CAROTENOIDS 2

Genetics and molecular biology of carotenoid pigment biosynthesis

GREGORY A. ARMSTRONG,*[,1] AND JOHN E. HEARST[†]

*Institute for Plant Sciences, Plant Genetics, Swiss Federal Institute of Technology, CH-8092 Zurich, Switzerland; and [†]Department of Chemistry, University of California, and Structural Biology Division, Lawrence Berkeley Laboratory, Berkeley, California 94720, USA

The two major functions of carotenoids in photosynthetic microorganisms and plants are the absorption of energy for use in photosynthesis and the protection of chlorophyll from photodamage. The synthesis of various carotenoids, therefore, is a crucial metabolic process underlying these functions. In this second review, the nature of these biosynthetic pathways is discussed in detail. In their elucidation, molecular biological techniques as well as conventional enzymology have played key roles. The reasons for some of the *cis-trans* isomerizations in the pathway are obscure, however, and much still needs to be learned about the regulation of carotenoid biosynthesis. Recent important findings, as summarized in this review, have laid the groundwork for such studies.

—James Olson, Coordinating Editor

ABSTRACT **The crucial roles of carotenoids and their metabolites in photooxidative protection and photosynthesis, not to mention nutrition, vision, and cellular differentiation, make them an important and complex class of biological pigments. Significant advances within the last few years have enhanced our understanding of the genetics and molecular biology of carotenoid biosynthesis in bacteria, fungi, algae, and plants. All of the genes involved in carotenoid biosynthesis from *Rhodobacter capsulatus*, an anoxygenic photosynthetic bacterium, and from several species of *Erwinia*, nonphotosynthetic bacteria, have been molecularly characterized. Recent studies have revealed that two early enzymes of carotenoid biosynthesis, geranylgeranyl pyrophosphate synthase and phytoene synthase, are structurally and functionally related in all carotenogenic organisms. In contrast, the subsequent conversion of phytoene, the first C_{40} carotenoid, to β-carotene requires two desaturases and one cyclase in oxygenic photosynthetic organisms (cyanobacteria, algae, and higher plants) but only one structurally distinct desaturase and a structurally distinct cyclase in other carotenogenic bacteria and in fungi. Studies of the enzymes that introduce oxygen-containing functional groups into carotenes to produce xanthophylls, the vast majority of all carotenoids, are still in their infancy. This review summarizes the most recent developments in carotenoid biosynthesis from a molecular genetic standpoint.—Armstrong, G. A., Hearst, J. E. Genetics and molecular biology of carotenoid pigment biosynthesis. *FASEB J.* 10, 228–237 (1996)**

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CAROTENOIDS REPRESENT ONE OF THE most fascinating, abundant, and widely distributed classes of natural pigments. Photosynthetic organisms from anoxygenic photosynthetic bacteria through cyanobacteria, algae, and higher plants, as well as numerous nonphotosynthetic bacteria and fungi, produce carotenoids (1). Among higher plants, these pigments advertise themselves in flowers, fruits, and storage roots exemplified by the yellow, orange, and red pigments of daffodils, carrots and tomatoes, respectively. In green plant tissues, carotenoids become evident only during the annual degradation of chlorophyll in the autumn. Both unmodified and metabolized dietary carotenoids also serve as natural colorants in organisms that are not themselves carotenogenic, such as crustaceans, insects, fish, and birds (2). A few prominent examples include the pigments found in egg yolks, lobster shells, salmon flesh, and flamingo plumage.

Wackenroder first proposed the name "carotene" in 1831 in describing the pigment he had isolated and crystallized from carrot roots. In 1837, Berzelius coined the term "xanthophyll" to denote chemically a yellow pigment he had extracted from senescent leaves. Tswett, recognizing the chemically related nature of the compounds known as carotenes and xanthophylls, created the designation "carotenoids" in 1911 to encompass both classes of pigments (3). Today, "carotene" is used to refer to a hydrocarbon carotenoid, and xanthophyll denotes a carotene derivatized with one or more oxygen-containing functional groups. A century and a half after Wackenroder's isolation of the first carotene, 563 structurally distinct carotenoids and their glycosides, not to mention isomers, had been chemically characterized (4) and approximately 60 new structures have since been described.

[1]To whom correspondence and reprint requests should be addressed, at: Institute for Plant Sciences, Plant Genetics, Swiss Federal Institute of Technology (ETH), CH-8092 Zürich, Switzerland.

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STRUCTURES OF CAROTENOIDS

Most carotenoids contain a linear C_{40} hydrocarbon backbone that includes between 3 and 15 conjugated double bonds (1, 5, 6). The number of double bonds largely determines the spectral properties of a given carotenoid, which typically absorbs light between 400 and 500 nm. A critical step in the formation of the first C_{40} acyclic hydrocarbon carotenoid, phytoene, is the tail-to-tail condensation of two molecules of the C_{20} intermediate geranylgeranyl pyrophosphate (GGPP).[2] This molecule arises from the head-to-tail condensations of four C_5 isoprene units derived from the general isoprenoid biosynthetic pathway (**Fig. 1**). Some organisms produce partially degraded pigments known as apocarotenoids or norcarotenoids, and a few bacteria synthesize C_{45} or C_{50} carotenoids by further isoprene additions to the C_{40} backbone (1, 5, 6). C_{30} carotenoids synthesized by the tail-to-tail condensation of two molecules of the C_{15} isoprenoid intermediate farnesyl pyrophosphate (FPP), rather than GGPP, also occur in some bacteria (7). In general, however, phytoene serves as the classical precursor for other carotenoids. Most organisms, particularly higher plants, algae, and fungi, synthesize 15, 15′-*cis*-phytoene, although some microbes produce mixtures of isomers that can include all-*trans*-and 9-*cis*-phytoene (5, 6, 8).

