Pure & Appl. Chem., Vol. 63, No. 1, pp. 101–108, 1991.
Printed in Great Britain.

The biosynthesis of carotenoids: a progress report

George Britton

Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX, U.K.

Abstract - A general review of carotenoid biosynthesis was presented at the Boston Symposium in 1987. This article gives a report on developments since then. The emphasis is placed on those areas where progress has been greatest, i.e. carotenogenic enzymes and especially molecular genetics. Recent work with carotenogenic enzyme systems from chromoplasts of Capsicum annuum fruit and daffodil (Narcissus pseudonarcissus) flowers is summarized. Genetic studies on carotenoid biosynthesis and its photoinduction in Myxococcus xanthus and on carotenogenesis in Phycomyces blakesleeanus are outlined. The main topic covered is the molecular genetics of carotenoid biosynthesis in the phototrophic bacteria, Rhodobacter capsulatus and Rb. sphaeroides. Work from several laboratories is reviewed, including progress on gene mapping and sequencing and deductions about the carotenogenic enzymes and their regulation. Recent developments in the cloning of carotenogenic enzymes in non-carotenogenic bacteria are also reported, and some ideas about the organization of the enzyme systems discussed.

INTRODUCTION

The Boston meeting in 1987 provided an opportunity for a general review of carotenoid biosynthesis (ref. 1) for the first time since the Bern Symposium in 1975 (refs. 2,3). Another broad review of carotenoid biosynthesis would not be appropriate for this meeting; the main aim of this presentation is to give a report of progress on the biosynthesis of carotenoids in the past three years, since Boston.

In this time, no new information has been published about the pathways and reaction mechanisms of carotenoid biosynthesis. There have been several reports describing further examples or details of the regulatory effects of light in various organisms, e.g. Gibberella fujikuroi (ref. 4), Aspergillus giganteus (ref. 5) and Scenedesmus obliquus (ref. 6), and a genetic study of the photoinduction of carotenoid biosynthesis in Myxococcus xanthus (refs.7-9) will be discussed later. Many chemicals also influence carotenoid biosynthesis. Most examples studied are inhibitory and the effects well known. In Phycomyces blakesleeanus, several compounds e.g. cinnamic alcohol, thymol, diphenylamine, that inhibit phytoene desaturase, also have a stimulatory effect on carotenogenesis overall, through disruption of normal feedback regulation (refs. 10,11).

This presentation will be selective rather than exhaustive and will concentrate on the two areas where the greatest progress has been made, i.e. the carotenogenic enzymes and, especially, the molecular genetics of carotenoid biosynthesis.

Other review articles which have been published since 1987 deal with carotenoid biosynthesis in general (ref. 12) and with the regulation of carotenoid biosynthesis (ref. 13).

ENZYMES OF CAROTENOID BIOSYNTHESIS

The main steps of carotenoid biosynthesis are outlined in Fig. 1. Progress with the enzymes that catalyse these steps has come mainly from the laboratories of Camara, Beyer and Kleinig, and Sandmann. Camara (ref. 14) used an affinity chromatography method to purify the isopentenyl diphosphate (IDP) isomerase and geranylgeranyl diphosphate (GGDP) synthase (prenyl transferase) enzymes from chromoplasts of Capsicum annuum, and has now used similar affinity procedures to purify to homogeneity phytoene synthase from the same source (ref. 15). This single monomeric protein (47.5kDa) carried two enzyme activities, catalysing both the coupling of two molecules of GGDP to yield prephytoene diphosphate (PPDP) and also the subsequent conversion of PPDP into phytoene. The enzymic activity was strictly dependent on Mn^{2+} and inhibited by inorganic pyrophosphate and by the arginine-specific reagent hydroxyphenylglyoxal. In no instance were the two reactions kinetically uncoupled, in agreement with the conclusion that phytoene synthase is a bifunctional enzyme.

