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MINIREVIEW

Eubacteria Show Their True Colors: Genetics of Carotenoid Pigment Biosynthesis from Microbes to Plants

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INTRODUCTION

Carotenoids compose a widely distributed class of structurally and functionally diverse yellow, orange, and red natural pigments. Prokaryotes and eukaryotes synthesize an estimated 10^8 tons of carotenoids yearly (54), composed of at least 600 structurally distinct compounds (90). These pigments typically consist of a C_{40} hydrocarbon backbone in the case of carotenes, often modified by various oxygen-containing functional groups to produce cyclic or acylic xanthophylls (21, 40). The degree of conjugation and the isomerization state of the backbone polyene chromophore determine the absorption properties of each carotenoid. Compounds with at least seven conjugated double bonds, such as ζ-carotene, absorb visible light. Some carotenoids occur naturally not only as all *trans* isomers but also as *cis* isomers (17, 40).

Carotenoids are derived from the general isoprenoid biosynthetic pathway, along with a variety of other important natural substances (see Fig. 1) (16, 20, 71, 72, 83, 89). The conversion of two molecules of geranylgeranyl pyrophosphate (GGPP) to phytoene, a compound common to all C_{40} carotenogenic organisms, constitutes the first reaction unique to the carotenoid branch of isoprenoid metabolism (21, 40). Anoxygenic photosynthetic bacteria, nonphotosynthetic bacteria, and fungi desaturate phytoene either three or four times to yield neurosporene or lycopene, respectively (see Fig. 2). In contrast, oxygenic photosynthetic organisms (cyanobacteria, algae, and higher plants) convert phytoene to lycopene via ζ-carotene in two distinct sets of reactions (15, 17, 48). At the level of neurosporene or lycopene, the carotenoid biosynthesis pathways of different organisms branch to generate the tremendous diversity of carotenoids found in nature.

In photosynthetic organisms and tissues, the lipophilic carotenoid and bacteriochlorophyll (Bchl) or chlorophyll (Chl) pigment molecules associate noncovalently but specifically with integral membrane proteins (22, 56). In nonphotosynthetic organisms and tissues, carotenoids, often protein bound, occur in cytoplasmic or cell wall membranes, oil droplets, crystals, and fibrils (21, 31, 40). Carotenoids provide crucial protection against photooxidative damage resulting from the combination of visible or near-UV light, singlet oxygen, and endogenous lipophilic photosensitizers, such as Bchl, Chl, heme, and protoporphyrin IX (22, 41, 92). This protective function explains the ubiquitous synthesis of carotenoids in photosynthetic organisms and their widespread distribution among nonphotosynthetic bacteria and fungi (21, 40). During photosynthesis, carotenoids also absorb light and transfer the energy to Bchl and Chl, dissipate excess radiant energy, and preserve the structural integrity of pigment-protein complexes (22, 56). In mammals, the cleavage products of several dietary carotenoids, particularly β-carotene, fulfill essential roles in nutrition (vitamin A), vision (retinal), and development (retinoic acid) (21, 35). Metabolism of certain cyclic epoxy-xanthophylls in higher plants yields abscisic acid, an important hormone (81). In addition, carotenoids and their derivatives provide pigmentation to many birds, fish, and crustaceans (21).

GENETICS OF CAROTENOID BIOSYNTHESIS IN EUBACTERIA

Studies performed with a few species of purple nonsulfur anoxygenic photosynthetic bacteria (*Rhodobacter capsulatus* and *Rhodobacter sphaeroides*), nonphotosynthetic bacteria (*Erwinia herbicola*, *Erwinia uredovora*, and *Myxococcus xanthus*), and cyanobacteria (*Synechococcus* sp. strain PCC7942, *Synechocystis* sp. strain PCC6803, *Anabaena* sp. strain PCC7120) have contributed enormously to our molecular-genetic understanding of carotenoid biosynthesis. To illustrate the rapid advances in this field, nucleotide sequences of carotenoid biosynthesis genes were first reported in *R. capsulatus* in 1989 (4, 12), *E. herbicola* and *E. uredovora* in 1990 (3, 67), *Synechococcus* sp. strain PCC7942 in 1991 (26), and *M. xanthus* in 1993 (33). The biosynthetic pathways used by these organisms, major carotenoid pigments accumulated, and assignments of gene and gene product functions are summarized here (Fig. 1 and 2 and Table 1) and are discussed in further detail elsewhere (2, 43, 46). To complement earlier surveys of biochemical and classical genetic experiments (20, 40), this minireview will focus on developments within the last five years from a molecular-genetic standpoint.

The *crt* nomenclature (Table 1) proposed in 1976 for the *R. capsulatus* genetic loci required for carotenoid biosynthesis (93) has been maintained in subsequent studies with *Rhodobacter* species, *Erwinia* species, and *Thermus thermophilus*. Genetic loci involved in carotenoid biosynthesis in *M. xanthus* have been designated *car* in a parallel nomenclature from 1987 (10). In cyanobacteria, a proposal to replace the current nomenclature that originated in 1991 with the *crt* nomenclature has recently been made (43). The new proposed gene designations (Table 1) will be employed throughout this minireview to reflect the similarities and differences between cyanobacteria and other eubacteria.

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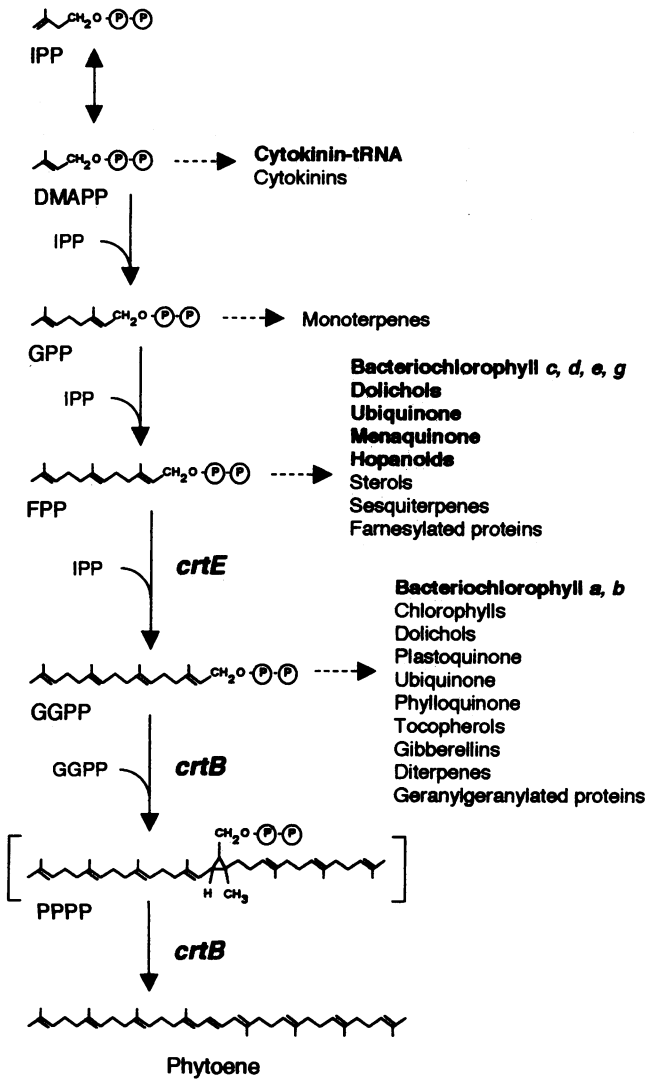

FIG. 1. General isoprenoid biosynthetic pathway. Branches in the pathway dependent on intermediates common to carotenoid biosynthesis are indicated on the right. The bold typeface highlights important compounds found in some or all eubacteria, while substances produced in eukaryotes appear in normal typeface. Abbreviations not given in the text are as follows: DMAPP, dimethylallyl pyrophosphate; GPP, geranyl pyrophosphate; IPP, isopentenyl pyrophosphate, PPPP, prephytoene pyrophosphate. PPPP is an unstable intermediate in the synthesis of phytoene. The genetic loci associated with specific biosynthetic conversions are discussed in the text and listed in Table 1.