A series of desaturation and cyclization reactions converts phytoene into cyclic carotenes, such as β-carotene (**Fig. 2**). The successive introduction of conjugated double bonds during this process lengthens the chromophore, producing colored carotenoids beginning with ζ-carotene. Lycopene, cyclic carotenes, and xanthophylls usually exist in the all-*trans* configuration, indicating that at least one isomerization step must occur (1, 5, 6). The cis to all-trans conversion does not, however, appear to require a distinct isomerase activity (9–12).

Carotenoid biosynthesis as a whole can be roughly thought of as an inverted tree, with the trunk representing the early reactions common to all organisms and the branches symbolizing the remarkable variety of xanthophylls that arise by the species-specific introduction of oxygen functionalities into acyclic (**Fig. 3**) and cyclic carotenes (**Fig. 4**). These oxygen functionalities, represented by hydroxy, methoxy, oxo, epoxy, carboxy, and aldehydic groups, thus provide most of the basis for the structural diversity observed among the carotenoids (1, 4).

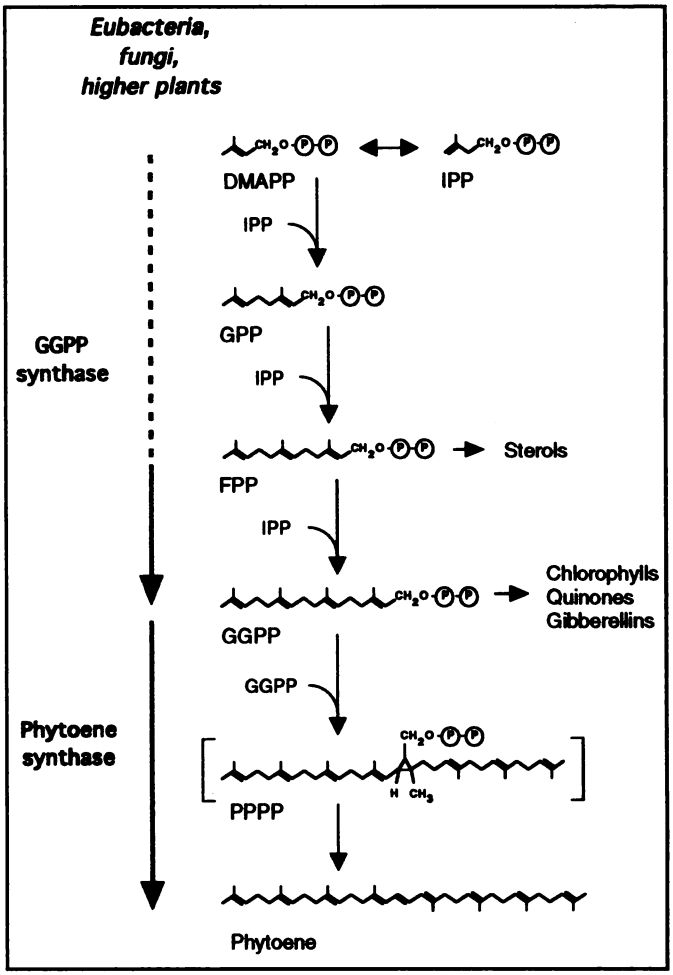

Figure 1. Biosynthesis of phytoene from the general isoprenoid biosynthetic pathway. Intermediates through GGPP serve as precursors for other important compounds. The conversion of two molecules of GGPP to phytoene, which occurs predominantly as the 15, 15′-*cis* isomer but is shown here as the all-*trans* isomer for convenience, is the first reaction unique to carotenoid biosynthesis. Prephytoene pyrophosphate (PPPP) is an unstable intermediate. GGPP synthases are structurally conserved in both prokaryotic and eukaryotic carotenogenic organisms, but can differ in their substrate specificities (dotted line). Phytoene synthases from various organisms are also structurally conserved. Genetic loci associated with specific enzymatic functions are listed in Table 1.

FUNCTIONS OF CAROTENOIDS

In addition to their obvious role as visually attractive natural pigments, carotenoids perform a variety of essential biological functions. Universally, colored carotenoids provide photooxidative protection against the effects of singlet oxygen and radicals generated in the presence of light and endogenous photosensitizers such as chlorophylls, heme, and protoporphyrin IX (13). During photosynthesis carotenoids can transfer absorbed radiant energy to chlorophyll molecules in a light-harvesting function, dissipate excess energy via the xanthophyll cycle in higher plants and certain algae, and quench excited-state chlorophylls directly (14, 15). Recently, the structural role of carotenoids as the molecular glue of certain photosynthetic pigment-protein complexes has become evident (16, 17). β-Carotene and structurally related compounds serve as the precursors for vitamin A, retinal, and retinoic acid in mammals, thereby playing essential roles in nutrition, vision, and cellular differentiation, respectively

[2]Abbreviations: DMAPP, dimethylallyl pyrophosphate; FPP, farnesyl pyrophosphate; GPP, geranyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; IPP, isopentenyl pyrophosphate, PPPP, prephytoene pyrophosphate.

