Plant Carotenoids: Pigments for Photoprotection, Visual Attraction, and Human Health

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INTRODUCTION

Plant carotenoids are red, orange, and yellow lipid-soluble pigments found embedded in the membranes of chloroplasts and chromoplasts. Their color is masked by chlorophyll in photosynthetic tissues, but in late stages of plant development these pigments contribute to the bright colors of many flowers and fruits and the carrot root. Carotenoids protect photosynthetic organisms against potentially harmful photooxidative processes and are essential structural components of the photosynthetic antenna and reaction center complexes. In plants, some of these compounds are precursors of abscisic acid (ABA), a phytohormone that modulates developmental and stress processes (Koornneef, 1986). Carotenoids with provitamin A activity are essential components of the human diet, and there is considerable evidence that many carotenoids have anti-cancer activity. Carotenoids absorbed through the diet, and often metabolized into other compounds, are responsible for the color of familiar animals such as lobster, flamingo, and fish. Often unaware of the chemical nature of food colorants, humans consume large amounts of carotenoids added to foods primarily to please the eye (Klaüü and Bauernfeind, 1981).

As with other natural pigments, carotenoids attracted the attention of 19th-century organic chemists. β-Carotene was isolated in 1817, and the pigments of autumn leaves were identified as xanthophylls in 1837 (Isler, 1971). Classical geneticists identified carotenoid mutants in maize, tomato, and Arabidopsis during the early part of this century. However, difficulties with the biochemical properties of the enzymes of the pathway and the generally poor viability of mutants affected in carotenoid biosynthesis during early stages of development conspired to delay development of the field. Genetic studies of carotenoid biosynthesis in bacteria (reviewed in Armstrong, 1994) have played a fundamental role in the cloning and characterization of plant carotenoid genes. Since 1991, when a bacterial probe was first used to clone a plant gene for an enzyme of the pathway, progress has been rapid. However, much remains to be learned about the environmental and developmental regulation of the pathway, and many classic mutants have not yet been characterized at the molecular level.

Recent reviews (Armstrong, 1994; Bartley et al., 1994; Sandmann, 1994) and two books on methods (Packer, 1992a, 1992b) have covered many aspects of the chemistry, biochemistry, and biology of carotenoids in bacteria and plants, and the classic Isler (1971) monograph on carotenoids is being updated (Britton et al., 1995). In this review, we summarize and update our knowledge of genes and enzymes for carotenoid biosynthesis in plants and focus on the current challenges for plant developmental biology and biotechnology.

THE CAROTENOID PATHWAY

The structures of some common plant carotenoids and a simplified biosynthetic pathway are shown in Figure 1. Carotenoids are generally C_{40} terpenoid compounds formed by the condensation of eight isoprene units (for a review of terpenoid metabolism, see McGarvey and Croteau, 1995, this issue). At the center of the molecule, the linkage order is reversed, so the molecule as a whole is symmetrical. A set of conjugated double bonds is responsible for the absorption of light in the visible region of the spectrum.

In the following sections we discuss the biosynthetic pathway, emphasizing those steps for which the corresponding genes have been cloned. The cloned plant genes are listed in Table 1. Genetic and molecular evidence indicate that all enzymes of the pathway are encoded by nuclear genes and imported post-translationally into chloroplasts. We mention genes from nonplant species only in those cases in which they served as probes for cloning plant genes or in which sequence comparisons revealed putative functions for the corresponding plant proteins.

Synthesis of Geranylgeranyl Pyrophosphate

In the first step of the pathway, the enzyme geranylgeranyl pyrophosphate synthase (GGPS) catalyzes three successive condensation reactions, the first of them being the condensation of isopentenyl pyrophosphate and dimethylallyl pyrophosphate (DMAPP) to form geranylgeranyl pyrophosphate (GGPP; see McGarvey and Croteau, 1995, this issue). GGPS from pepper has been purified to homogeneity and shown to catalyze the
Figure 1. Carotenoid Biosynthesis in Plants.

(A) A simplified biosynthetic pathway. The colors indicate the prevalent color of the compounds in nature. Compounds before \( \zeta \)-carotene do not absorb light in the visible region of the spectrum. The color of compounds in nature does not necessarily correspond to the colors of purified compounds in solution due to interactions with other components of chromoplast membranes and to concentration effects. The first steps of the pathway are condensation reactions that result in the formation of geranylgeranyl diphosphate (geranylgeranyl pyrophosphate, GGPP). Phytoene synthase (PSY) catalyzes the condensation of two molecules of GGPP into prephytoene pyrophosphate (not shown) and then into phytoene. A series of desaturation reactions results in the synthesis of lycopene, which is then cyclized into \( \beta \)-carotene. The circular arrow shows the interconversion of zeaxanthin and violaxanthin, the two main compounds of the xanthophyll cycle. The phytohormone ABA is derived from xanthophylls.