Rhodobacter capsulatus and *Rhodobacter sphaeroides*

The first classical genetic studies of eubacterial carotenoid biosynthesis were conducted with *R. sphaeroides* in the 1950s (41, 42). This facultative phototroph and its close relative *R. capsulatus* photosynthesize anoxygenically, obviating the need for carotenoids and thus facilitating the isolation of biosynthetic mutants. Low light intensities or oxygen tensions stimulate photosynthetic membrane formation and carotenoid pigment accumulation in *Rhodobacter* species (2). Research in the subsequent 40 years, summarized below, has revealed that carotenoid biosynthesis requires the products of seven clustered genes (*crtA*, *crtB*, *crtC*, *crtD*, *crtE*, *crtF*, and *crtI*) (Table 1). The corresponding enzymes convert farnesyl pyrophosphate (FPP) to spheroidene in strictly anoxygenic cultures or to spheroidenone in the presence of oxygen (Fig. 1 and 2).

Taking advantage of a genetic recombination system in *R. capsulatus*, mapping of distinct classes of photopigment mutants demonstrated the tight genetic linkage of *crt* and Bchl *a* (*bch*) biosynthetic loci (88, 93). Analysis by conjugation-mediated marker rescue and transposon and interposon mutagenesis of a cloned 46-kb photosynthesis gene supercluster produced an integrated genetic-physical map of the clustered *crtA*, *crtB*, *crtC*, *crtD*, *crtE*, *crtF*, and *crtI* genes and the physically separated *crtJ* gene (7, 38, 39, 60, 91, 95). Nucleotide sequencing of the *R. capsulatus crt* gene cluster (4) (Fig. 3) revealed the presence of an additional open reading frame (ORF) that was designated *crtK* on the basis of an earlier mutational analysis (39). *R. sphaeroides* was also shown to contain a similarly organized *crt* gene cluster (29, 36, 74), which has been partially characterized molecularly (37, 53). In contrast to earlier indications, recent mutational analyses demonstrate that neither *crtJ* (18) (now termed ORF 469) nor *crtK* (53) (now termed ORF 160) participate directly in *Rhodobacter* carotenoid biosynthesis. ORF 469, which may be involved in suppressing Bchl and carotenoid levels (76), encodes a product with some sequence features found in known bacterial transcription factors (1, 2).

Inhibitor and mutant studies led to proposals for a *Rhodobacter* carotenoid biosynthetic pathway from phytoene to the end products (Fig. 2) (38, 40, 88). Analysis of *R. capsulatus crtB* and *crtE* mutants blocked in phytoene accumulation demonstrated that both mutations permitted the synthesis of Bchl in vivo and accumulation of GGPP in vitro, indicating an active isoprenoid biosynthetic pathway through the latter compound (Fig. 1) (7). Partly on the basis of these data, functions were suggested for CrtB in prephytoene pyrophosphate synthesis and CrtE in phytoene synthesis. Subsequent proposals that eubacterial CrtB and CrtE might instead be the phytoene and GGPP synthases, respectively (23, 57, 64), were confirmed by in vivo complementation studies with *Erwinia* and *Synechococcus crt* genes in an *Escherichia coli* host (25, 64, 85) and with a tomato phytoene synthase cDNA in an *R. capsulatus crtB* mutant (14). Interestingly, all *Rhodobacter crtE* mutants, including those containing gross gene disruptions, synthesize Bchl-containing pigment-protein complexes (7, 39, 95). Thus, the effective branchpoint between Bchl *a* and carotenoid biosynthesis may occur even earlier than previously thought (Fig. 1), and carotenoid- and Bchl *a*-specific pools of GGPP may exist.

Of the *Rhodobacter crt* genes, *crtIB* and *crtEF* form multigene operons (Fig. 3), the latter of which also contributes to a superoperon that includes *bch* and other photosynthesis genes (4, 5, 7, 29, 36, 39, 94). The levels of the *R. capsulatus crtA*, *crtC*, *crtD*, *crtE*, and *crtF* mRNAs and the activities of the *crtA* and *crtEF* promoters increase moderately and transiently in response to anaerobiosis, while the *crtIB* operon is unaffected. These changes in gene expression may reflect an increased demand for carotenoid biosynthesis (2). (Over)expression of *R. capsulatus* CrtI in a *crtI* mutant restores the normal carotenoid complement but has no quantitative effect on carotenoid levels (12). Although the *R. capsulatus crt* genes do not direct carotenoid synthesis in *E. coli* (60), introduction of the *R. sphaeroides crt* gene cluster into phylogenetically related noncarotenogenic eubacteria (*Paracoccus denitrificans*, *Agrobacterium tumefaciens*, *Agrobacterium radiobacter*, and *Azotomonas insolita*) leads to carotenoid production (75).