(2). Cleavage of specific cyclic epoxy-xanthophylls serves as the starting point for the biosynthesis of abscisic acid, an important plant hormone (18). Zeaxanthin has been proposed to serve as a blue light photoreceptor in corn coleoptiles (19).

CLASSICAL GENETIC STUDIES

The variety of carotenoid structures and isomers found in nature has posed many challenges for chemists, biochem-

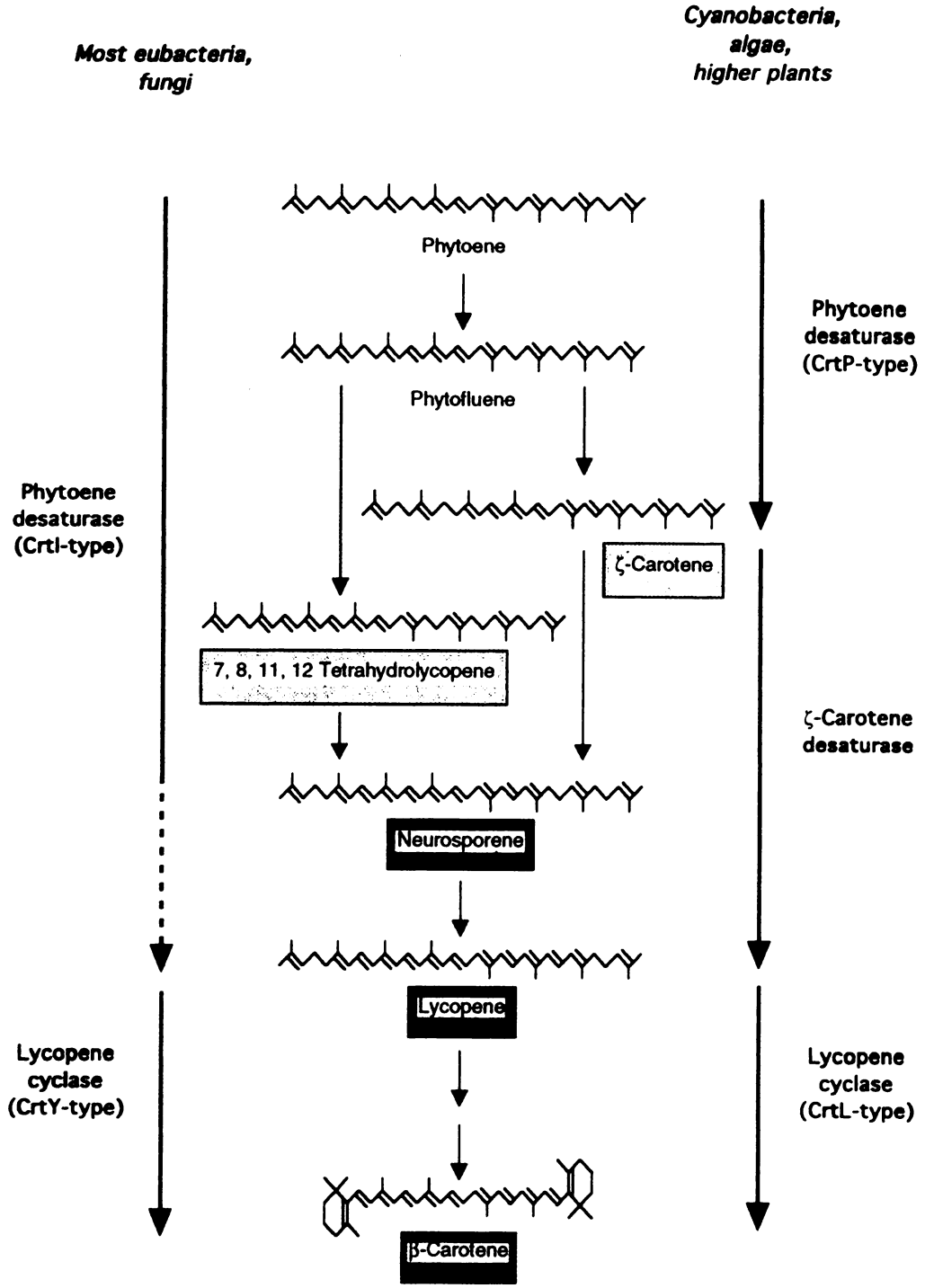

Figure 2. Simplified scheme for the conversion of phytoene to β-carotene. In general, nonenzymatic isomerization of 15, 15′-*cis*-phytoene, shown here as the all-*trans* isomer for convenience, occurs during the desaturations to yield all-*trans*-lycopene. Two structurally and functionally distinct classes of phytoene desaturase have been described. Similarly, two structurally distinct types of lycopene cyclase exist. In contrast to other eubacteria and fungi, CrtI-type phytoene desaturase from the *Rhodobacter* species converts phytoene to neurosporene rather than to lycopene (dotted line). β-Carotene occurs as a major pigment in cyanobacteria, plants, and fungi. The colors of carotenoids that absorb visible light are highlighted.