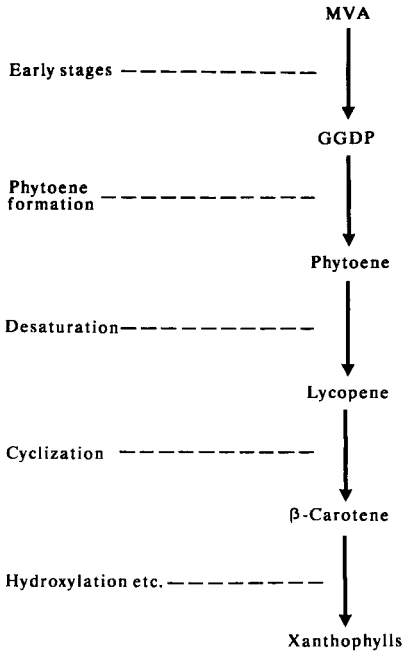

Fig. 1 The main steps of carotenoid biosynthesis

Beyer and Kleinig have studied carotenoid biosynthesis in chromoplast membrane preparations from daffodil, Narcissus pseudonarcissus. The intact chromoplasts synthesize ß-carotene, together with some α-carotene, from IDP. Further work on the early enzymes in this system concentrated on the relationship between IDP isomerase and the phytoene synthase complex (ref. 16). An extensive paper describes detailed studies on the desaturase and cyclase enzyme activities in these chromoplasts (ref. 17). Homogenization of the chromoplasts gave a membrane-bound enzyme preparation in which three distinct segments or phases of the biosynthetic sequence could be identified, though the different enzyme activities have not been isolated. In the first phase, desaturation of (15Z)-phytoene to (15Z)-ζ-carotene occurred in the dark, with O_2 as an essential cofactor. The second phase of the sequence required light to bring about the photoisomerization of the (15Z)-ζ-carotene to (all-E)-ζ-carotene and the desaturation of this to (7Z,9Z,7'Z,9'Z)-lycopene (prolycopene) in preference to the (all-E)-isomer. The involvement of an associated redox system (cytochrome, ferredoxin or similar) as suggested some years ago (ref. 18), was confirmed. The description of the desaturations in these two phases as trans and cis hydrogen elimination, respectively, is misleading; there is no evidence of the stereochemistry of the actual hydrogen loss. The third segment, cyclization, occurred only under strictly anaerobic conditions. The cyclization proceeded efficiently only with prolycopene or (7Z,7'Z)-lycopene as substrates; (all-E)-lycopene was not a substrate, yet the products of cyclization, ß-carotene and some α-carotene, were exclusively in the (all-E) form. The absolute requirement for O_2 for the desaturation phases and for strictly anaerobic conditions for cyclization means that the microenvironment of the successive enzymes must be strictly controlled.

A compound with the properties of prolycopene is found in the mutant strain C-6D of the green alga Scenedesmus obliquus. From the tentative identification of the isomers of the biosynthetic intermediates present, a pathway for the biosynthesis of prolycopene has been proposed (ref. 19), somewhat similar to that previously suggested from studies with the tangerine mutant of tomato (ref. 20). Another green alga, Dunaliella bardawil, can accumulate large amounts of a mixture of (all-E)-and (9Z)-a-carotene. It has been suggested that the two isomers are biosynthesized independently, via all-E and 9Z intermediates, respectively (ref. 21).

Sandmann and coworkers have reported studies of carotenoid biosynthesis in vitro in preparations of the cyanobacterium Anacystis, concentrating on the phytoene desaturase reaction (ref. 22), and have also described investigations of the kinetics of interactions of the well-known desaturation inhibitor norflurazon with phytoene desaturase (ref. 23).

GENETICS OF CAROTENOID BIOSYNTHESIS

Some aspects of the genetics of carotenoid biosynthesis have been covered in lectures at two previous meetings. At Madison, in 1978, Cerda-Olmedo and Torres-Martinez (ref. 24) discussed the genetics and regulation of carotene biosynthesis in Phycomyces and, at Liverpool in 1981, Marrs (ref. 25) introduced the exciting potential of genetic work with phototrophic bacteria, in particular Rhodopseudomonas capsulata, now renamed Rhodobacter capsulatus. Since Boston, work on the genetics of carotenoid biosynthesis in several organisms has been published. Thus Nelson et al. (ref. 26) reported the cloning of the al-3 gene (for geranylgeranyl diphosphate synthase) of Neurospora crassa. Murillo and colleagues (refs. 7-9) have described work on transposon mutagenesis of Myxococcus xanthus. Carotenoid biosynthesis is photoinduced in the wild type, but six mutants were obtained which did not require light for carotenoid production.