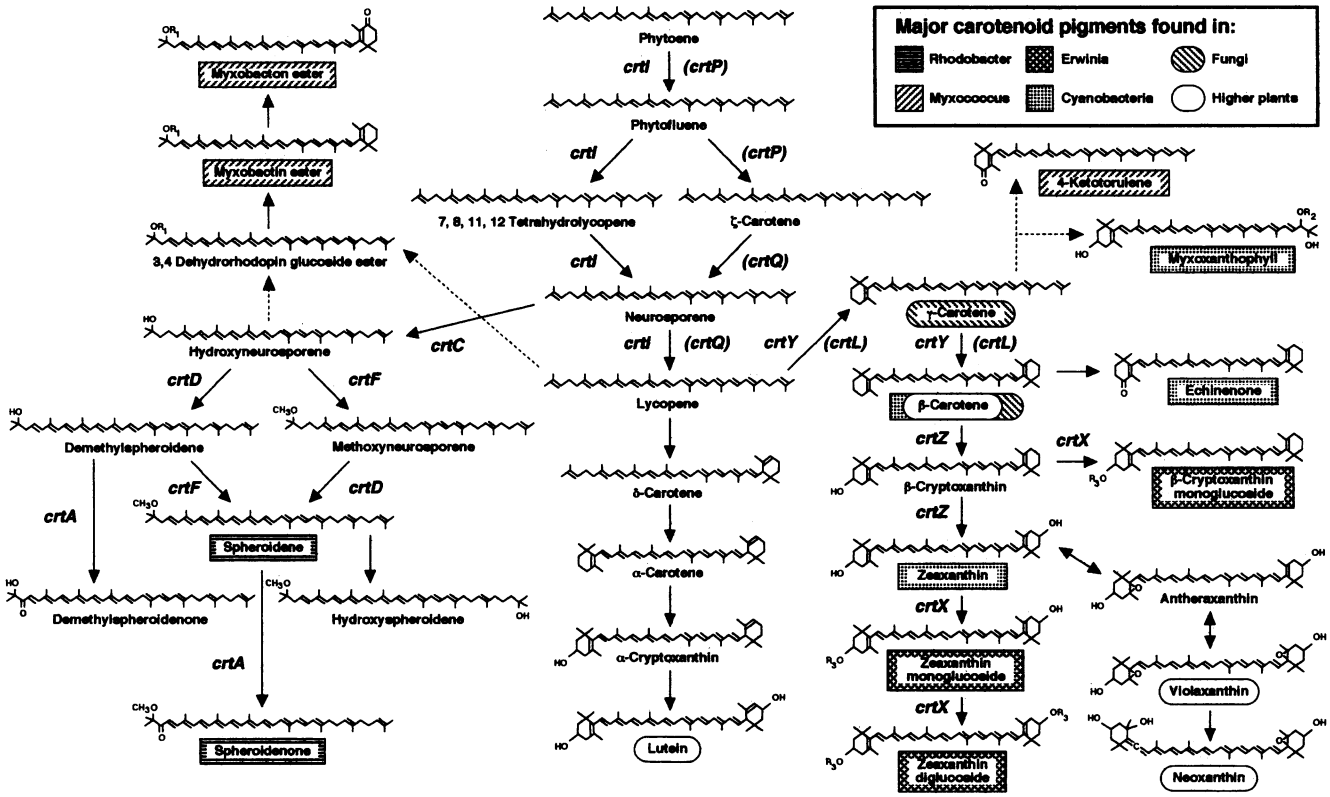

FIG. 2. Major carotenoid biosynthesis pathways. The normal carotenoids accumulated in various classes of organisms are indicated. β-Carotene, for example, occurs as a major pigment in cyanobacteria, plants, and fungi. Structures are presented as all-*trans* isomers for convenience. R_1, glucoside fatty acid ester; R_2, rhamnoside; R_3, glucoside. The dotted lines indicate postulated conversions involving an undetermined number of reactions. The genetic loci associated with specific eubacterial biosynthetic conversions are discussed in the text and listed in Table 1. Parentheses indicate unique cyanobacterial *crt* genes that replace the *crt* genes present in other eubacteria.

Erwinia herbicola and *Erwinia uredovora*

crt genes from yellow-pigmented nonphotosynthetic *Erwinia* species, *E. herbicola* and *E. uredovora*, have been identified by their expression in *E. coli*, a normally noncarotenogenic host (49, 58, 67, 77). The main pigments produced both in these *Erwinia* species and *E. coli* correspond to β-cryptoxanthin monoglucoside and zeaxanthin mono- and diglucosides (Fig. 2) (50, 67). Accumulation of carotenoids in *E. coli* carrying the *E. herbicola* Eho10 *crt* genes requires cyclic AMP and is repressed by glucose (77, 92).

Nucleotide sequencing, mutagenesis, and identification of carotenoid intermediates accumulated in the *E. coli* host have defined six clustered genes (*crtB*, *crtE*, *crtI*, *crtX*, *crtY*, and *crtZ*) (Table 1) involved in the biosynthetic pathway from FPP to the carotenoid glycosides (3, 49, 50, 64, 67, 85). *E. herbicola* Eho10 and *E. uredovora* contain almost identical *crt* gene clusters, with the exception of an intervening ORF in *E. herbicola* (49) (Fig. 3). A minimum of two operons, *crtZ* and *crtE*(ORF 6)*XYIB*, thus encode carotenoid biosynthetic enzymes.

(Over)expression of *Erwinia* CrtB, CrtE, CrtI, CrtX, CrtY, and CrtZ in *E. coli* or *A. tumefaciens* has confirmed their proposed biosynthetic activities (Table 1) (34, 51, 52, 64, 85). Purified *E. uredovora* CrtI can convert 15-*cis*-phytoene to all-*trans*-lycopene, suggesting that *cis-trans* isomerization of carotenoids in vivo occurs nonenzymatically (34). An *E. herbicola crtI* gene transferred to an *R. sphaeroides crtI* mutant directed the synthesis of novel xanthophylls, presumably because *R. sphaeroides* CrtI would normally generate neurosporene rather than lycopene (Fig. 2) (9). Expression of *E. uredovora crt* genes in noncarotenogenic *Zymomonas mobilis* and *A. tumefaciens* has been used to produce β-carotene accumulation in these eubacteria (68).

Myxococcus fulvus and *Myxococcus xanthus*

The nonphotosynthetic bacterium *Myxococcus fulvus* synthesizes 4-ketotorulene, and fatty acid esters of the carotenoid glucosides myxobacton and myxobactin as its major red pigments (Fig. 2) (55). The *M. fulvus* biosynthetic pathway, postulated on the basis of carotenoids accumulated in wild-type and chemically inhibited bacterial cultures, also functions in *M. xanthus* (84).

Classical genetic studies with *M. xanthus* have identified two phenotypic classes of pigmentation mutants, namely, constitutive carotenoid-producing strains and completely carotenoid-deficient strains, and defined several unlinked loci, *carBA*, *carC*, *carD*, and *carR* associated with these phenotypes (46). *carB* encodes an enzyme involved in phytoene synthesis (63), and *carC* encodes phytoene desaturase (33). The linked *carBA* loci have been cloned and this region is being analyzed molecularly (84). Thus far, biosynthetic genes encoding GGPP synthase, phytoene synthase, hydroxyneurosporene synthase, and hydroxyneurosporene desaturase have been identified (Table 1) (70). The total number of genes involved in *M. xanthus* carotenoid biosynthesis remains to be established. In contrast to the *Rhodobacter* and *Erwinia crt* gene clusters, in *M.*

TABLE 1. Eubacterial carotenoid biosynthesis genes and gene products

Gene[a]	Demonstrated or proposed gene product or function	Species
crtA	Spheroidene monooxygenase	*R. capsulatus*,[b,c] *R. sphaeroides*
crtB	Phytoene synthase	*E. herbicola*,[b] *E. uredovora*,[b] *M. xanthus*,[d] *R. capsulatus*,[b] *R. sphaeroides*, *Synechococcus* sp. strain PCC7942,[b,e] *Synechocystis* sp. strain PCC6803,[b,e] *T. thermophilus*[b]
crtC	Hydroxyneurosporene synthase	*M. xanthus*,[d] *R. capsulatus*,[b] *R. sphaeroides*
crtD	Methoxyneurosporene desaturase	*M. xanthus*,[d] *R. capsulatus*,[b,f] *R. sphaeroides*[b]
crtE	GGPP synthase	*E. herbicola*,[b] *E. uredovora*,[b] *M. xanthus*,[d] *R. capsulatus*,[b] *R. sphaeroides*
crtF	Hydroxyneurosporene-*O*-methyltransferase	*R. capsulatus*,[b] *R. sphaeroides*
crtI/*carC*	Phytoene desaturase (CrtI type)	*E. herbicola*,[b] *E. uredovora*,[b] *M. xanthus*,[b] *R. capsulatus*,[b] *R. sphaeroides*
crtL	Lycopene cyclase (CrtL type)	*Synechococcus* sp. strain PCC7942[e]
crtP	Phytoene desaturase (CrtP type)	*Synechococcus* sp. strain PCC7942,[b,e] *Synechocystis* sp. strain PCC6803[b,e]
crtQ	ζ-Carotene desaturase	*Anabaena* sp. strain PCC7120[b,e]
crtX	Zeaxanthin glucosylase	*E. herbicola*,[b] *E. uredovora*[b]
crtY	Lycopene cyclase (CrtY type)	*E. herbicola*,[b] *E. uredovora*[b]
crtZ	β-Carotene hydroxylase	*E. herbicola*,[b] *E. uredovora*[b]
carA	Regulatory	*M. xanthus*
carB	Required for phytoene synthesis	*M. xanthus*[g]
carD	Regulatory	*M. xanthus*
carQ	Regulatory	*M. xanthus*[b]
carR	Regulatory	*M. xanthus*[b]
carS	Regulatory	*M. xanthus*[b]