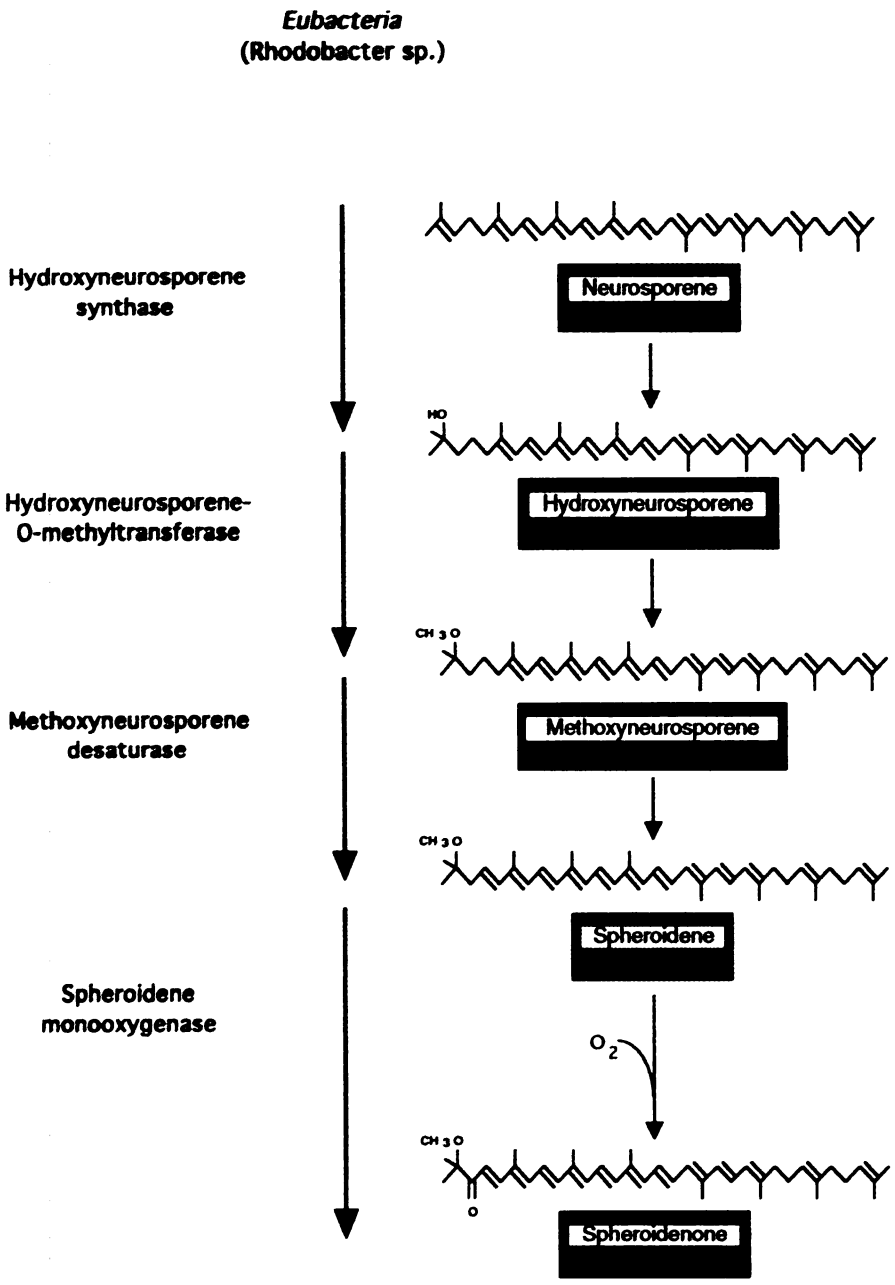

Figure 3. Simplified scheme for the conversion of neurosporene to acyclic xanthophylls in *Rhodobacter* species, as determined in *R. capsulatus*. The distribution of spheroidene and spheroidenone as the major end products is determined by the availability of molecular oxygen in the growth medium. The colors of carotenoids that absorb visible light are highlighted.

ists, and geneticists in the construction of postulated biosynthetic pathways. Before the advent of molecular genetics, evidence for specific biochemical conversions was assembled primarily through the use of labeled carotenoid precursors, carotenogenesis inhibitors and the characterization of bacterial, fungal, and plant mutants impaired in pigment synthesis. These topics have been thoroughly reviewed elsewhere (1, 5, 6) and will be discussed here only briefly.

Biochemical dissection of carotenoid biosynthesis pathways has historically been hindered by the hydrophobic, membrane-bound nature of the enzymes involved in the conversion of phytoene into colored carotenoids. Classical genetics, by comparison, has offered a relatively easy means to obtain information about biosynthetic conversions. Many early mutant studies were performed with nonphotosynthetic or anoxygenic photosynthetic bacteria, and with fungi. Carotenoids are dispensable in these organisms under certain conditions, thus facilitating the isolation of abnormally pigmented colonies after mutagenesis. Determination of the chemical structures of carotenoid intermediates accumulated by pigment mutants