(B) Four biological systems that have contributed to the development of the genetics of carotenoid biosynthesis in plants. Basic information provided by cyanobacteria (Synechocystis) and photosynthetic bacteria (Rhodobacter) was instrumental in identifying plant genes for carotenoid biosynthesis. Maize and soybean are two of the plants from which carotenoid biosynthesis genes have been cloned.

(C) and (D) Plant carotenoid mutants affect plastid development. In the case of the tomato ghost mutant (C) and the Arabidopsis immutans mutant (D), a block in the desaturation of phytoene results in variegated green/white leaves. White sectors accumulate phytoene, and green sectors accumulate wild-type carotenoids.
**Table 1. Cloned Plant Genes for Enzymes of the Carotenoid Biosynthesis Pathway**

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*a + indicates the type of clone (genomic or cDNA) that has been isolated.

*b + indicates a sequence has been identified in the expressed sequence tags data base.

*c B. Buckner (personal communication).

d J. Hirschberg (personal communication).

corresponding reactions in vitro (Dogbo and Camara, 1987). An antibody to the purified enzyme was used to isolate a cDNA, which was shown to encode a protein with GGPS enzymatic activity (Kuntz et al., 1992). An Arabidopsis GGPS cDNA was cloned using information derived from the pepper protein sequence (Scolnik and Bartley, 1994). GGPS proteins from different organisms, including plants, share conserved regions that are likely to correspond to catalytic centers (Carattoli et al., 1991; Chen and Poulter, 1994).

In addition to its role in carotenoid biosynthesis, GGPP is a precursor of other plant compounds, such as chlorophyll phytoyl chains, phyloquinones, plastoquinones, taxol, tocopherols, abietic acid, gibberellic acid, diterpenoid phytoalexins, and diterpenes (see McGarvey and Croteau, 1995, this issue).
Therefore, it is likely that the synthesis of this branch compound is subject to complex regulation and, possibly, compartmentalization. There is evidence for multiple GGPS genes in Arabidopsis. In addition to the aforementioned cDNA, we have reported the DNA sequence of a genomic clone (GenBank accession number L22947) for which a cDNA has not yet been identified, two cDNAs for additional GGPS genes (G.E. Bartley and P.A. Scolnik, unpublished data), and a fourth cDNA that may encode a more divergent GGPS (GenBank accession number L40577; Scolnik and Bartley, 1995a). One possibility is that these genes encode dedicated enzymes for different branches of the pathway.

### Synthesis of Phytoene

In the first dedicated step of carotenoid biosynthesis, the enzyme phytoene synthase (PSY) catalyzes the two-step conversion of two molecules of GGPP into prephytoene pyrophosphate (PPPP) and then into phytoene (Dogbo et al., 1988). The possible mechanism of this enzyme, including regions of substrate recognition, have been reviewed recently (Sandmann, 1994). Two molecules of GGPP are joined in a condensation reaction with the loss of hydrogen and the diphosphate group from C-1' of the same molecule. Cleavage of the C-1 diphosphate group of the resulting PPPP, followed by a 1-1' rearrangement, results in the formation of phytoene, which, depending on the stereochemistry of the reaction, may be 15-cis or all-trans. Phytoene has the basic C_{40} skeleton of carotenoids, and all subsequent reactions in the pathway involve chemical conversions of this basic structure.

Clone pTOM5, a cDNA for a tomato ripening-specific gene of unknown function (Ray et al., 1987), was later shown by genetic approaches to correspond to a PSY gene. The first evidence for this assignment was the observation that the predicted protein encoded by pTOM5 is more than 20% identical to PSY enzymes encoded by the crtB genes of the carotenoid-producing bacteria Erwinia herbicola and Rhodobacter capsulatus (Armstrong et al., 1990). The function suggested by this structural similarity was confirmed by studies in plants and bacteria. Fruit and flower carotenoids are greatly reduced in transgenic tomato plants carrying a pTOM5 antisense construct (Bird et al., 1991), and the block is in the conversion of GGPP to phytoene (Bramley et al., 1992). When driven by bacterial transcription and translation signals, the pTOM5 cDNA complements R. capsulatus crtB mutants (Bartley and Scolnik, 1992). A cDNA for a second PSY gene, now named PSY2, has been identified in tomato leaves (Bartley and Scolnik, 1993). The gene corresponding to the pTOM5 cDNA has been renamed PSY1.

