene desaturase; crtE, GGDP synthase; crtF, hydroxyneurosporene-O-methyltransferase; crtI , crtP and Pds, phytoene desaturase; CrtL-e, lycopene ε-cyclase; crtQ and Zds, ζ-carotene desaturase; crtU, β-carotene desaturase; crtW, crtO and Bkt, β-carotene C(4) oxygenase; crtX, zeaxanthin glucosylase; crtY and CrtL-b, lycopene β-cyclase; crtZ and crtR-b, β-carotene hydroxylase; Vep, violaxanthin deepoxidase; Zep, zeaxanthin epoxidase.

Various techniques have been applied to clone crt genes [11•]. In particular, functional color complementation in *Escherichia coli* cells expressing crt genes from *Erwinia* species (which produce carotenoids that serve as substrates for the enzyme to be cloned) has been used successfully for the cloning of a variety of additional microbial and plant crt genes [12–15]. Recent advances in plant (including cyanobacteria) genomics and the use of cyanobacteria as models of plant carotenogenesis resulted in the identification of nearly all enzymes involved in plant carotenoid biosynthesis (reviewed in [8,16•]). In contrast, cloned enzymes of bacterial carotenoid biosynthesis cover only the main routes.

Genes encoding the early carotenoid biosynthetic enzymes GGDP synthase, phytoene synthase and phytoene desaturase account for more than half of all cloned crt genes. Different phytoene desaturase genes are available that introduce two, three, four or five double bonds into phytoene to produce ζ-carotene (plant, cyanobacteria, algae), neurosporene (*Rhodobacter*), lycopene (most eubacteria and fungi) or 3,4-didehydrolycopene (*Neurospora crassa*) [12,17•,18–20], respectively.

Lycopene-β-cyclases, catalyzing β-ring formation, have been cloned from a number of bacteria and plants. More recently, genes encoding lycopene-ε-cyclases have been isolated from plants [21,22]. Whereas dicyclic products are formed by the β-lycopene cyclase, plant ε-lycopene cyclases usually synthesize monocyclic ε, ψ-carotene with the exception of lettuce ε-cyclase, which forms ε,ε-carotene [16•]. To date, only β-ring modifying enzymes have been cloned, including a number of β-carotene C(3) hydroxylases [23,24] and β-carotene C(4) ketolase or oxygenases [14,25–28]. In