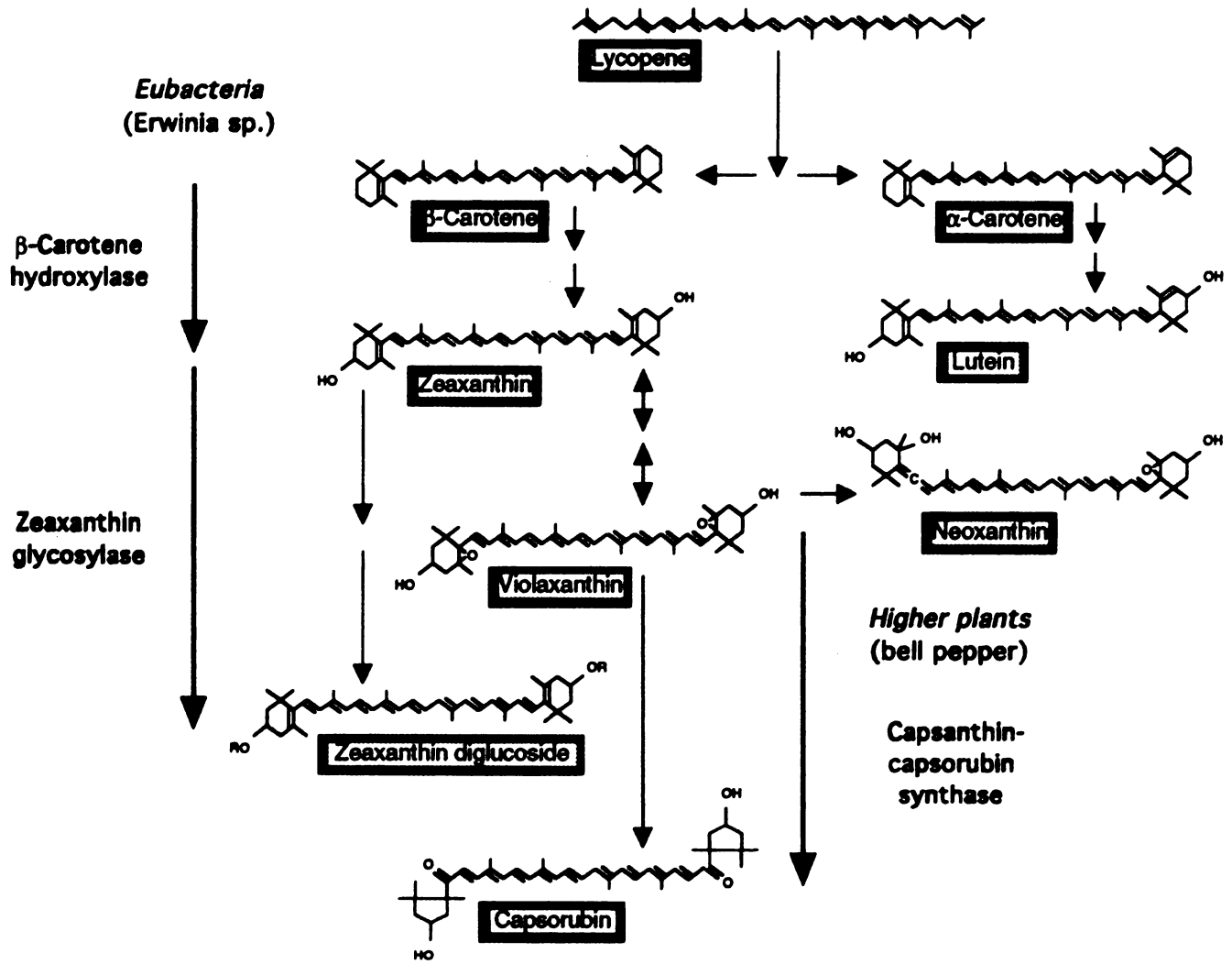

Figure 4. Simplified scheme for the conversion of cyclic carotenes to cyclic xanthophylls. Xanthophyll glycosides are found in some bacteria. Zeaxanthin diglucoside (R = glucoside) is a typical end product found in *Erwinia* species, zeaxanthin accumulates in cyanobacteria, and lutein, violaxanthin and neoxanthin are major end products found in higher plants. Enzymes and genes involved in many of these biosynthetic conversions have not yet been characterized. The colors of carotenoids that absorb visible light are highlighted.

helped greatly in the development of biosynthetic schemes. General genetic aspects of microbial carotenoid biosynthesis have recently been summarized (20).

In oxygenic photosynthetic organisms, identification of mutants blocked early in carotenoid biosynthesis has proved difficult because chlorophyll-containing cells that are simultaneously exposed to light and oxygen in the absence of colored carotenoids suffer photooxidative damage and destruction. In addition, mutations late in the biosynthetic pathway, although not necessarily lethal, would be masked by the endogenous chlorophylls. For these reasons, relatively few carotenoid biosynthesis mutants of cyanobacteria and algae have been isolated. In higher plants the situation has been more encouraging because potentially lethal mutations manifested in green photosynthetic tissues can be maintained in the heterozygous state, and fruit and flower pigmentation mutations do not necessarily affect viability (1, 5, 21). In particular, a collection of maize mutants affected in leaf and/or endosperm carotenoid biosynthesis has been assembled.

MOLECULAR GENETIC STUDIES

The advent of molecular genetics has allowed the further characterization of genetic loci required for carotenoid biosynthesis, and has indirectly yielded valuable structural information about the corresponding enzymes. **Table 1** updates recent reviews in this area (20, 21) in listing carotenoid biosynthesis cDNAs and genes that have been cloned and sequenced. More detailed information on the biosynthesis of bacterial and fungal carotenoids in specific groups of organisms can be obtained from several other current reviews (32–35).