[a] See text for references. The gene designations *crtG*, *crtH*, *crtJ*, and *crtK* are obsolete (2).
[b] Gene sequence has been reported.
[c] The 3′ portion of the originally reported *crtA* sequence encodes BchI (4, 5, 7), a likely component of an evolutionarily conserved enzyme required for Bchl and Chl synthesis (16, 32).
[d] Identified in a preliminary characterization of the region containing *carBA* (70).
[e] Previous cyanobacterial gene designations were *psy* and *pys* (*crtB*), *lcy* (*crtL*), *pds* (*crtP*), and *zds* (*crtQ*) (43).
[f] The sequence of the *crtD223* mutant allele was reported (3, 4).
[g] May correspond to *crtB* or *crtE*.

xanthus at least two physically unlinked operons, *carBA* and *carC*, encode biosynthetic enzymes (Fig. 3).

The *carA*, *carD*, and *carR* loci exert regulatory functions (46). The translationally coupled and positively light-regulated *carQ*, *carR*, and *carS* regulatory genes have recently been cloned and sequenced from the *carR* region (65). A series of elegant genetic experiments has led to a model for a complex regulatory circuit that controls the blue light-induced accumulation of carotenoids in *M. xanthus* (46). Induction of carotenoid accumulation in *M. xanthus* may involve the generation of singlet oxygen by photoactivated membrane-localized protoporphyrin IX. Singlet oxygen is thought to interact with CarR, which in turn initiates a regulatory cascade involving CarQ, CarS, and CarD that ultimately activates the respective 20- and 400-fold light-inducible *carBA* and *carC* promoters. CarA represses the *carBA* promoter in the dark and stimulates the *carC* promoter in the light. Interestingly, light induces the *carC* promoter only under conditions of carbon starvation (33).

Cyanobacteria

Cyanobacteria typically synthesize β-carotene, zeaxanthin, echinenone, and myxoxanthophyll as their major carotenoid pigments (Fig. 2) (27, 40). *crt* genes encoding the enzymes that convert GGPP to β-carotene (*crtB*, *crtL*, *crtP*, and *crtQ*) (Table 1) have been analyzed by a combination of genetic and molecular techniques (43). Cyanobacterial *crtL* and the combination of *crtP* and *crtQ* replace the functions encoded by *crtY*

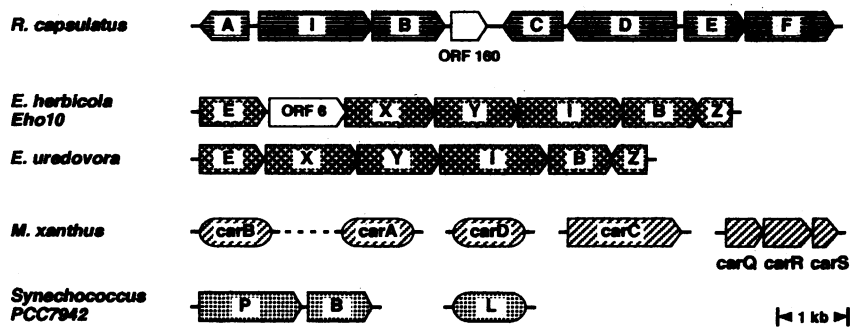

FIG. 3. Organization of eubacterial carotenoid biosynthesis genes. The orientations of *crt* genes (single letters), *car* genes, and ORFs are given when known. Cloned genes for which sequences have not been published are represented by ovals of arbitrary size. Other sequenced *crt* genes not shown are listed in Table 1. The shading of the genes corresponds to that used to highlight specific carotenoids from the same class of organisms (see Fig. 2). The 7-kb region encompassing *carBA* is not drawn to scale.

and *crtI*, respectively, in other eubacteria. The *Synechococcus* sp. strain PCC7942 and *Synechocystis* sp. strain PCC6803 *crtP* genes and the physically linked *crtB* genes were cloned by identifying mutant DNA sequences that conferred resistance to norflurazon (25–27, 61, 62). This bleaching herbicide inhibits phytoene desaturation and causes photooxidative cell death in Chl-containing organisms (22). A lycopene cyclase inhibitor-resistant mutant was similarly used to clone the *Synechococcus crtL* gene (30). Heterologous in vivo complementation of *E. coli* carrying eubacterial *crt* genes has recently been used to identify the *Anabaena* sp. strain PCC7120 *crtQ* gene for ζ-carotene desaturase and to characterize CrtQ (59). This method has also been employed to confirm the enzymatic functions of cyanobacterial CrtB, CrtP, and CrtL (25, 30, 61). The physical arrangement of the cyanobacterial *crtP* and *crtB* genes mirrors that observed for *Rhodobacter* and *Erwinia crtIB* (Fig. 3), although *Synechocystis crtP* and *crtB* are not cotranscribed (25, 26, 61, 62). Whether either *crtL* or *crtQ* is physically linked to *crtP* and *crtB* has not been reported.

EVOLUTIONARY CONSERVATION OF EUBACTERIAL CAROTENOID BIOSYNTHESIS ENZYMES

Carotenoid biosynthetic pathways found in eubacteria, in particular *Erwinia* species, overlap significantly with those of fungi and higher plants (Fig. 1 and 2). Comparison of the predicted amino acid sequences of eubacterial and putative eukaryotic carotenoid biosynthesis enzymes (2, 6, 15, 23, 57, 59, 64, 73) and heterologous hybridization with cyanobacterial DNA probes (15, 73) have helped to identify eukaryotic cDNAs or genes encoding GGPP synthase, phytoene synthase, and phytoene desaturase. In vivo complementation with eukaryotic cDNAs of *R. capsulatus* mutants or *E. coli* strains carrying *Erwinia crt* genes has been used to study the conservation of enzyme functions (11, 13–15, 73, 86). Conversely, tobacco and noncarotenogenic yeast cells have served as hosts for expression of *Erwinia crt* genes (9, 69).

Two distinct types of evolutionarily conserved prenyltransferases, CrtE and CrtB, mediate the early reactions of carotenoid biosynthesis from FPP to phytoene (Fig. 1). Structurally, eubacterial CrtE (GGPP synthase) belongs to a group of eubacterial, archaebacterial, and eukaryotic isoprenyl pyrophosphate synthases that includes other GGPP, as well as FPP and hexaprenylpyrophosphate synthases (2, 6, 8, 28). Eukaryotic CrtE homologs include Al-3 in *Neurospora crassa* (23, 86) and Ggps in bell pepper (57). The genomes of the *Cyanophora paradoxa* cyanelle (66) and the *Porphyra purpurea* red algal plastid (80) encode gene products that may represent homologs of CrtE or rather of structurally related but functionally distinct isoprenyl pyrophosphate synthases (6). Comparing the eubacterial and eukaryotic enzymes, *E. herbicola* Eho10 CrtE can use FPP as an allylic substrate (64), while Al-3 accepts dimethylallyl pyrophosphate (86) and Ggps can convert dimethylallyl pyrophosphate, geranyl pyrophosphate, or FPP into GGPP (Fig. 1) (57).