Transgenic tobacco plants expressing high levels of PSY1 show a reduction in size and in levels of gibberellic acid (GA) and chlorophyll, suggesting that overproduction of PSY diverts GGPP from a pool common to the GA and phytol biosynthetic pathways (R. Fray, personal communication).

### Desaturation of Phytoene

A series of desaturation reactions converts the colorless compound phytoene into yellow, orange, and red carotenoids by creating the conjugated double bonds that form the chromophore. There are four desaturation steps between phytoene and lycopene (Figure 1). A higher plant gene for phytoene desaturase (PDS) was cloned from soybean (Bartley et al., 1991) and subsequently from several other species (Table 1). Expression of plant PDS genes in bacteria leads to the formation of ζ-carotene (Bartley et al., 1991; Pecker et al., 1992). This result, plus the existence of mutants of maize and tomato that accumulate ζ-carotene and of herbicides that primarily block the conversion of ζ-carotene into lycopene, suggests that, like some bacteria (Linden et al., 1993b; Armstrong, 1994), plants have two carotenoid desaturases. The gene for the plant ζ-carotene desaturase, however, has not yet been cloned, and it is possible that interactions with other proteins and/or modifications of PDS alter the specificity of the enzyme to accept ζ-carotene as a substrate.

Phytoene is the first lipophilic compound in the carotenoid pathway. Accordingly, whereas PSY is peripherally associated with plastid membranes (Bartley et al., 1992), PDS is an intrinsic membrane protein in both chloroplasts and chromoplasts (Bartley et al., 1991; Hugueney et al., 1992; Linden et al., 1993a). Bacterial, fungal, and plant PDS proteins contain a typical pyridine dinucleotide binding motif generally associated with the binding of FAD (Armstrong et al., 1990; Bartley et al., 1990), and this cofactor has been found to bind to purified pepper PDS (Hugueney et al., 1992). Other studies with purified protein have demonstrated that, depending on the species, either NAD(P) or FAD is sufficient to catalyze the desaturation reaction, supporting the idea of an enzymatic mechanism involving a dehydrogenation reaction with an oxidized dinucleotide as electron and hydrogen mediator (Sandmann, 1994).

As in other redox reactions, the desaturation of phytoene requires ancillary redox proteins. The mode of action of the bleaching herbicide sulcotrione supports the concept that PDS is part of a multiprotein redox complex in which quinones play an important role. Plants treated with sulcotrione are blocked in the desaturation of phytoene, but this herbicide is not an inhibitor of PDS (Schulz et al., 1993). Further characterization of sulcotrione revealed that it is a potent inhibitor of the enzyme (S)-hydroxyphenylpyruvate dioxygenase, which converts (S)-hydroxyphenylpyruvate to homogentisic acid, an intermediate in the biosynthesis of plastoquinones, tocopherols, and related compounds. The most likely interpretation of this result is that sulcotrione inhibits PDS indirectly through a block in the biosynthesis of quinones required for PDS activity (Schulz et al., 1993). Supporting this biochemical evidence, two Arabidopsis phytoene-accumulating mutants that map to genetic loci other than PDS were found blocked at two different steps of the plastoquinone biosynthesis pathway (D. DellaPenna, personal communication).
electrons derived from double-bond formation, and an NAD(P)H-dependent quinone oxidoreductase regulates PDS activity (Mayer et al., 1992). The gene for this oxidoreductase has been cloned, and the deduced sequence shows a high level of conservation with the 23-kD protein of plant oxygen-evolving complexes (P. Beyer, personal communication).

Cyclization of Lycopene

Lycopene, the product of the sequential desaturations of phytoene, is converted into β-carotene by the enzyme lycopene cyclase (LYC). This enzyme can accept either of the acyclic compounds neurosporene or lycopene as substrates to form cyclic carotenoids such as α- and β-carotene. The biochemistry of carotenoid cyclization has been reviewed by Sandmann (1991). Synthesis of β-carotene from lycopene by extracts from spinach chloroplasts or tomato chloroplasts requires FAD (Kushwaha et al., 1989). The cyanobacterial gene for LYC (crtL) has recently been cloned by genetic complementation from a mutant strain resistant to the LYC inhibitor 2-(4-methylphenoxyl)triethylamine (Cunningham et al., 1994). A computer search of the data base of expressed sequence tags (dbest) revealed the existence of an Arabidopsis cDNA with extensive sequence conservation to the cyanobacterial gene (GenBank accession number L40176; Scolnik and Bartley, 1995b). The predicted protein product of this gene contains an apparent plastid transit peptide and a conserved pyridine dinucleotide binding domain at the N terminus of the mature protein. Expression of this cDNA in a lycopene-producing Escherichia coli strain confirms that it encodes a protein with LYC activity (Scolnik and Bartley, 1995b). The LYC gene has also been cloned from tobacco and tomato based on sequence similarity with the cyanobacterial crtL gene (J. Hirschberg, personal communication).