Clusters of prokaryotic genes devoted to carotenoid biosynthesis (*crt*) were originally identified and cloned from *Rhodobacter capsulatus*, an anoxygenic photosynthetic bacterium, by in vivo complementation of mutants (36) and from the nonphotosynthetic plant epiphyte *Erwinia herbicola* Eho10 by heterologous gene expression leading to pigment production in *Escherichia coli* (37). The complete nucleotide sequences of *crt* gene clusters

TABLE 1. *Carotenoid biosynthesis enzymes and the corresponding sequenced genes*

Enzymatic function	Gene	Organisms	References[a]
Formation of phytoene/dehydrosqualene:			
GGPP synthase	*crtE*	Various eubacteria	Reviewed in ref 20
	gds	Archaebacterium (*S. acidocaldarius*)	22
	idsA	Archaebacterium (*M. thermoautotrophicum*)	23
	al-3	Fungus (*N. crassa*)	Reviewed in ref 20
	GGPS	Various higher plants	24; reviewed in ref 21
Phytoene synthase[b]	*crtB*	Various eubacteria (including cyanobacteria)	25; reviewed in ref 20
	al-2	Fungus (*N. crassa*)	26
	PSY1, PSY2, Y1	Various higher plants	Reviewed in ref 21
Dehydrosqualene synthase[b]	*crtM*	Eubacterium (*S. aureus*)	27
Formation of β-carotene/ diaponeurosporene:[d]			
Phytoene desaturase (CrtI-type)	*crtI*	Various eubacteria[c] (excluding cyanobacteria)	25; reviewed in ref 20
	al-1	Fungus (*N. crassa*)	Reviewed in ref 20
	PDH	Fungus (*C. nicotianae*)	28
Phytoene desaturase (CrtP-type)	*crtP*	Various cyanobacteria	Reviewed in ref 20
	PDS	Various higher plants	Reviewed in ref 21
Dehydrosqualene desaturase[d]	*crtN*	Eubacterium (*S. aureus*)	27
ζ-Carotene desaturase	*crtQ*	Cyanobacterium (*Anabaena* sp. Strain PCC7120)	Reviewed in ref 20
Lycopene cyclase (CrtY-type)	*crtY*	Eubacteria (*Erwinia sp.*)	Reviewed in ref 20
Lycopene cyclase (CrtL-type)	*crtL*	Cyanobacterium (*Synechococcus* sp. Strain PCC7942)	
Formation of acyclic xanthophylls:			
Hydroxyneurosporene synthase	*crtC*	Eubacterium (*R. capsulatus*)	Reviewed in ref 20
Methoxyneurosporene desaturase	*crtD*	Eubacteria (*Rhodobacter* sp.)	Reviewed in ref 20
Hydroxyneurosporene-O-methyltransferase	*crtF*	Eubacterium (*R. capsulatus*)	Reviewed in ref 20
Spheroidene monooxygenase	*crtA*	Eubacterium (*R. capsulatus*)	Reviewed in ref 20
Formation of cyclic xanthophylls and xanthophyll glycosides:			
β-Carotene hydroxylase	*crtZ*	Eubacteria (*Erwinia* sp.)	Reviewed in ref 20
Zeaxanthin glucosylase	*crtX*	Eubacteria (*Erwinia* sp.)	Reviewed in ref 20
β-C-4-oxygenase	*crtW*	Eubacteria (*A. aurantiacum, Alcaligenes* PC-1)	30
Capsanthin-capsorubin synthase	CCS	Bell pepper (*C. annuum*)	31

[a]See reviews for further references. [b]C_{30} carotenoid biosynthesis enzyme analogous to phytoene synthase (Fig. 1). [c]Referred to as *carC* in *M. xanthus*. [d]C_{30} carotenoid biosynthesis enzyme analogous to CrtI-type phytoene desaturase (Fig. 2).

from *R. capsulatus* (38) and from several species of *Erwinia* have since been published (10, 11, 39, 40). Studies with *R. sphaeroides* (25, 41) and with nonphotosynthetic bacteria including *Myxococcus xanthus*, in which carotenogenesis is light-induced (42), *Mycobacterium aurum* (43), and *Thermus thermophilus*, a thermophile (44), have defined crt gene clusters that have been partially characterized. Unlike the other bacteria mentioned, the *T. thermophilus crt* gene cluster is plasmid-borne. Clustering, however, is not a universal rule as carotenoid biosynthesis genes are dispersed in the genomes of carotenogenic eukaryotes and are not confined to a single cluster in cyanobacteria (29, 34). In vivo complementation of pigment mutants in the homologous host or of a partially deleted *Erwinia crt* gene cluster expressed in *E. coli* have been the most commonly used approaches to obtain other prokaryotic *crt* genes. Because of the difficulties in directly selecting for carotenoid biosynthesis mutants of cyanobacteria, the second approach was applied to isolate the *crtQ* gene encoding ζ-carotene desaturase (Fig. 2) (45). In addition, resistances to carotenoid biosynthesis herbicides that target phytoene desaturation or lycopene cyclization have also been used to select mutations that have allowed the isolation of the corresponding cyanobacterial genes (29, 34, 46).

The molecular characterization of eukaryotic carotenoid biosynthesis genes started innocently with the isolation of a tomato cDNA known to be expressed during fruit ripening, a process typically associated with the massive accumulation of lycopene (Fig. 2) (47). Subsequent to the

characterization of the *crt* gene cluster from *R. capsulatus* (38), it became possible to propose a functional relationship between this tomato cDNA and the bacterial CrtB protein based on sequence homology (39). Elegant experiments involving plant transformation with an antisense construct (48) and heterologous in vivo complementation of a bacterial mutant (49) have confirmed that the tomato cDNA (now known as *PSY1*) encodes the enzyme phytoene synthase (Fig. 1).