Eubacterial CrtB (phytoene synthase) corresponds structurally and functionally to Psy in higher plants (2, 3, 6, 13, 14, 19, 78, 79, 82). The sequences of CrtB proteins display 25 to 30% identity with that of the tomato pTOM5 protein (3, 6), encoded by a fruit ripening-associated cDNA (78). Biochemical analysis of transgenic tomato plants expressing antisense pTOM5 mRNA (19) and in vivo complementation of an *R. capsulatus crtB* mutant with a pTOM5-related cDNA confirmed the role of this protein as a phytoene synthase (14). CrtB also shares conserved residues with eukaryotic squalene synthase (64), which condenses two molecules of FPP to produce squalene for sterol biosynthesis (Fig. 1) in a reaction resembling that catalyzed by CrtB.

Phytoene desaturases in eubacteria can be divided into two structurally and functionally distinct groups (Table 1): CrtI type (including CrtI and CarC) and CrtP type. CrtI- and CrtP-type phytoene desaturases are homologous to Al-1 in *N. crassa* (11, 87), and Pds in algae and higher plants, respectively (15, 48, 73). The two enzyme classes, which are thought to have evolved independently (73), differ with respect to their specificities for substrates and products and sensitivities to chemical inhibitors. CrtI-type enzymes synthesize neurosporene or lycopene from phytoene but cannot accept ζ-carotene as a substrate (11, 24, 34), while CrtP-type desaturases produce ζ-carotene from phytoene (15, 48, 73). The differential inhibition of CrtI- and CrtP-type enzymes by norflurazon has been exploited to create herbicide-resistant tobacco by introduction of the gene encoding norflurazon-insensitive *E. uredovora* CrtI (69). The primary structures of eubacterial CrtD (methoxyneurosporene desaturase) and CrtQ (ζ-carotene desaturase) also display significant similarity to those of the CrtI-type enzymes, despite their differences in substrate specificities (Fig. 2 and Table 1) (4, 6, 11, 59). One small region conserved in all carotenoid desaturases corresponds to a βαβ dinucleotide-binding fold predicted to interact with flavin adenine nucleotide (FAD) or NADP (2, 3, 6, 11, 15, 48, 73). In support of this observation, a mutation in this region destroys the activity of *R. capsulatus* CrtD (1, 3). FAD also stimulates the activity of purified *E. uredovora* CrtI (34), and bell pepper Pds contains bound FAD (48).

Erwinia CrtY (49, 52, 67) and *Synechococcus* CrtL (30) represent two separate classes of lycopene cyclases on the basis of their deduced sequences (44), although both catalyze β-ring cyclizations (Fig. 2). Furthermore, a distinct lycopene cyclase probably catalyzes the synthesis of the ε-ring of δ-carotene (20, 22). DNA-DNA hybridization suggests the existence of algal and higher plant homologs of CrtL (30).

Several other eubacterial carotenoid biosynthesis enzymes not found in eukaryotes also share conserved sequence motifs with other proteins. *R. capsulatus* CrtF catalyzes an *S*-adenosylmethionine-dependent methylation reaction restricted to a few species of anoxygenic photosynthetic bacteria (Fig. 2) (40, 88). The primary sequences of a number of noncarotenogenic eubacterial, plant, and animal *O*-methyltransferases display significant identity with that of CrtF, including conservation of a putative *S*-adenosylmethionine-binding site (2). *E. herbicola* Eho10 CrtX shares a conserved region that may be a UDP-binding site with noncarotenogenic eukaryotic enzymes that also interact with UDP-glucosyl moieties (51).

SUMMARY AND CONCLUSIONS

The opportunities to understand eubacterial carotenoid biosynthesis and apply the lessons learned in this field to eukaryotes have improved dramatically in the last several years. On the other hand, many questions remain. Although the pigments illustrated in Fig. 2 represent only a small fraction of the carotenoids found in nature, the characterization of eubacterial genes required for their biosynthesis has not yet been completed. Identifying those eukaryotic carotenoid biosynthetic mutants, genes, and enzymes that have no eubacterial counterparts will also prove essential for a full description of the biochemical pathways (81). Eubacterial *crt* gene regulation has not been studied in detail, with the notable exceptions of *M. xanthus* and *R. capsulatus* (5, 33, 39, 45, 46, 84). Determination of the rate-limiting reaction(s) in carotenoid biosynthesis has thus far yielded species-specific results (12, 27, 47, 69),

and the mechanisms of many of the biochemical conversions remain obscure. Predicted characteristics of some carotenoid biosynthesis gene products await confirmation by studying the purified proteins.

Despite these challenges, (over)expression of eubacterial or eukaryotic carotenoid genes in heterologous hosts has already created exciting possibilities for the directed manipulation of carotenoid levels and content. Such efforts could, for example, enhance the nutritional value of crop plants or yield microbial production of novel and desirable pigments. In the future, the functional compatibility of enzymes from different organisms will form a central theme in the genetic engineering of carotenoid pigment biosynthetic pathways.

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ADDENDUM IN PROOF

The heterologous expression of *E. herbicola* Eho10 *crt* genes in *R. sphaeroides* (C. N. Hunter, B. S. Hundle, J. E. Hearst, H. P. Lang, A. T. Gardiner, S. Takaichi, and R. J. Cogdell, J. Bacteriol. **176:**3692–3697, 1994), the characterization of an *R. sphaeroides* photopigment biosynthesis regulatory gene (R. J. Penfold and J. M. Pemberton, J. Bacteriol. **176:**2869–2876, 1994), and the nucleotide sequence of the *E. herbicola* Eho13 *crt* gene cluster (K.-Y. To, E.-M. Lai, L.-Y. Lee, T.-P. Lin, C.-H. Hung, C.-L. Chen, Y.-S. Chang, and S.-T. Liu, J. Gen. Microbiol. **140:**331–339, 1994) have recently been reported.