**Table 1**

**Selection of genes encoding carotenoid biosynthesis enzymes.**

| Enzyme | Gene | Organism | Accession number |
|---|---|---|---|
| **Assembly of carotenoid backbone** | | | |
| GGDP-synthase | *crtE* | *Erwinia uredevora* | D90087 |
| | *crtE* | *Synechocystis* PCC6803 | D90899 |
| | *al-3* | *Neurospora crassa* | X53979 |
| | *Ggp* | *Arabidopsis thaliana* | L25813 |
| Phytoene synthase | *crtB* | *Agrobacterium aurantiacum* | D58420 |
| | *crtB* | *Synechocystis* PCC6803 | X69172 |
| | *al-2* | *Neurospora crassa* | L27652 |
| | *Psy* | *Arabidopsis thaliana* | L25812 |
| Dehydrosqualene synthase (C30 carotenoids) | *crtM* | *Staphylococcus aureus* | X73889 |
| **Biosynthesis of acyclic carotenoids** | | | |
| Phytoene desaturase | | | |
| two desaturations | *crtP* | *Synechocystis* PCC6803 | X62574 |
| | *Pds1* | *Arabidopsis thaliana* | L16237 |
| three desaturations | *crtI* | *Rhodobacter capsulatus* | Z11165 |
| four desaturations | *crtI* | *Erwinia uredevora* | D90087 |
| up to five desaturations | *al-1* | *Neurospora crassa* | M57465 |
| ζ-carotene desaturase | *crtQ* | *Synechocystis* PCC6803 | X62574 |
| | *crtQ* | *Capsicum annuum* | X68058 |
| Dehydrosqualene desaturase (C30 carotenoids) | *crtN* | *Staphylococcus aureus* | X73889 |
| Hydroxyneurosporene synthase | *crtC* | *Rhodobacter sphaeroides* | X82458 |
| | *crtC* | *Rubrivivax gelatum* | U73944 |
| Methoxyneurosporene desaturase | *crtD* | *Rhodobacter capsulatus* | Z11165 |
| | *crtD* | *Rubrivivax gelatum* | U73944 |
| Hydroxyneurosporene-O-methyltransferase | *crtF* | *Rhodobacter sphaeroides* | X82458 |
| Spheroidene monooxygenase | *crtA* | *Rhodobacter capsulatus* | Z11165 |
| **Biosynthesis of cyclic carotenoids** | | | |
| Lycopene-β-cyclase | *crtY* | *Erwinia uredevora* | D90087 |
| | *crtY* | *Synechocystis* PCC6803 | X74599 |
| | *CrtL-b* | *Arabidopsis thaliana* | Z29211 |
| Lycopene-ε-cyclase | *CrtL-e* | *Arabidopsis thaliana* | U50738 |
| β-carotene hydroxylase | *crtZ* | *Agrobacterium aurantiacum* | D58420 |
| | *CrtR-b1* | *Capsicum annuum* | Y09225 |
| Zeaxanthin glucosylase | *crtX* | *Erwinia herbicola* EhoI | M87280 |
| β-carotene C(4) oxygenase | *crtW* | *Agrobacterium aurantiacum* | D58420 |
| | *crtO* | *Synechocystis* PCC6803 | D64004 |
| | *crtW* | *Alcaligenes PC1* | D58422 |
| | *CrtO/Bkt* | *Haematococcus pluvialis* | X86782/D45881 |
| Zeaxanthin epoxidase | *Zep1* | *Arabidopsis thaliana* | T45502 |
| Violaxanthin deepoxidase | *Vde1* | *Arabidopsis thaliana* | N37612 |
| Violaxanthin cleavage | *Vp14* | *Zea mays* | U95953 |
| Capsanthin/capsorubin synthase | *Ccs* | *Capsicum annuum* | X77289 |
| β-carotene desaturase | *crtU* | *Streptomyces griseus* | X95596 |

the past few years, plant genes were identified encoding zeaxanthin C(5,6) epoxidase, violaxanthin C(5,6) deepoxidase and pepper capsanthin/capsorubin synthase catalyzing κ-ring formation from the 3-hydroxy-5,6-epoxy-β-rings in violaxanthin and antheraxanthin (reviewed in [16•]).

Enzymes involved in acyclic carotenoid biosynthesis have so far only been cloned from phototrophic bacteria for the synthesis of acyclic xanthophylls [29–32]. Recent additions to the collection of crt genes are dehydrosqualene synthase and desaturase from *Staphylococcus aureus* [33] for 4,4′-diaponeurosporene (a C30 carotenoid) synthesis and β-carotene desaturase from *Streptomyces griseus* [34,35••] for isorenieratene (contains aromatic end groups) synthesis.

## Engineering carotenoid biosynthesis pathways by gene combination

To broaden the range of structurally diverse carotenoids that can be produced in recombinant microorganisms, crt genes can be combined in new biosynthetic pathways. Examples for gene combinations in *E. coli* and the produced carotenoids are shown in Table 2. Prerequisites for this approach are that crt enzymes from different species can function cooperatively in a heterologous host and display enough promiscuity regarding the structure of their substrates.