These mutants showed red phenotypes, in contrast to the yellow wild-type. Four genes were identified, designated carA,B,C and R. Mutation in carB or carC blocked carotenogenesis, whereas mutations in carA or carR removed the requirement for light. The gene carA was identified as a cis-acting element for the control of carB. The carotenoid analyses that are necessary to complement the genetic work and allow detailed study of the biosynthetic pathway have not yet been done. Another important development is the expression of the carotenoid biosynthesis genes from Erwinia species in the normally non-carotenogenic E. coli. Work with this system, including results reported in Kyoto, will be commented upon later.

Further work has been published on the biosynthesis of ß-carotene in Phycomyces blakesleeanus, mainly from the laboratory of Cerda-Olmedo (refs. 10,11, 27). The production of ß-carotene is greatly increased by exposure of the mycelia to blue light or to chemicals such as retinol or dimethyl phthalate and by interactions between mycelia of opposite sex. These four agents all cause increased gene expression but appear to have different modes of action. Several structural genes for carotenoid biosynthesis have been recognised. In addition to the well-known genes carB and carR, mutants in which accumulate phytoene and lycopene, respectively, mutation in carS removes the normal feedback inhibition so that ß-carotene accumulation is increased 100-fold. The carS function is influenced by two antagonistic genes, carD and carC, whereas carA is essential for the expression of the entire pathway. Mutants in carI are much less sensitive to chemicals that enhance carotene biosynthesis in the wild-type.

It has been proposed that expression of the carotenoid biosynthesis genes requires a product, pA, of carRA. The product pS of gene carS binds and inactivates pA and the normal end-product, ß-carotene, enhances the inactivation by forming a pS:ß-carotene:pA complex. Retinol, by competition with ß-carotene prevents the formation of the complex, as does absence of the pS function (carS or carD mutants) and interruption of the pathway so that ß-carotene is not produced.

The major advances over the past three years have been made with the phototrophic bacteria, following on from the work of Marrs (ref. 25).

Molecular genetics of carotenoid biosynthesis in phototrophic bacteria

The phototrophic bacteria, especially the purple, non-sulphur bacteria of the Rhodospirillaceae, have been studied extensively for many years. Their carotenoids are almost all acyclic compounds which usually have a long chromophore and have tertiary hydroxy- and methoxy-groups at C-1 and C-1', e.g. hydroxyspheroidene. Mainly as a result of kinetic studies of the transformations that occur on removal of inhibitors such as diphenylamine or nicotine, pathways have been proposed for the biosynthesis of spirilloxanthin either from lycopene, or from neurosporene via spheroidene and hydroxyspheroidene (Fig. 2). Although these are frequently referred to as 'alternative pathways', they use the same reactions, but at different relative rates in the two halves