REFERENCES

1. **Alberti, M., D. H. Burke, and J. E. Hearst.** Structure and sequence of the photosynthesis gene cluster. *In* R. E. Blankenship, M. T. Madigan, and C. E. Bauer (ed.), Anoxygenic photosynthetic bacteria; advances in photosynthesis, in press. Kluwer Academic Publishers, Dordrecht, The Netherlands.
2. **Armstrong, G. A.** Genetic analysis and regulation of carotenoid biosynthesis. *In* R. E. Blankenship, M. T. Madigan, and C. E. Bauer (ed.), Anoxygenic photosynthetic bacteria; advances in photosynthesis, in press. Kluwer Academic Publishers, Dordrecht, The Netherlands.
3. **Armstrong, G. A., M. Alberti, and J. E. Hearst.** 1990. Conserved enzymes mediate the early reactions of carotenoid biosynthesis in nonphotosynthetic and photosynthetic prokaryotes. Proc. Natl. Acad. Sci. USA **87:**9975–9979.
4. **Armstrong, G. A., M. Alberti, F. Leach, and J. E. Hearst.** 1989. Nucleotide sequence, organization, and nature of the protein products of the carotenoid biosynthesis gene cluster of *Rhodobacter capsulatus*. Mol. Gen. Genet. **216:**254–268.
5. **Armstrong, G. A., D. N. Cook, D. Ma, M. Alberti, D. H. Burke, and J. E. Hearst.** 1993. Regulation of carotenoid and bacteriochlorophyll biosynthesis genes and identification of an evolutionarily conserved gene required for bacteriochlorophyll accumulation. J. Gen. Microbiol. **139:**897–906.
6. **Armstrong, G. A., B. Hundle, and J. E. Hearst.** 1993. Evolutionary conservation and structural similarities of carotenoid biosynthesis gene products from photosynthetic and nonphotosynthetic organisms. Methods Enzymol. **214:**297–311.
7. **Armstrong, G. A., A. Schmidt, G. Sandmann, and J. E. Hearst.** 1990. Genetic and biochemical characterization of carotenoid biosynthesis mutants of *Rhodobacter capsulatus*. J. Biol. Chem. **265:**8329–8338.
8. **Ashby, M. N., and P. A. Edwards.** 1990. Elucidation of the deficiency in two yeast coenzyme Q mutants. J. Biol. Chem. **265:**13157–13164.
9. **Ausich, R. L. (Amoco Technology Co., Naperville, Ill.).** 1994. Personal communication.
10. **Balsalobre, J. M., R. M. Ruiz-Vázquez, and F. J. Murillo.** 1987. Light induction of gene expression in *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA **84:**2359–2362.
11. **Bartley, G. E., T. J. Schmidhauser, C. Yanofsky, and P. A. Scolnik.** 1990. Carotenoid desaturases from *Rhodobacter capsulatus* and *Neurospora crassa* are structurally and functionally conserved and contain domains homologous to flavoprotein disulfide oxidoreductases. J. Biol. Chem. **265:**16020–16024.
12. **Bartley, G. E., and P. A. Scolnik.** 1989. Carotenoid biosynthesis in photosynthetic bacteria. J. Biol. Chem. **264:**13109–13113.
13. **Bartley, G. E., and P. A. Scolnik.** 1993. cDNA cloning, expression during development, and genome mapping of *Psy2*, a second tomato gene encoding phytoene synthase. J. Biol. Chem. **268:**25718–25721.
14. **Bartley, G. E., P. V. Viitanen, K. O. Bacot, and P. A. Scolnik.** 1992. A tomato gene expressed during fruit ripening encodes an enzyme of the carotenoid biosynthesis pathway. J. Biol. Chem. **267:**5036–5039.
15. **Bartley, G. E., P. V. Viitanen, I. Pecker, D. Chamovitz, J. Hirschberg, and P. A. Scolnik.** 1991. Molecular cloning and expression in photosynthetic bacteria of a soybean cDNA coding for phytoene desaturase, an enzyme of the carotenoid biosynthesis pathway. Proc. Natl. Acad. Sci. USA **88:**6532–6536.
16. **Bauer, C. E., D. W. Bollivar, and J. Y. Suzuki.** 1993. Genetic analysis of photopigment biosynthesis in eubacteria: a guiding light for algae and plants. J. Bacteriol. **175:**3919–3925.
17. **Beyer, P., M. Mayer, and H. Kleinig.** 1989. 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:**141–150.
18. **Bollivar, D. W., J. Y. Suzuki, J. T. Beatty, J. M. Dobrowolski, and C. E. Bauer.** 1994. Directed mutational analysis of bacteriochlorophyll *a* biosynthesis in *Rhodobacter capsulatus*. J. Mol. Biol. **237:**622–640.
19. **Bramley, P., C. Teulieres, I. Blain, C. Bird, and W. Schuch.** 1992. Biochemical characterization of transgenic tomato plants in which carotenoid synthesis has been inhibited through the expression of antisense RNA to pTOM5. Plant J. **2:**343–349.
20. **Bramley, P. M., and A. Mackenzie.** 1988. Regulation of carotenoid biosynthesis. Curr. Top. Cell. Regul. **29:**291–343.
21. **Britton, G.** 1983. The biochemistry of natural pigments. Cambridge University Press, Cambridge.
22. **Britton, G.** 1993. Carotenoids in chloroplast pigment-protein complexes, p. 447–484. *In* C. Sundqvist and M. Ryberg (ed.), Pigment-protein complexes in plastids: synthesis and assembly. Academic Press, San Diego, Calif.
23. **Carattoli, A., N. Romano, P. Ballario, G. Morelli, and G. Macino.** 1991. The *Neurospora crassa* carotenoid biosynthetic gene (albino 3) reveals highly conserved regions among prenyltransferases. J. Biol. Chem. **266:**5854–5859.
24. **Chamovitz, D.** 1993. Ph.D. thesis. The Hebrew University of Jerusalem, Jerusalem, Israel.
25. **Chamovitz, D., N. Misawa, G. Sandmann, and J. Hirschberg.** 1992. Molecular cloning and expression in *Escherichia coli* of a cyanobacterial gene coding for phytoene synthase, a carotenoid biosynthesis enzyme. FEBS Lett. **296:**305–310.
26. **Chamovitz, D., I. Pecker, and J. Hirschberg.** 1991. The molecular basis of resistance to the herbicide norflurazon. Plant Mol. Biol. **16:**967–974.
27. **Chamovitz, D., G. Sandmann, and J. Hirschberg.** 1993. Molecular and biochemical characterization of herbicide-resistant mutants of cyanobacteria reveal that phytoene desaturation is a rate-limiting step in carotenoid biosynthesis. J. Biol. Chem. **268:**17348–17353.
28. **Chen, A., P. A. Kroon, and C. D. Poulter.** 1994. Isoprenyl diphosphate synthases: protein sequence comparisons, a phylogenetic tree, and predictions of secondary structure. Protein Sci. **3:**600–607.