With a few exceptions, such as, for example, zeaxanthin C(5,6)epoxidase [36] and β-carotene desaturase [35••], almost all cloned carotenoid biosynthetic enzymes are functionally expressed in *E. coli*. The success of functional color

**Table 2**

**Combination of carotenoid biosynthesis genes in *E. coli* and the resulting major carotenoids accumulated.**

| Gene combination* | Major carotenoids accumulated | References |
|---|---|---|
| $crtE,B_{EU} + crtI_{XD} + crtX_{EU}$ | Lycopene | [12] |
| $crtE,B_{EU} + crtI_{EU}$ | Lycopene | [46] |
| *crtE,BES +PdsAT + ZdsAT* | Pro-lycopene | [17•] |
| $crtE.B_{EU} + crtI_{EU} + crtY_{EU} + crtZ_{EU}$ | Zeaxanthin | [46] |
| $crtE,B_{EU} + crtI_{EU} + crtY_{EU} + crtZ_{AA}$ | Zeaxanthin | [28] |
| $crtE,B_{EU} + crtI_{EU} + crtY_{EU} + CrtZ_{HP}$ | Zeaxanthin, β-cryptoxanthin | [23] |
| $crtE,B_{EU} + crtI_{XD} + crtY_{EU} + crtX_{EU} + crtZ_{EU}$ | Zeaxanthin > zeaxanthin-glucoside | [12] |
| $crtE,B_{EU} + crtI_{EU} + crtY_{EU} + crtZ_{EU} + crtX_{EU}$ | zeaxanthin-β-diglucoside | [46] |
| $crtE,B_{EU} + crtI_{EU} + crtY_{EU} + crtO_{SY}$ | Echinenone > canthaxanthin, β-carotene | [26] |
| $crtE,B_{EU} + crtI_{EU} + crtY_{EU} + Bkt_{HP}$ | Canthaxanthin | [14,23,27] |
| $crtE,B_{EU} + crtI_{EU} + crtY_{EU} + crtW_{AA}$ *or* $crtW_{EU}$ | Canthaxanthin | [25] |
| $crtE,B_{EU} + crtI_{EU} + crtY_{EU} + crtW_{AA}$ | Canthaxanthin | [28] |
| $crtE,B_{EU} + crtI_{EU} + crtY_{EU} + CrtZ_{HP} + Bkt_{HP}$ | Canthaxanthin, β-cryptoxanthin > zeaxanthin, Adonixanthin, astaxanthin | [23] |
| $crtE,B_{EU} + crtI_{EU} + crtY_{EU} + crtZ_{EU} + crtX_{EU} + crtW_{AA}$ | Astaxanthin-β-glucoside, astaxanthin-β-D-diglucoside | [45] |
| $crtE,B_{EU} + crtI_{EU} + crtY_{EU} + crtZ_{AA} + crtX_{EU} + crtW_{AA}$ | Astaxanthin, adonixanthin 3′-β-D-glucoside | [45] |
| $crtE,B_{EU} + crtI_{EU} + crtY_{EU} + crtZ_{EU} + Bkt_{HP}$ | Astaxanthin, canthaxanthin, zeaxanthin | [14] |
| $crtE,B_{EU} + crtI_{EU} + crtY_{EU} + crtZ_{EU} + crtW_{AA}$ | Astaxanthin > phoenicoxanthin, adonixanthin canthaxanthin | [28] |
| $crtE,B_{EU} + crtI_{EU} + crtY_{EU} + crtZ_{AA} + crtW_{AA}$ | Adonixanthin, astaxanthin >canthaxanthin | [28] |
| $crtE,B_{EU} + crtI_{EU} + crtY_{EU}$ | β-carotene | [46] |
| $crtE,B_{EH} + crtI_{EH} + CrtL\text{-}b_{AT}$ | β-carotene | [21] |
| $crtE,B_{EH} + crtI_{EH} + crtY_{EH} + CrtL\text{-}e_{AT}$ | β-carotene >> β,ε-carotene | [21] |
| $crtE,B_{EH} + crtI_{EH} + CrtL\text{-}e_{AT}$ | ε,ψ-carotene | [21] |
| $crtE,B_{EH} + crtI_{SY} + CrtL\text{-}b_{AT}$ *or* $CrtL\text{-}e_{AT}$ | ζ-carotene | [21] |
| $crtE,B_{EU} + crtI_{Sy} + crtY_{EU}$ | ζ-carotene > tetrahydro-β-carotene, dihydro-β-zeacarotene | [41] |
| $crtE,B_{EH} + crtI_{EH} + crtL\text{-}b_{AT} + CrtL\text{-}e_{AT}$ | ε,ψ-carotene > β,ε-carotene | [21] |
| $crtE,B_{EH} + crtI_{RC} + CrtL\text{-}b_{AT}$ | β-zeacarotene, neurosporene | [21] |
| $crtE,B_{EH} + crtI_{RC} + CrtL\text{-}e_{AT}$ | Neurosporene, α-zeacarotene | [21] |
| $crtE,B_{EU} + crtI_{RC} + crtY_{EU}$ *or* $CrtL\text{-}b_{CA}$ | Dihydro-β-carotene | [41] |
| $crtE,B_{EU} + crtI_{RC} + crtY_{EU} + crtZ_{EU}$ | Dihydrozeaxanthin, dihydro-β-caroten-3/3′-ol, β-zeacaroten-3-ol | [41] |
| $crtE,B_{EU} + crtI_{RC} + crtY_{EU} + crtZ_{EU}$ | 7,8-dihydrozeaxanthin > 3-hydroxy-β-zeacarotene, 3/3′-hydroxy-7,8-dihydro-β-carotene | [42] |
| $crtE,B_{EU} + crtI_{EU} + crtY_{EU} + crtC_{RC}$ | β-carotene >1,1′-dihydroxylycopene, 1-hydroxylycopene, 1′-hydroxy-γ-carotene | [42] |
| $crtE,B_{EU} + crtI_{EU} + crtC_{RC}$ | 1,1′-dihydroxylycopene, 1-hydroxylycopene | [42] |
| $crtE,B_{EU} + crtI_{RC} + crtC_{RC}$ | Hydroxyneurosporene | [26,42] |
| $crtE,B_{EU} + crtI_{RC} + crtC_{RC} + crtD_{RS}$ | Demethylspheroidene, 1-hydroxylycopene | [42] |
| $crtM_{SA} + crtN_{SA}$ | 4,4′-diaponeurosporene | [33] |