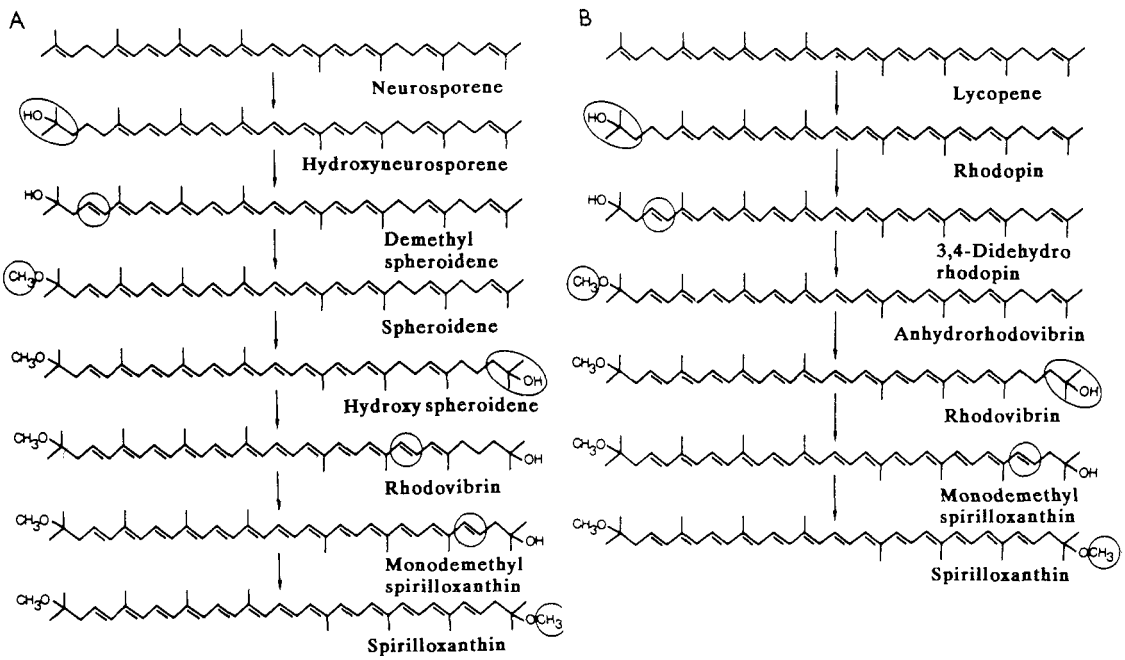

Fig. 2 Pathways for the biosynthesis of spirilloxanthin
A. From neurosporene, via spheroidene; B. From lycopene

of the carotenoid molecule. The carotenoids accumulate in the cells under anaerobic conditions. With several species, exposure to oxygen leads to the rapid formation of the corresponding 2-oxo-derivatives, e.g. spheroidene is rapidly converted into spheroidenone in Rhodobacter sphaeroides and Rb. capsulatus. Details of the localization and functioning of carotenoids in the bacteria are discussed in another article in this volume (ref. 28). The bacteria contain photosynthetic membranes in which there is one photosystem consisting of a reaction centre and one or two associated light-harvesting antennae. These pigment-protein complexes contain peptides, bacteriochlorophylls (and bacteriophaeophytin) and the carotenoids. In the Rhodobacter species, the biosynthesis of all these components is encoded on a tight gene cluster (46 kilobase in Rb. capsulatus, 45 kilobase in Rb. sphaeroides). Marrs (ref. 29) discovered that this gene cluster can be inserted into a plasmid and is therefore amenable to molecular genetic manipulations such as transposon or interposon mutagenesis. In the past 2-3 years, a great deal of exciting progress has been made in the laboratories of Marrs, Scolnik, Giuliano and Hearst with Rb. capsulatus and of Harding and Pemberton, and Hunter with Rb. sphaeroides.