29. **Coomber, S. A., M. Chaudri, A. Connor, G. Britton, and C. N. Hunter.** 1990. Localized transposon Tn5 mutagenesis of the photosynthetic gene cluster of *Rhodobacter sphaeroides*. Mol. Microbiol. **4**:977–989.
30. **Cunningham, F. X., Jr., D. Chamovitz, N. Misawa, E. Gantt, and J. Hirschberg.** 1993. Cloning and functional expression in *Escherichia coli* of a cyanobacterial gene for lycopene cyclase, the enzyme that catalyzes the biosynthesis of β-carotene. FEBS Lett. **328**:130–138.
31. **Deruère, J., S. Römer, A. d'Harlingue, R. A. Backhaus, M. Kuntz, and B. Camara.** 1994. Fibril assembly and carotenoid overaccumulation in chromoplasts: a model for supramolecular lipoprotein structures. Plant Cell **6**:119–133.
32. **Falbel, T. (University of Colorado, Boulder).** 1994. Personal communication.
33. **Fontes, M., R. Ruiz-Vázquez, and F. J. Murillo.** 1993. Growth phase dependence of the activation of a bacterial gene for carotenoid synthesis by blue light. EMBO J. **12**:1265–1275.
34. **Fraser, P. D., N. Misawa, H. Linden, S. Yamano, K. Kobayashi, and G. Sandmann.** 1992. Expression in *Escherichia coli*, purification, and reactivation of the recombinant *Erwinia uredovora* phytoene desaturase. J. Biol. Chem. **267**:19891–19895.
35. **Frickel, F.** 1985. Retinoids: an overview of some natural carotenoid metabolites and their synthetic analogs. Pure Appl. Chem. **57**:709–716.
36. **Garí, E., I. Gibert, and J. Barbé.** 1992. Spontaneous and reversible high-frequency frameshifts originating a phase transition in the carotenoid biosynthesis pathway of the phototrophic bacterium *Rhodobacter sphaeroides* 2.4.1. Mol. Gen. Genet. **232**:74–80.
37. **Garí, E., J. C. Toledo, I. Gibert, and J. Barbé.** 1992. Nucleotide sequence of the methoxyneurosporene dehydrogenase gene from *Rhodobacter sphaeroides*: comparison with other bacterial carotenoid dehydrogenases. FEMS Microbiol. Lett. **93**:103–108.
38. **Giuliano, G., D. Pollock, and P. A. Scolnik.** 1986. The *crtI* gene mediates the conversion of phytoene into colored carotenoids in *Rhodopseudomonas capsulata*. J. Biol. Chem. **261**:12925–12929.
39. **Giuliano, G., D. Pollock, H. Stapp, and P. A. Scolnik.** 1988. A genetic-physical map of the *Rhodobacter capsulatus* carotenoid biosynthesis gene cluster. Mol. Gen. Genet. **213**:78–83.
40. **Goodwin, T. W.** 1980. The biochemistry of the carotenoids, vol. 1. Plants. Chapman and Hall, New York.
41. **Griffiths, M., W. R. Sistrom, G. Cohen-Bazire, and R. Y. Stanier.** 1955. Function of carotenoids in photosynthesis. Nature (London) **176**:1211–1214.
42. **Griffiths, M., and R. Y. Stanier.** 1956. Some mutational changes in the photosynthetic pigment system of *Rhodopseudomonas spheroides*. J. Gen. Microbiol. **14**:698–715.
43. **Hirschberg, J., and D. Chamovitz.** Carotenoids in cyanobacteria. *In* D. Bryant (ed.), The molecular biology of the cyanobacteria, in press. Kluwer Academic Publishers, Dordrecht, The Netherlands.
44. **Hirschberg, J. (The Hebrew University of Jerusalem, Jerusalem, Israel).** 1994. Personal communication.
45. **Hodgson, D. A.** 1993. Light-induced carotenogenesis in *Myxococcus xanthus*: genetic analysis of the *carR* region. Mol. Microbiol. **7**:471–488.
46. **Hodgson, D. A., and F. J. Murillo.** 1993. Genetics of regulation and pathway of synthesis of carotenoid, p. 157–181. *In* M. Dworkin and D. Kaiser (ed.), Myxobacteria II. American Society for Microbiology, Washington, D.C.
47. **Hoshino, T., R. Fujii, and T. Nakahara.** 1993. Molecular cloning and sequence analysis of the *crtB* gene of *Thermus thermophilus* HB27, an extreme thermophile producing carotenoid pigments. Appl. Environ. Microbiol. **59**:3150–3153.
48. **Hugueney, P., S. Römer, and B. Camara.** 1992. Characterization and molecular cloning of a flavoprotein catalyzing the synthesis of phytofluene and ζ-carotene in *Capsicum* chromoplasts. Eur. J. Biochem. **209**:399–407.
49. **Hundle, B., M. Alberti, V. Nievelstein, P. Beyer, H. Kleinig, G. A. Armstrong, D. Burke, and J. E. Hearst.** Functional assignment of *Erwinia herbicola* Eho10 carotenoid genes expressed in *Escherichia coli*. Mol. Gen. Genet., in press.
50. **Hundle, B. S., P. Beyer, H. Kleinig, G. Englert, and J. E. Hearst.** 1991. Carotenoids of *Erwinia herbicola* and an *Escherichia coli* HB101 strain carrying the *Erwinia herbicola* carotenoid gene cluster. Photochem. Photobiol. **54**:89–93.
51. **Hundle, B. S., D. A. O'Brien, M. Alberti, P. Beyer, and J. E. Hearst.** 1992. Functional expression of zeaxanthin glucosyltransferase from *Erwinia herbicola* and a proposed uridine diphosphate binding site. Proc. Natl. Acad. Sci. USA **89**:9321–9325.
52. **Hundle, B. S., D. A. O'Brien, P. Beyer, H. Kleinig, and J. E. Hearst.** 1993. In vitro expression and activity of lycopene cyclase and β-carotene hydroxylase from *Erwinia herbicola*. FEBS Lett. **315**:329–334.
53. **Kaplan, S. (University of Texas, Houston).** 1994. Personal communication.
54. **Kläui, H.** 1982. Industrial and commercial uses of carotenoids, p. 309–317. *In* G. Britton and T. W. Goodwin (ed.), IUPAC carotenoid chemistry and biochemistry. Pergamon Press, Oxford.
55. **Kleinig, H.** 1975. On the utilization *in vivo* of lycopene and phytoene as precursors for the formation of carotenoid glucoside ester and on the regulation of carotenoid biosynthesis in *Myxococcus fulvus*. Eur. J. Biochem. **57**:301–308.
56. **Kühlbrandt, W., D. N. Wang, and Y. Fujiyoshi.** 1994. Atomic model of plant light-harvesting complex by electron crystallography. Nature (London) **367**:614–621.
57. **Kuntz, M., S. Römer, C. Suire, P. Hugueney, J. H. Weil, R. Schantz, and B. Camara.** 1992. Identification of a cDNA for the plastid-located geranylgeranyl pyrophosphate synthase from *Capsicum annuum* correlative increase in enzyme activity and transcript level during fruit ripening. Plant J. **2**:25–34.
58. **Lee, L.-Y., and S.-T. Liu.** 1991. Characterization of the yellow-pigment genes of *Erwinia herbicola*. Mol. Microbiol. **5**:217–224.
59. **Linden, H., N. Misawa, T. Saito, and G. Sandmann.** 1994. A novel carotenoid biosynthesis gene coding for ζ-carotene desaturase: functional expression, sequence and phylogenetic origin. Plant Mol. Biol. **24**:369–379.
60. **Marrs, B.** 1981. Mobilization of the genes for photosynthesis from *Rhodopseudomonas capsulata* by a promiscuous plasmid. J. Bacteriol. **146**:1003–1012.
61. **Martínez-Férez, I. M., B. Fernández-González, G. Sandmann, and A. Vioque.** 1994. Cloning and expression in *Escherichia coli* of the gene coding for phytoene synthase from the cyanobacterium *Synechocystis* sp. PCC6803. Biochim. Biophys. Acta **1218**:145–152.
62. **Martínez-Férez, I. M., and A. Vioque.** 1992. Nucleotide sequence of the phytoene desaturase gene from *Synechocystis* sp. PCC6803 and characterization of a new mutation which confers resistance to the herbicide norflurazon. Plant Mol. Biol. **18**:981–983.
63. **Martínez-Laborda, A., J. M. Balsalobre, M. Fontes, and F. J. Murillo.** 1990. Accumulation of carotenoids in structural and regulatory mutants of the bacterium *Myxococcus xanthus*. Mol. Gen. Genet. **223**:205–210.
64. **Math, S. K., J. E. Hearst, and C. D. Poulter.** 1992. The *crtE* gene in *Erwinia herbicola* encodes geranylgeranyl diphosphate synthase. Proc. Natl. Acad. Sci. USA **89**:6761–6764.
65. **McGowan, S. J., H. C. Gorham, and D. A. Hodgson.** 1993. Light-induced carotenogenesis in *Myxococcus xanthus*: DNA sequence analysis of the *carR* region. Mol. Microbiol. **10**:713–735.
66. **Michalowski, C. B., W. Löffelhardt, and H. J. Bohnert.** 1991. An ORF323 with homology to *crtE*, specifying prephytoene pyrophosphate dehydrogenase, is encoded by cyanelle DNA in the eukaryotic alga *Cyanophora paradoxa*. J. Biol. Chem. **266**:11866–11870.
67. **Misawa, N., M. Nakagawa, K. Kobayashi, S. Yamano, Y. Izawa, K. Nakamura, and K. Harashima.** 1990. Elucidation of the *Erwinia uredovora* carotenoid biosynthetic pathway by functional analysis of gene products expressed in *Escherichia coli*. J. Bacteriol. **172**:6704–6712.
68. **Misawa, N., S. Yamano, and H. Ikenaga.** 1991. Production of β-carotene in *Zymomonas mobilis* and *Agrobacterium tumifaciens* by introduction of the biosynthesis genes from *Erwinia uredovora*. Appl. Environ. Microbiol. **57**:1847–1849.
69. **Misawa, N., S. Yamano, H. Linden, M. R. de Felipe, M. Lucas, H. Ikenaga, and G. Sandmann.** 1993. Functional expression of the *Erwinia uredovora* carotenoid biosynthetic gene *crtI* in transgenic plants showing an increase in β-carotene biosynthesis activity and resistance to the bleaching herbicide norflurazon. Plant J. **4**:833–840.