These types of approaches as well as isolation of plant PCR products based on cyanobacterial gene sequences (50), screening of plant protein expression libraries with specific antibodies (31, 51, 52), transposon tagging in maize (53), and chromosome walking from a nearby selectable gene in the fungus *Neurospora crassa* (54) have also been used to obtain and characterize other eukaryotic cDNAs and genes of carotenoid biosynthesis (20, 21). The eukaryotic sequences described thus far (Table 1) appear to represent single-copy genes, with the exceptions of the *PSY1* and *PSY2* genes encoded in tomato (21) and the possible existence of multiple *GGPS* genes in bell pepper (24). Specific information regarding enzymes involved in different portions of the carotenoid biosynthesis pathway is summarized below.

FORMATION OF PHYTOENE

The formation of phytoene by the tail-to-tail condensation of two molecules of GGPP, catalyzed by phytoene synthase (Fig. 1), has generally been regarded as the first reaction unique to carotenoid biosynthesis. It is therefore unexpected that in *Rhodobacter* species and in *N. crassa* mutations in genes encoding GGPP synthase also block carotenoid accumulation (Table 1), in the former case without concomitant disruption of bacteriochlorophyll biosynthesis (Fig. 1). These observations, as well as the presence of the gene encoding the eubacterial GGPP synthase (CrtE) within the *crt* gene clusters of *Rhodobacter* and *Erwinia* species, suggest that a carotenoid biosynthesis-specific enzyme may exist (20, 35). The homodimeric Mg^{2+}-dependent GGPP synthases from archaebacteria, eubacteria, and eukaryotes are structurally related and also belong to an enzyme superfamily that includes FPP and other isoprenyl pyrophosphate synthases (23, 55, 56). There are, however, some functional differences among GGPP synthases in substrate and product preferences. Archaebacterial enzymes can use different allylic substrates and synthesize not only GGPP but also FPP in substantial amounts (22, 23). In contrast, eubacterial and eukaryotic GGPP synthases produce only GGPP, but individual enzymes vary in their stringencies with respect to the chain length of the allylic substrate (52, 57, 58).

The eukaryotic and eubacterial phytoene synthases (CrtB) described thus far are both structurally and functionally conserved (20, 21, 26, 39, 55). The bell pepper enzyme requires Mn^{2+} for activity and acts as a monomer during the tail-to-tail condensation of two molecules of GGPP (Fig. 1) (59). Structurally, phytoene synthases display sequence similarities with dehydrosqualene synthase (CrtM), the analogous enzyme of C30 carotenoid biosynthesis (27), and squalene synthase, a key enzyme of sterol biosynthesis (57). The common feature of dehydrosqualene and squalene synthases is that both condense two molecules of FPP to yield a C_{30} product (Fig. 1) (7). In addition, several protein sequence motifs likely to be involved in pyrophosphate substrate or product binding and/or enzyme catalysis are common to phytoene, GGPP, and FPP synthases (52, 55–58). Because the earliest reactions of carotenoid biosynthesis are common to all carotenogenic organisms, the structures and, for them most part, the functions of GGPP and phytoene synthases have thus been conserved throughout evolution.

PHYTOENE DESATURATION

The structural and functional features of the enzymes involved in the conversion of phytoene into more desaturated acylic and cyclic carotenes (Fig. 2) depend on whether or not the organism survives by oxygenic photosynthesis. At first glance this seems surprising because successive desaturations of phytoene, albeit in different isomeric forms, are common to all C40 carotenogenic organisms. These desaturations ultimately lead to the production of lycopene, usually in the all-*trans* configuration. The immediate products arising from phytoene desaturation differ, however, between nonphotosynthetic and anoxygenic photosynthetic microbes on the one hand and oxygenic photosynthetic organisms on the other. In the former category, CrtI-type phytoene desaturases (Table 1) produce lycopene or neurosporene (12, 60) as the result of three or four consecutive desaturations that require ATP (25). The structurally related dehydrosqualene desaturase (CrtN) performs the analogous reaction during C_{30} carotenoid biosynthesis (27). In cyanobacteria, algae, and higher plants, CrtI-type enzymes have been replaced by structurally and functionally distinct CrtP-type phytoene desaturases (Table 1). CrtP-type enzymes catalyze two desaturations to convert phytoene into ζ-carotene (50, 51, 61), a positional isomer of the nonaccumulating desaturation intermediate produced by CrtI-type enzymes (Fig. 2). The exact nature of the FAD/NADP cofactor requirements for CrtP- and CrtI-mediated desaturations remains to be established. The bell pepper CrtP-type enzyme does, however, contain bound FAD (51). Indeed, a putative ADP-binding motif is the only region structurally conserved between the two types of enzymes (39, 50, 55, 60, 61). In organisms that contain CrtP-type phytoene desaturases, the ζ-carotene produced by CrtP is converted to lycopene through two further desaturations catalyzed by the ζ-carotene desaturase (CrtQ) (45). Unexpectedly, the single CrtQ protein described to date is structurally similar to CrtI-type enzymes and to methoxyneurosporene desaturase (CrtD) (20, 35), a ca-

rotenoid desaturase involved in acyclic xanthophyll biosynthesis in *Rhodobacter* species (Fig. 3).