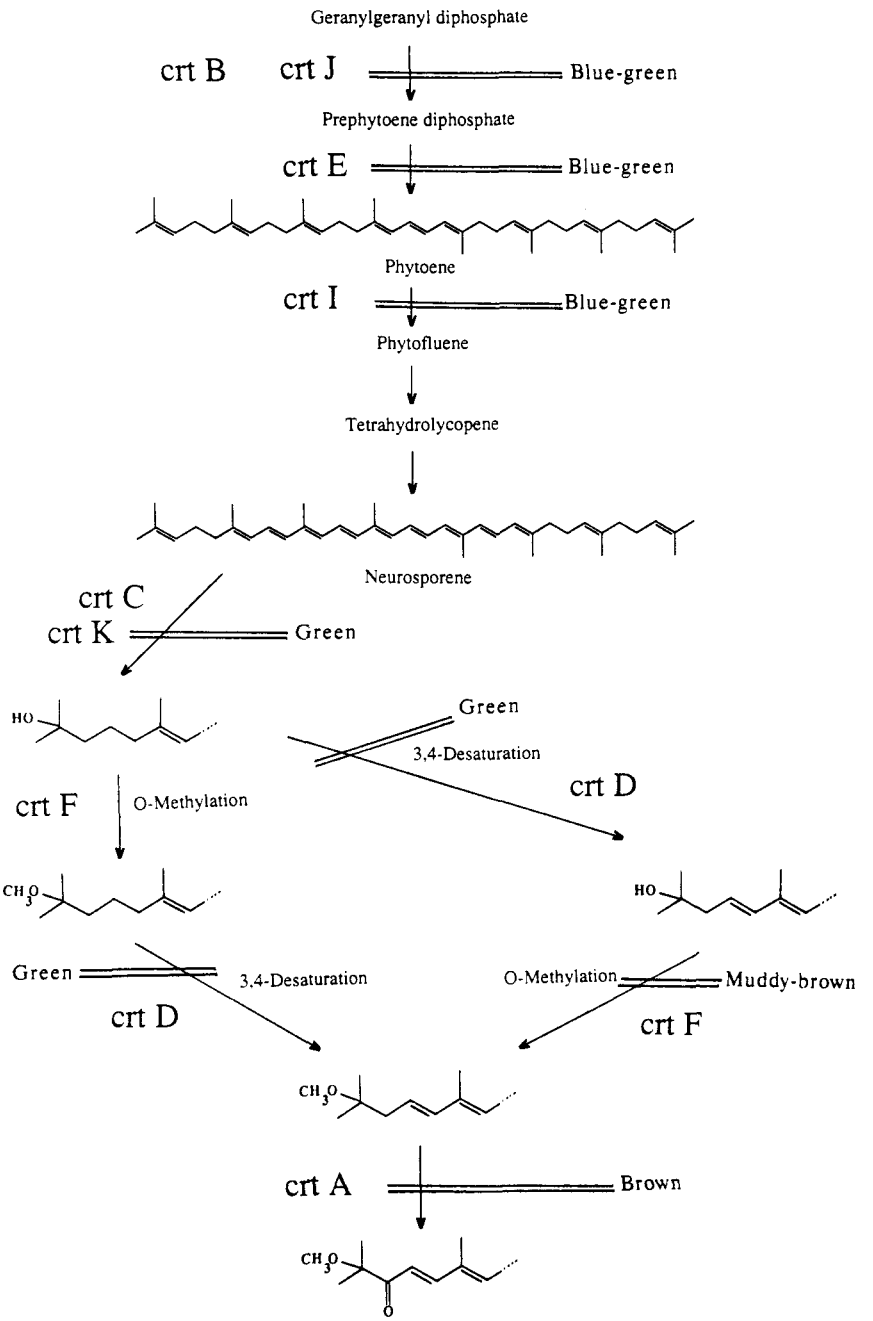

Fig. 3 Sequence of reactions in the biosynthesis of carotenoids in Rhodobacter species. The reactions catalysed by the known gene products are shown, and the colour phenotypes given by mutations in the various crt genes are indicated.

Rhodobacter capsulatus. In the case of Rb. capsulatus, all the known carotenoid genes lie within the 46kb photosynthetic gene cluster which is derived from the bacterial chromosome and which includes at least 9 carotenoid (crt) and 11 bacteriochlorophyll (bch) genes. A variety of transposon, interposon and point mutants are available which are easily identified by their altered pigmentation. Phenotype selection has sometimes been made simply on the basis of the colour of the colonies, but it is essential that the characterization should be supported by rigorous identification of the carotenoids present, by HPLC, UV-visible and mass specta. By the use of such mutants, the crt genes, introduced into plasmid pRPS404, have been mapped by genetic and physical techniques (refs. 30,31) and biochemical functions have been assigned to the gene products by identification of the intermediates that accumulate in the mutants. The sequence of reactions in the biosynthesis of the carotenoids in Rb. capsulatus is illustrated in Fig. 3; also indicated are the sites at which the various gene products act, and the colour phenotype given by a mutation in each crt gene.