Synthesis of Xanthophylls

Xanthophylls are hydroxy, epoxy, furanoxy, and oxy derivatives of the carotenes formed in the late stages of the pathway. One of the many different plant xanthophylls, violaxanthin, is shown in Figure 1. The first xanthophylls are formed from cyclic carotenoids such as β-carotene by the introduction of hydroxy groups in positions C3 and C3' of the ionone rings, followed by epoxidation at the 5,6 and 5,6' positions. Little is known about the mechanisms of the enzymes for xanthophyll biosynthesis, and the corresponding genes have not yet been cloned. Xanthophylls undergo light-dependent epoxidation/de-epoxidation cycles, represented in Figure 1 by the interconversion of zeaxanthin and violaxanthin. As discussed later, the xanthophyll cycle is likely to play an important role in photoprotection under conditions of excess excitation energy. The epoxidase and de-epoxidase enzymes responsible for the xanthophyll cycle have been detected in chloroplast fractions, but they have not yet been purified to homogeneity (Yamamoto, 1979; Gruszczek and Krupa, 1993; Hager and Holocher, 1994). The enzyme for the conversion of the 5,6-epoxyxanthene antheraxanthin and violaxanthin into the pepper carotenoids capsanthin and capsorubin has been purified to homogeneity, and antibodies raised against this enzyme were used to isolate a full-length cDNA. The monomeric molecular mass of this enzyme is 50 kD, and the primary structure reveals the presence of a consensus dinucleotide-binding site (Bouvier et al., 1994).

Synthesis of Abscisic Acid

The plant hormone ABA participates in the control of water relations in plants and is a general inhibitor of growth and, in some species, seed dormancy. According to the currently accepted hypothesis, ABA forms from products of the oxidative cleavage of xanthophylls (Perry and Horgan, 1992; Perry et al., 1992; for review, see Zeevaart et al., 1991). Therefore, all steps involving C4 compounds are common to both carotenoids and ABA, whereas steps after xanthophyll cleavage are specific for ABA biosynthesis. Mutants in carotenoid biosynthetic steps up to zeaxanthin have low levels of ABA; they also have sharply reduced levels of chlorophyll, due to the alterations in chloroplast differentiation induced by carotenoid deficiency. The connection between carotenoids and ABA was supported by the analysis of maize mutants with reduced levels of colored carotenoids and ABA in seedlings and endosperms (Fong et al., 1983) and the observation that the mutant phenotype can be mimicked by application of the PDS inhibitor fluridone (Smith et al., 1983). Because ABA is required for dormancy, these mutants often show vivipary, that is, premature germination of the embryo while it is still attached to the plant. The Arabidopsis aba mutant has greatly reduced levels of violaxanthin and neoxanthin and accumulates zeaxanthin at levels higher than wild type, thus contributing to the biochemical evidence suggesting that epoxyxanthenes are precursors of ABA (Rock and Zeevaart, 1991; Rock et al., 1992). The tomato mutants flacca and sitiens, thought to carry genetic blocks at the level of ABA–aldehyde oxidation, have contributed to our understanding of the late stages of ABA biosynthesis (for review, see Zeevaart et al., 1991).

FUNCTIONS OF CAROTENOIDS

Plant carotenoids play fundamental roles as accessory pigments for photosynthesis, as protection against photooxidation, and as structural determinants in plastid pigment–protein complexes. The role of these pigments is determined primarily by whether the tissue is photosynthetic or nonphotosynthetic. In photosynthetic tissues, photoprotection against harmful oxygen species is their most important function. In nonphotosynthetic
tissues, carotenoids determine or contribute to the color of flowers and fruits.

**Carotenoids in Chloroplasts**

Carotenoids, chlorophylls, and various apoproteins form the photosystem complexes of the chloroplast thylakoid membranes (Thornber et al., 1987; Herrin et al., 1992; see von Wettstein et al., 1995, this issue). Carotenoids absorb light in the blue region of the spectrum (400 to 600 nm), and the energy absorbed can be transferred to chlorophylls. Therefore, carotenoids serve as accessory pigments by harvesting radiant light in a region of the spectrum not covered by the chlorophylls.