LYCOPENE CYCLIZATION

Lycopene is the typical acyclic carotene used as a substrate for the synthesis of β- and α-carotenes (Fig. 2, Fig. 4). The structural differences between carotenoid biosynthesis enzymes from nonphotosynthetic bacteria and oxygenic photosynthetic organisms are further reflected are the distinct protein sequences of CrtY-type and CrtL-type lycopene cyclases (Table 1), both of which produce β-carotene by two successive β-cyclizations. Species of *Erwinia* contain CrtY enzymes (10, 11, 62), whereas cyanobacteria possess CrtL' enzymes (29). By analogy to the CrtI- and CrtP-type phytoene desaturases, CrtY and CrtL are structurally unrelated with the exception of a putative ADP-binding fold that may be involved in the interaction with an FAD/NADP cofactor (29, 55). No information is currently available about the presumably distinct lycopene cyclase that introduces the ε-ring into α-carotene, a precursor of the highly abundant xanthophyll lutein in higher plants (Fig. 4).

FORMATION OF ACYCLIC XANTHOPHYLLS

Although most carotenogenic organisms synthesize cyclic carotenoids, certain species of bacteria, including *Rhodobacter* and *Myxococcus* species (20), accumulate acyclic xanthophylls (Fig. 3). In *R. capsulatus* the entire pathway for acyclic xanthophyll biosynthesis starting with neurosporene, an acyclic carotene, has been genetically defined and molecularly characterized (Table 1). The biochemistry of these reactions is, however, poorly understood. It is known that hydroxyneurosporene-O-methyltransferase (CrtF) exhibits sequence similarities that may define the S-adenosylmethionine cofactor-binding site with other O-methyltransferases not involved in carotenoid biosynthesis (35). As noted earlier, CrtD is structurally related to the CrtI-type phytoene desaturases and to CrtQ, and retains the putative ADP-binding fold found in these enzymes (20, 35, 45, 55).

FORMATION OF CYCLIC XANTHOPHYLLS AND XANTHOPHYLL GLYCOSIDES

Even though cyclic xanthophylls and their glycosides make up the vast majority of carotenoids (4), surprisingly little is known about their biosynthesis. *Erwinia* species produce carotenoid intermediates equivalent to those found in higher plants up to the production of the cyclic xanthophyll zeaxanthin (Fig. 4) (9–11, 20). β-Carotene hydroxylase (CrtZ) derivatizes each β-ring once to yield zeaxanthin via cryptoxanthin (62). Subsequently, *Erwinia* species produce unique glycosides through the action of the zeaxanthin glucosylase (CrtX), which contains a putative UDP-binding site also present in noncarotenogenic enzymes that recognize substrates substances containing UDP-glucosyl moieties (63). The isolation and molecular cloning of a eukaryotic cDNA encoding the bifunctional bell pepper capsanthin-capsorubin synthase (Fig. 4) have recently been reported (31). The enzyme is a monomer and contains a putative ADP-binding fold. Recently, genes encoding β-C-4-oxygenase (CrtW), the enzyme that converts β-carotene to the cyclic diketocarotenoid canthaxanthin, have been isolated from several species of marine bacteria (30) and from a green alga (64). No biochemical information about CrtW has yet been reported.

REGULATION OF CAROTENOID BIOSYNTHESIS GENES AND ENZYMES

A discussion of the regulation of carotenoid biosynthesis by endogenous and exogenous factors cannot be presented here due to space limitations. Instead, interested readers are referred to recent reviews that include sections on this topic (5, 21, 32–35).

CONCLUSIONS AND OUTLOOK

One of the most intriguing aspects in recent studies of carotenoid biosynthesis has been the emerging pattern, exemplified by phytoene desaturases and lycopene cyclases, that oxygenic photosynthetic organisms contain a set of membrane-associated or -bound enzymes that are distinct from those found in nonphotosynthetic bacteria, anoxygenic photosynthetic bacteria, and fungi. How and why these parallel classes of enzymes have evolved, what their mechanisms of catalysis are, and whether this parallelism has been preserved among functionally related enyzmes of xanthophyll biosynthesis from different organisms remain to be determined. Our expanding recognition of the complexity of carotenoid biosynthesis will increasingly proceed hand in hand with attempts to address defined applied problems by directed manipulation of carotenoid production through genetic engineering. FJ

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Note added in proof: In addition to the carotenoid biosynthesis genes listed in Table 1, a number of additional cDNA and gene sequences have recently been characterized from bacteria [Botella et al. (1995) *Eur. J. Biochem.* **233**, 238–248; Lang et al. (1995) *J. Bacteriol.* **177**, 2064–2073; Misawa et al. (1995) *J. Bacteriol.* **177**, 6575–6584), an alga (Kajiwara et al. (1995) *Plant Mol. Biol.* **29**, 343–352), and higher plants (Albrecht et al. (1995) *FEBS Lett.* **372**, 199–202; Hable and Oishi (1995) *Plant Physiol.* **108**, 1329–1330; Hugueney et al. (1995) *Plant J.* **8**, 417–424; Karvouni et al. (1995) *Plant Mol. Biol.* **27**, 1153–1162; Norris et al. (1995) *Plant Cell* **7**, 2139–2149; Al-Babili et al. (1996) *Plant J.*, in press; Li et al. (1996) *Plant Mol. Biol.*, in press].

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