*Subscripts indicate the organisms from which the crt genes (refer to Table 1 for gene designations) have been cloned:
AA, *Agrobacterium aurantiacum*; AL, *Alcaligenes sp.*; AT, *Arabidopsis thaliana*; CA, *Capsicum annuum*; EH, *Erwinia herbicola*; ES, *Erwinia stewartii*; EU, *Erwinia uredevora*; HP, *Haematococcus pluvialis*; RC, *Rhodobacter capsulatus*; RS, *Rhodobacter sphaeroides*; SA, *Staphylococcus aureus*; SY, *Synechocystis sp.*; XD, *Xanthophyllomyces dendrorhous*.

complementation in transgenic *E. coli* for the cloning of a number of carotenoid biosynthesis genes demonstrates that enzymes from phylogenetically distant species can assemble into a functional membrane-bound multi-enzyme complex at which carotenoid biosynthesis presumably takes place [6••,16•].

Xanthophyll biosynthesis in phototrophic bacteria provides an excellent example for the intrinsic broad substrate specificity of the later enzymes in carotenoid biosynthesis, as well as the modular role of phytoene desaturation for achieving carotenoid diversity. In phototrophic bacteria, the same enzymes crtC, crtD and crtF (see Table 1 for crt gene names) synthesize spheroidene from neurosporene and spirilloxanthin from lycopene [29–32]. *In vitro* analysis of the substrate specificity of crtD from *Rhodobacter sphaeroides* showed that both lycopene and neurosporene are good substrates for the enzyme [37]. The order of end-group modifications catalyzed by crtC, crtD and crtF is also not completely fixed. Deletion of *crtD* in *Rhodospirillum rubrum* resulted in the accumulation of the non-natural 3,4,3′,4′-tetradehydrospirilloxanthin and its precursors, demonstrating that crtF can act as well on substrates lacking the conjugated double bond introduced by crtD [32].