In addition to their light-harvesting functions, carotenoids are essential for photoprotection. In the absence of colored carotenoids, plants suffer severe photooxidative damage, which generally results in the death of the organism. Based on work with photosynthetic bacteria and with in vitro assays containing porphyrins, the likely mechanism for photoprotection is the quenching, by colored carotenoids, of chlorophyll triplets that would otherwise lead to the generation of oxygen singlets that can react with lipids, proteins, and other macromolecules, causing irreparable damage (Krinsky, 1979; Davidson and Cogdell, 1981). The light-harvesting and photoprotective functions of carotenoids have been reviewed by Koyama (1991).

Unless treated with herbicides that inhibit carotenoid biosynthesis, plants are not likely to suffer severe depletion of carotenoids under field conditions. A more physiologically relevant situation, particularly for plants growing in full sunlight, is when light levels exceed the maximum that can be used productively by the photosynthetic apparatus. There is considerable evidence in support of a photoprotective role of the xanthophyll cycle in the removal of excess excitation energy from the photosynthetic antennae (reviewed in Demmig-Adams and Adams, 1993). Under conditions of excess excitation energy, zeaxanthin accumulates from violaxanthin via a two-step enzymatic de-epoxidation, and this accumulation correlates with rapid nonphotochemical quenching of chlorophyll fluorescence.

Recent studies on the xanthophyll cycle point to a complex light-mediated process that takes place in the chloroplast membranes. An analysis of the pigment stoichiometry of barley pigment–protein complexes revealed that the xanthophyll cycle intermediates occur mainly in the light-harvesting complexes of photosystem I and photosystem II, but the amounts of carotenoid per polypeptide are nonstoichiometric (Iue-chih and Thornber, 1995). The sites of xanthophyll epoxidation and de-epoxidation within the chloroplasts appear to be different (Gruszeczki and Krupa, 1993), and recent results suggest that the de-epoxidase is mobile within the thylakoid lumen at the neutral pH values expected to occur in vivo in the dark but becomes attached to the membranes at the low pH values induced by strong illumination, thus gaining access to its substrate, violaxanthin (Hager and Holocher, 1994). A working hypothesis postulates that excess light captured by the photosystem II antenna system triggers an acidification of the lumen that in turn activates the xanthophyll cycle de-epoxidase and may alter the conformation of antenna pigment–protein complexes to facilitate interaction with xanthophylls, resulting in nonphotochemical quenching (Gilmore et al., 1995). Clearly, much remains to be learned about the mechanistic aspects of the xanthophyll cycle, and, as pointed out by Iue-chih et al. (1995), mutants lacking parts of the photosynthetic apparatus may help elucidate the arrangement of carotenoids within their protein complexes and their role in photoprotection.

**Carotenoids in Chromoplasts**

Chromoplasts are carotenoid-containing plastids responsible for the yellow, orange, and red colors of many flower petals and fruits and of some roots. In the absence of chlorophyll, the photoprotective function of carotenoids is no longer essential, and it is likely that the main, and perhaps only, function of chromoplast carotenoids is the attraction of pollinating insects and animals. One possible exception is the proposed structural role of chromoplast carotenoids in the tapetum during pollen development (Brooks and Shaw, 1968). To provide the intense color of flowers and fruits, carotenoids have to accumulate in chromoplasts at very high levels. Chromoplasts contain specialized structures composed of carotenoids, lipids, and proteins that function to sequester large amounts of carotenoids. These structures can be classified as globular, membranous, fibrillar, crystalline, and tubular (Emter et al., 1990). The isolation and identification of the individual components of these structures, together with the development of in vitro reconstitution systems, are required for understanding the molecular interactions governing their assembly. Several carotenoid binding chromoplast proteins have been identified (Hansmann and Sitte, 1984; Bathgate et al., 1985; Cervantes-Cervantes et al., 1990; Smirra et al., 1993), but detailed structural information is still lacking.

In addition to high levels of the most common apolar carotenoids and xanthophylls, chromoplasts accumulate secondary carotenoids such as acylated xanthophylls that are thought to be required for the formation of chromoplast structures. A study with chemical inhibitors of protein and carotenoid biosynthesis confirmed the essential role of these components in chromoplast formation (Emter et al., 1990). Changes in carotenoid esterification during pepper fruit ripening have been reported recently, and the fatty acids esterifying yellow and red xanthophylls have been characterized (Mínguez-Mosquera and Hornero-Méndez, 1994).

Recently, Deruère et al. (1994a) studied the fibril assembly process in bell pepper chromoplasts. The fibrils contain carotenoids, galactolipids, phospholipids, and fibrillin, a 32-kD carotenoid binding protein. A cDNA for fibrillin was cloned and shown to encode a protein that is post-translationally imported into plastids (Deruère et al., 1994b). Fibrils were reconstituted in vitro by mixing purified fibrillin, carotenoids, and polar lipids in the same stoichiometric ratio found in vivo. Therefore, fibril
assembly is another example of cellular processes that prevent potential destabilizing or toxic effects by targeting excess lipids to storage structures (Deruère et al., 1994a).