70. **Murillo, F. J. (Universidad de Murcia, Murcia, Spain).** 1994. Personal communication.
71. **Omer, C. A., and J. B. Gibbs.** 1994. Protein prenylation in eukaryotic microorganisms: genetics, biology and biochemistry. Mol. Microbiol. **11:**219–225.
72. **Ourisson, G., M. Rohmer, and K. Poralla.** 1987. Prokaryotic hopanoids and other polyterpenoid sterol surrogates. Annu. Rev. Microbiol. **41:**301–333.
73. **Pecker, I., D. Chamovitz, H. Linden, G. Sandmann, and J. Hirschberg.** 1992. A single polypeptide catalyzing the conversion of phytoene to ζ-carotene is transcriptionally regulated during tomato fruit ripening. Proc. Natl. Acad. Sci. USA **89:**4962–4966.
74. **Pemberton, J. M., and C. M. Harding.** 1986. Cloning of carotenoid biosynthesis genes from *Rhodopseudomonas sphaeroides*. Curr. Microbiol. **14:**25–29.
75. **Pemberton, J. M., and C. M. Harding.** 1987. Expression of *Rhodopseudomonas sphaeroides* carotenoid photopigment genes in phylogenetically related nonphotosynthetic bacteria. Curr. Microbiol. **15:**67–71.
76. **Penfold, R. J., and J. M. Pemberton.** 1991. A gene from the photosynthetic gene cluster of *Rhodobacter sphaeroides* induces *trans* suppression of bacteriochlorophyll and carotenoid levels in *R. sphaeroides* and *R. capsulatus*. Curr. Microbiol. **23:**259–263.
77. **Perry, K. L., T. A. Simonitch, K. J. Harrision-Lavoie, and S.-T. Liu.** 1986. Cloning and regulation of *Erwinia herbicola* pigment genes. J. Bacteriol. **168:**607–612.
78. **Ray, J., C. Bird, M. Maunders, D. Grierson, and W. Schuch.** 1987. Sequence of pTOM5, a ripening related cDNA from tomato. Nucleic Acids Res. **15:**10587.
79. **Ray, J., P. Moureau, C. Bird, A. Bird, D. Grierson, M. Maunders, M. Truesdale, P. Bramley, and W. Schuch.** 1992. Cloning and characterization of a gene involved in phytoene synthesis from tomato. Plant Mol. Biol. **19:**401–404.
80. **Reith, M., and J. Munholland.** 1993. A high-resolution gene map of the chloroplast genome of the red alga *Porphyra purpurea*. Plant Cell **5:**465–475.
81. **Rock, C. D., and J. A. D. Zeevaart.** 1991. The *aba* mutant of *Arabidopsis thaliana* is impaired in epoxy-carotenoid biosynthesis. Proc. Natl. Acad. Sci. USA **88:**7496–7499.
82. **Römer, S., P. Hugueney, F. Bouvier, B. Camara, and M. Kuntz.** 1994. Expression of the genes encoding the early carotenoid biosynthetic enzymes in *Capsicum annuum*. Biochem. Biophys. Res. Commun. **196:**1414–1421.
83. **Rüdiger, W., and S. Schoch.** 1991. The last steps of chlorophyll biosynthesis, p. 451–464. *In* H. Scheer (ed.), Chlorophylls. CRC Press, Boca Raton, Fla.
84. **Ruiz-Vázquez, R., M. Fontes, and F. J. Murillo.** 1993. Clustering and co-ordinated activation of carotenoid genes in *Myxococcus xanthus* by blue light. Mol. Microbiol. **10:**25–34.
85. **Sandmann, G., and N. Misawa.** 1992. New functional assignment of the carotenogenic genes *crtB* and *crtE* with constructs of these genes from *Erwinia* species. FEMS Microbiol. Lett. **90:**253–258.
86. **Sandmann, G., N. Misawa, M. Wiedemann, P. Vittorioso, A. Carattoli, G. Morelli, and G. Macino.** 1993. Functional identification of *al-3* from *Neurospora crassa* as the gene for geranylgeranyl pyrophosphate synthase by complementation with *crt* genes, *in vitro* characterization of the gene product and mutant analysis. J. Photochem. Photobiol. B Biol. **18:**245–251.
87. **Schmidhauser, T. J., F. R. Lauter, V. E. A. Russo, and C. Yanofsky.** 1990. Cloning, sequence, and photoregulation of *al-1*, a carotenoid biosynthetic gene of *Neurospora crassa*. Mol. Cell. Biol. **10:**5064–5070.
88. **Scolnik, P. A., M. A. Walker, and B. L. Marrs.** 1980. Biosynthesis of carotenoids derived from neurosporene in *Rhodopseudomonas capsulata*. J. Biol. Chem. **255:**2427–2432.
89. **Sherman, M. M., L. A. Petersen, and C. D. Poulter.** 1989. Isolation and characterization of isoprene mutants of *Escherichia coli*. J. Bacteriol. **171:**3619–3628.
90. **Straub, O.** 1987. List of carotenoids, p. 11–296. *In* H. Pfander (ed.), Key to carotenoids, 2nd ed. Birkhäuser Verlag, Basel.
91. **Taylor, D. P., S. N. Cohen, W. G. Clark, and B. L. Marrs.** 1983. Alignment of the genetic and restriction maps of the photosynthesis region of the *Rhodopseudomonas capsulata* chromosome by a conjugation-mediated marker rescue technique. J. Bacteriol. **154:** 580–590.
92. **Tuveson, R. W., R. A. Larson, and J. Kagan.** 1988. Role of cloned carotenoid genes expressed in *Escherichia coli* in protection against inactivation by near-UV light and specific phototoxic molecules. J. Bacteriol. **170:**4675–4680.
93. **Yen, H. C., and B. Marrs.** 1976. Map of genes for carotenoid and bacteriochlorophyll biosynthesis in *Rhodopseudomonas capsulata*. J. Bacteriol. **126:**619–629.
94. **Young, D. A., C. E. Bauer, J. C. Williams, and B. L. Marrs.** 1989. Genetic evidence for superoperonal organization of genes for photosynthetic pigments and pigment-binding proteins in *Rhodobacter capsulatus*. Mol. Gen. Genet. **218:**1–12.
95. **Zsebo, K. M., and J. E. Hearst.** 1984. Genetic-physical mapping of a photosynthetic gene cluster from *R. capsulata*. Cell **37:**937–947.