The first experiments to reroute carotenoid biosynthesis were carried out by splicing the pathways from *Erwinia herbicola* and *Rb. sphaeroides*. The *Erwinia crtB*, *crtI*, *crtY*, and *crtZ* genes for zeaxanthin biosynthesis were transferred into *Rb. sphaeroides* mutant strains bearing either a *crtI* or a *crtC* deletion. When crtC was present, carotenoid biosynthesis was directed to the synthesis of the *Rb. sphaoroides* carotenoids

derived from neurosporene. In the absence of crtC and the presence of the *Erwinia* four-step desaturase crtI though, *Erwinia* carotenoids were produced from lycopene [38]. Introduction of only the four-step desaturase gene *crtI* from *Erwinia* into a *Rb. sphaeroides crtI*- strain led to the synthesis of spirilloxanthin and ketospirilloxanthin from lycopene, which are not produced in wild-type *Rb. sphaeroides* but are synthesized in other phototrophic bacteria [39•,40].

Modulation of phytoene desaturation also allowed the synthesis of novel cyclic carotenoids in *E. coli*. Lycopene cyclases from *Erwinia uredevora* and *Capsicum annuum* have been shown to cyclize neurosporene and ζ-carotene in addition to lycopene. Synthesis of the respective monocyclic and dicyclic products from neurosporene and ζ-carotene in *E. coli* was achieved by combining *crtE* and *crtB* from *E. uredevora*, and *crtI* from *Rhodobacter capsulatus* (three-step desaturase) or *Synechoccus sp.* (two-step desaturase) together with the lycopene cyclase genes [41]. Extension of the pathway for the synthesis of cyclic carotenoids from neurosporene with the gene for β-carotene hydroxylase, *crtZ*, enabled the production of additional novel hydroxy-carotenoids in *E. coli*. Combination of *crtI* from *Rb. capsulatus* or *E. uredevora* with *crtC* and *crtD*, or *crtY*, *crtZ*, and *crtC* allowed synthesis in *E. coli* of various unusual acyclic and cyclic hydroxy-carotenoids, respectively [42]. Both lycopene and neurosporene, but not ζ-carotene, are cyclized by the β- and ε-lycopene cyclases from *Arabidopsis thaliana*, enabling synthesis in *E. coli* of β- and ε,ψ-carotene and α- and β-zeacarotene, respectively. Co-expression of both plant β- and ε-lycopene cyclases in lycopene-producing *E. coli* resulted in the synthesis of α-carotene [21].

The broad substrate specificity of β-carotene hydroxylase (crtZ) and β-carotene C(4) oxygenase (crtW, Bkt, crtO) allows different synthesis routes for astaxanthin formation *in vitro* [43•]. *In vivo*, however, combinations of genes sufficient for astaxanthin biosynthesis in *E. coli* [14,28] or cyanobacteria [44] resulted in the production of a mixture of intermediates together with astaxanthin. Enzyme activities and substrate affinities of a biosynthetic pathway therefore determine accumulation of intermediates and terminal product formation.

Combination of seven different genes (*crtE, crtB*, *crtI*, *crtY*, and *crtX* from *E. uredevora*, *crtW* from *Agrobacterium aurantiacum*, and *crtZ* from *E. uredevora* or *A. aurantiacum*) in one pathway enabled synthesis of two new carotenoid glucosides, astaxanthin-β-D-diglucoside and adonixanthin 3′-β-D-glucoside, and the known astaxanthin glucoside in *E. coli* [45]. Although crtZ from *E. uredevora* and *A. aurantiacum* share a high amino acid sequence homology (56%), they have different hydroxylating activities, which accounts for the synthesis of different carotenoid glucosides in *E. coli* [46].