**REGULATION OF CAROTENOIDs**

Carotenoid biosynthetic enzymes and carotenoid binding proteins are encoded by nuclear genes, and the corresponding precursor proteins are post-translationally imported into plastids. Therefore, transcriptional and post-transcriptional steps are potential targets for regulation of carotenoid biosynthesis. At present, some information is available on the steady state mRNA levels for genes encoding apoproteins and some biosynthetic enzymes. The mRNA levels for tomato PSY1 and PDS are highest in flowers and ripening fruits. Transcript levels are elevated in seedlings treated with herbicides that inhibit carotenoid biosynthesis and in leaves of the tomato ghost mutant, which is blocked in phytoene desaturation. Therefore, expression of PSY1 and PDS in tomato is controlled by both environmental and developmental signals (Maunders et al., 1987; Pecker et al., 1992; Giuliano et al., 1993). In contrast to PSY1, whose transcripts accumulate primarily in flowers and fruits, PSY2 transcripts accumulate in seedlings, leaves, and fruits, and the expression of this gene is not significantly affected by fruit ripening (Bartley and Scoulnik, 1993). The existence of more than one active PSY gene in tomato is consistent with results obtained with PSY1 antisense tomato plants, which show a sharp decline in carotenoid levels in fruits but not in leaves (Bird et al., 1991; Bramley et al., 1992). In contrast to tomato, expression of PSY is not induced by ripening in pepper fruits (Römer et al., 1993). As indicated later, this result may point to differences in the control of gene expression between climacteric and nonclimacteric fruits.

**DEVELOPMENTAL BIOLOGY**

Chloroplast Differentiation and Leaf Variegation

Loss of function mutations in genes critical for carotenoid biosynthesis generally result in conditional albino or pale green mutants that accumulate some chlorophyll only at very low light. Exceptions to this general rule are the tomato mutant ghost and the Arabidopsis mutant immutans, in which genetic blocks in the desaturation of phytoene result in variegated green/white leaf phenotypes. Several similarities in the phenotypes of these mutants suggest a possible common basis, although definite molecular evidence is still lacking. Both ghost and immutans accumulate phytoene in their white leaf sectors but map genetically to loci other than PDS, suggesting that the structural gene is not affected (Giuliano et al., 1993; Wetzel et al., 1994). Plastids in the green sectors of both mutants are phenotypically wild type, whereas in white sectors they lack thylakoids and are highly vacuolated. In addition to these unpigmented plastids, the white sectors of the immutans mutant also contain some small green plastids and even some normal chloroplasts, indicating that this nuclear mutation does not affect equally all plastids in white sectors (Wetzel et al., 1994).

The nuclear-encoded functions affected in ghost and immutans offer the opportunity to explore unknown areas of nucleo-cytoplasmic interactions. Until these mutants are characterized at the molecular level, we can only speculate about the possible functions of the corresponding genes. It is important to consider that although these mutants accumulate phytoene, the primary effect may be unrelated to carotenoid biosynthesis. For example, because phytoene synthase is a peripheral membrane protein but subsequent enzymes are part of a multiprotein integral membrane complex, disruptions in the development of plastid membranes may lead to an accumulation of phytoene. Alternatively, the ghost or immutans gene product may regulate the expression of PDS, the activity of PDS, or the assembly of complexes required for PDS activity. The basis for variegation is still unknown, but, as discussed by Wetzel et al. (1994), immutans (and by extension ghost) are clear examples of the metabolic heterogeneity of cells within an organ.

Chromoplast Differentiation in Flowers, Fruits, and Endosperms

As discussed previously, carotenoids accumulate in chromoplasts of flowers and fruits, old leaves, and some roots. Color development in flowers, which is important for attracting insect pollinators, is part of the floral morphogenesis program. Flower carotenoids are generally yellow, but the combination of carotenoids and anthocyanins may result in a different final color. Novel colors of orchid flowers have been obtained by genetically altering the composition of carotenoids and anthocyanins (Griesbach, 1984; see also Holton and Cornish, 1995, this issue).

During the development of tomato flowers, transcripts for PSY and PDS reach maximum levels right before anthesis. In organs of mature flowers, expression of both genes is maximal in petals and anthers (Giuliano et al., 1993). A PDS promoter fragment has been shown to confer chromoplast-specific expression to a reporter gene (G. Giuliano, personal communication). In a cucumber in vitro bud culture system, gibberellin A3 (GA3) was shown to promote the rapid induction of both carotenoids and a carotenoid binding protein (Vainstein et al., 1994). Although transcript levels for carotenoid biosynthesis enzymes were not measured, this work opens the possibility that GA3 participates in the modulation of carotenoid biosynthesis and accumulation during flower development. Interestingly, GA3 also induces anthocyanin biosynthesis during petal development (Weiss et al., 1992), suggesting a possible link between the induction of carotenoids and anthocyanins during flower development.
The developmental process of tomato fruit ripening has been well characterized genetically and biochemically. It involves tissue softening, conversion of chloroplasts into chromoplasts, and metabolic changes that lead to the accumulation of sugars and acids, and it results in fruits with appealing color and taste (Gillaspy et al., 1993). In contrast to mutations that affect chloroplast development, genetic blocks in fruit chromoplast differentiation do not affect the viability of the organism and are therefore easy to propagate and analyze (Rick, 1991). Several tomato mutants affected in chromoplast development during fruit ripening appear to result from defects in carotenoid biosynthesis (for review, see Bartley et al., 1994). To date, only a yellow flesh (also known as the \( R \) locus) has been shown to correspond to a cloned gene (\( \text{PSY} \); Fray and Grierson, 1993). As indicated previously, expression of this gene is induced more than 25-fold during ripening (Bartley and Scolnik, 1993; Giuliano et al., 1993). The molecular basis for the remaining mutants is not currently known.

Ethylene promotes ripening in climacteric fruits such as tomato (Theologis, 1992). Expression of \( \text{PSY} \) and other ripening-specific genes was previously reported as induced by ethylene (Grierson, 1986), but more recent results indicate that the control of expression is not at the level of transcription (reviewed in Bartley et al., 1994). Fruit ripening in pepper, unlike in tomato, is a nonclimacteric process and is thus useful for elucidating the roles in ripening of signals other than ethylene. Pepper \( \text{PSY} \), in contrast with the corresponding tomato gene, is minimally induced by ripening (Römer et al., 1993). However, a cDNA clone (\( \text{P541} \)) for a gene that was shown to encode a plastid protein is expressed at high levels in late stages of pepper fruit ripening (Houlé et al., 1994). The fruits of a mutant pepper line homozygous for the \( y \) locus are yellow due to the accumulation of violaxanthin, a precursor of the red ketocarotenoids capsanthin and capsorubin, and the \( \text{P541} \) transcript is absent. A computer database search (Bouvier et al., 1994; G.E. Bartley and P.A. Scolnik, unpublished data) revealed that \( \text{P541} \) encodes the capsanthin-capsorubin synthase reported by Derèure et al. (1994b), a possibility contemplated by Houlé et al. (1994) based on the sequence of the protein and the timing of expression. Therefore, pepper fruit ripening involves the induction of a gene encoding an enzyme that catalyzes the synthesis of the keto-carotenoids characteristic of pepper fruit. The expression of this gene is under genetic control by the \( y \) gene, which also controls the synthesis or accumulation of the carotenoid binding protein ChrA (Oren-Shamir et al., 1993). Thus, \( y \) may be a general regulator of keto-carotenoid complexes in pepper fruits.

Although carotenoids accumulate during maize endosperm development, little work has been done on the developmental characterization of chromoplasts in this organism. Several carotenoid mutants of maize have been identified (Robertson, 1975), many of which exhibit vivipary due to the lack of ABA (Fong et al., 1983). The \( \text{yellow-f (Yf)} \) locus was cloned by transposon tagging and shown to correspond to \( \text{PSY} \) (Buckner et al., 1990, 1993). The viviparous-5 (\( \text{vp5} \)) locus has been recently shown by genetic mapping to correspond to \( \text{PDS} \) (W. Hable and K. Oishi, unpublished data; GenBank accession number L39266; E. Wurtzel, personal communication).

### BIOTECHNOLOGY

Some carotenoids are precursors of vitamin A, an essential component of the mammalian diet. In the Western diet there is no lack of pro-vitamin A compounds, but in some underdeveloped countries, particularly those whose diets are based primarily on rice, inadequate supplies of vitamin A can lead to night blindness and, in extreme cases, to xerophthalmia, an eye disease that primarily affects children (Toenniessen, 1991). The availability of cloned genes for carotenoid biosynthesis opens the possibility of genetically engineering rice to produce more \( \beta \)-carotene in the endosperm. A consortium of plant biologists sponsored by the Rockefeller Foundation is attempting to modify the carotenoid content of rice without altering the useful agronomical traits of this staple crop.