## Molecular breeding of carotenoid biosynthesis pathways

A different approach to expand the recombinant production of known carotenoids and to synthesize completely new structures is to breed novel pathways by combining available biosynthetic genes and evolving new enzyme functions through random mutagenesis, recombination (DNA-shuffling [47]) and selection. The phytoene desaturase (crtI) and the lycopene cyclase (crtY) have been targeted for *in vitro* evolution [48•] to achieve synthesis of novel carotenoids in *E. coli* (C Schmidt-Dannert, D Umeno, FH Arnold, unpublished data). Both enzymes are located at important branch points of biosynthetic pathways for acyclic and cyclic carotenoids. Changing the substrate specificity of crtI and crtY, combination with additional end-group modifying enzymes and, if necessary, changing the catalytic properties of those subsequent enzymes through additional rounds of *in vitro* evolution should therefore allow extensive branching of carotenoid biosynthesis for the synthesis of numerous novel carotenoids.

DNA-shuffling of *crtI* and *crtY* genes from *Erwinia* species within assembled carotenoid biosynthetic pathways allowed the production of novel acyclic and cyclic carotenoids in *E. coli*. In addition, a number of pathways were identified that resulted in the accumulation of different ratios and amounts of acyclic and cyclic carotenoids indicating that this approach can be used to direct synthesis towards intermediates and to optimize carotenoid biosynthesis.

## Metabolic engineering of microbial carotenoid production

Most crt genes and gene clusters have been cloned and expressed in the genetically easy to manipulate non-carotenogenic host *E. coli*; however, *E. coli* has only a limited supply of the common isoprenoid precursors IDP, DMADP and GGDP, needed for carotenoid biosynthesis. The production levels of carotenoids in recombinant *E. coli* are therefore low (10–500 μg $g^{-1}$ cell dry weight) compared to commercially employed carotenogenic microbial strains such as *Dunalliella*, *Haematococcus* and *Xanthophyllomyces dendrorhous* (formerly *Phaffia rhodozyma*), where production levels of up to 50 mg $g^{-1}$ dry weight are obtained [1].

Efforts to increase the isoprenoid central flux in *E. coli* (reviewed in [49]) have been directed at increasing the production of IDP and of GGDP from IDP. Overexpression of the IDP isomerase (*idi*) that catalyzes the isomerization of IDP to DMAP together with an archaebacterial multifunctional GGDP synthase (*gps*) that converts IDP and DMADP directly to GGDP, resulted in a 50-fold increase of astaxanthin production [50•]. Comparable production levels in *E. coli* of about 1500 μg $g^{-1}$ dry weight could be obtained for β-carotene and zeaxanthin by overexpression of the 1-deoxy-D-xylose 5-phosphate synthase (*dxs*) involved in IDP synthesis and *idi* [51••]. Further increase of IDP synthesis by co-expression of *idi*, *dxs* and a 1-deoxy-D-xylose 5-phosphate reductase (*dxr*) was toxic for *E. coli*, possibly due to overloading of the membranes with carotenoids. Maximal production levels of carotenoids in *E. coli* may therefore have been already reached without additional increase of carotenoid storage capacities in *E. coli*.

Yeasts, however, are capable of accumulating large quantities of the isoprenoid derivative ergosterol. Ergosterol biosynthesis has been successfully diverted for the production of carotenoids in the non-carotenogenic food yeasts *Saccharomyces cerevisiae* and *Candida utilis* (reviewed in [49]). Recently, overexpression of the 3-hydroxy methylglutaryl coenzyme A reductase and blockage of ergosterol synthesis by disruption of the *ERG9* gene, encoding squalene synthase, yielded a lycopene overproducing *C. utilis* strain (7.8 mg $g^{-1}$ dry weight) with commercial potential [52••]. It should be noted that transgenic plants have been successfully engineered for the synthesis of carotenoids as well (reviewed in [53•]).

## Conclusions

Successful assembly of crt genes into new pathways and molecular breeding of novel biosynthesis routes, together with the large pool of available carotenoid biosynthesis genes, have opened numerous possibilities for the synthesis of structurally diverse carotenoids. Recent accomplishments in metabolic engineering of microbial carotenoid production should allow synthesis of novel high-value carotenoids for medical and pharmaceutical purposes.

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## References and recommended reading

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