Epidemiological studies have shown that carotenoid-rich diets are correlated with a significant reduction in the risk for lung cancer, other forms of cancer, coronary heart disease, and some degenerative diseases (Bendich, 1994). Cancer prevention is generally attributed to the antioxidant activities of carotenoids (see, for example, Seis et al., 1994). However, even in developed countries, dietary choices result in a 4.5-mg difference between the actual and recommended daily intake of \( \beta \)-carotene (Lachance, 1988). This deficiency can be compensated for by either taking vitamin supplements or ingesting more carotenoid-rich foods. Carotenoids used as vitamin supplements are currently extracted from plants or produced by organic synthesis (Pfander, 1992). More recently, a biotechnology approach has been used to engineer carotenoid-overproducing yeast strains, which can now be used as a source of natural carotenoids (Ausch, 1994). It is likely that the genetic manipulation of plant carotenoid biosynthesis genes could be used to increase the carotenoid content of widely consumed fruits and vegetables.

In addition to the genetic engineering of plants with improved carotenoid content, genes for carotenoid biosynthesis can be used to generate plants resistant to herbicides that inhibit carotenoid biosynthesis. Introduction of a recombinant DNA construct containing the \( E. uredovora \) gene for \( \text{PDS(CrtI)} \) fused to a chloroplast transit peptide and under control of the cauliflower mosaic virus 3SS promoter into tobacco plants resulted in plants resistant to several herbicides that target carotenoid biosynthesis and show altered metabolism of xanthophylls (Misawa et al., 1993, 1994). Expression of bacterial carotenoid biosynthesis genes in plants may therefore extend the use of carotenoid inhibitors as herbicides for crops that are currently sensitive to these compounds.
CONCLUSIONS

A knowledge of the biology of plant carotenoids is required both to understand such basic plant processes as photoprotection and plastid differentiation during the development of reproductive organs and to develop biotechnology approaches to improve the nutritional value of crops. Recent advances in the molecular biology of the carotenoid biosynthesis pathway in plants have been fueled by previous breakthroughs in the biochemistry and genetics of the pathway in carotenogenic bacteria. Although there are examples of purification of carotenoid biosynthetic enzymes to the degree of homogeneity required to identify the corresponding genes, most of the cloned plant genes were isolated either by using DNA probes of bacterial origin or by identifying entries in sequence data bases by searching with bacterial queries. Although we can anticipate that this approach will still yield interesting genes, many components of the pathway will not be accessible by this methodology. Thus, to isolate the remaining genes for carotenoid biosynthetic enzymes and ancillary proteins, including redox proteins and regulators, it is also necessary to pursue the purification of the corresponding proteins and the study of mutants disrupted at specific biosynthetic steps. In planning these studies, it will be important to take into account the fact that a holistic approach to the study of the biology of carotenoids in plants will require parallel examinations of the genes and proteins for biosynthetic enzymes, apoproteins, and redox components and will regard complexes, and not just carotenoids, as final products of the pathway.

With the isolation of plant biosynthetic genes, it has been possible to study their regulation in response to environmental and developmental factors. Based on the few plant carotenoid genes that have been cloned, a picture has begun to emerge in which this process is divided into two major areas: biosynthesis in photosynthetic tissues, which appears to be under the control of environmental signals, primarily light, and biosynthesis in nonphotosynthetic tissues, which is controlled by yet-unidentified developmental signals. The genes for carotenoid binding proteins are also likely to be regulated by environmental signals in green tissues and by developmental signals in reproductive organs.

In addition to understanding the control of gene expression, it is important to elucidate the molecular interactions that govern the assembly of carotenoid complexes. Carotenoids accumulate in plastids bound to specific proteins and in complexes that include other components, such as chlorophylls and various lipids. Although there is evidence for binding specificity, the basis for molecular recognition between carotenoids and carotenoid binding proteins is poorly understood.

Combined biochemical and genetic approaches to understanding the mechanistic links among morphogenesis, plastid differentiation, carotenoid biosynthesis, and complex assembly are likely to dominate the next decade of research in this field.

ACKNOWLEDGMENTS

We apologize to many colleagues in the field who were not cited in this article due to space limitations and the need to reach a non-specialized audience. We thank Steve Rodermel (University of Iowa, Ames, IA) for providing the photograph of *immutans* shown in Figure 1. We are also indebted to those colleagues who communicated unpublished results and to Rebecca Chasan, Giovanni Giuliani, and Peter Beyer for critical reading of the manuscript.

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*Plant Cell* 1995;7:1027-1038

DOI 10.1105/tpc.7.7.1027

This information is current as of November 6, 2017