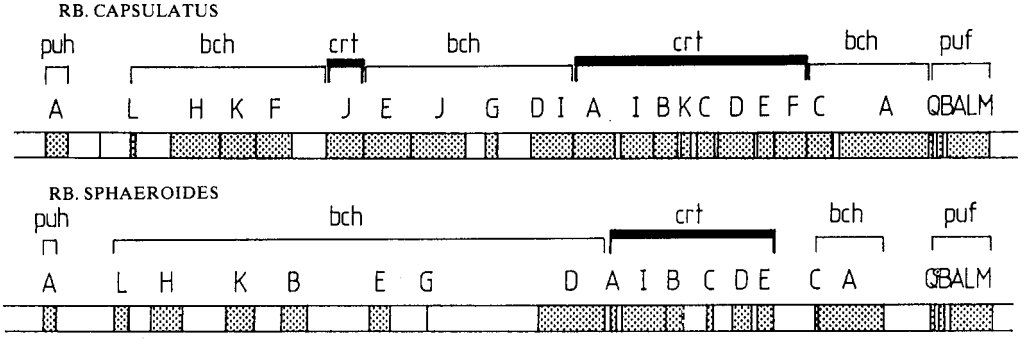

Fig. 4 Maps of the photosynthetic gene clusters of (a) Rb. capsulatus, (b) Rb. sphaeroides. [Data from refs. 33, 36]

A segment of contiguous DNA from the photosynthetic gene cluster has now been cloned and sequenced. This segment, consisting of 11039 base pairs, contains eight of the nine crt genes that have been recognized, namely crtA, B, C, D, E, F, I and a newly detected gene, crtK. The sequence of 11039bp has been published (ref. 30) and the sequence of each crt gene deduced. The locations of the crt genes in the photosynthetic cluster are illustrated in Fig. 4. The region between crtB and crtC is considered to be a previously undetected gene, designated crtK. The function of this gene is not known but its product, CrtK, appears to be associated, together with CrtC, with the C-1,2-hydration reaction. The crt genes are not all transcribed in the same direction (Fig. 5). This work reverses the direction of transcription of crtD from that proposed earlier (ref. 31).

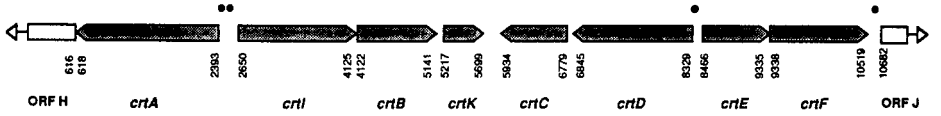

Fig. 5 Direction of transcription of the crt genes in Rb. capsulatus [Data from ref. 33]

Some promoter sequences similar to E. coli σ^{70} and some highly conserved palindromic motifs that are homologous to a recognition site for DNA-binding regulatory proteins have been located and their possible involvement in the regulation of carotenoid biosynthesis in Rb. capsulatus has been suggested.

From the base sequence, and from a consideration of codon usage in Rb. capsulatus, which shows a strong bias against codons having A or T in the third position, sites of translational starts have been proposed for the crt genes, some possible ribosome-binding sites identified and possible transcription termination signals detected for crtI, B, C, F and K.

From the base sequences the amino acid sequences, molecular masses and mean hydropathy values have been deduced for the crt gene products, the biosynthetic enzymes (Table 1). Confirmation of the sequences and properties awaits the isolation of the proteins themselves. Except for CrtK, the proteins are not particularly hydrophobic. The enzymes CrtI and CrtD which catalyse rather similar reactions, namely 7,8- and 11,12-desaturation (CrtI) and 3,4-desaturation (CrtD), show a remarkably high degree of amino acid homology.

The conclusions of Armstrong et al. (ref. 30) do not agree fully with those of Bartley and Scolnik (ref. 32) who concentrated on the crtI gene, for which they identified an earlier initiation signal, and assigned a 1658bp sequence against the 1473bp of Armstrong et al. (ref. 30). According to Bartley and Scolnik, the desaturase enzyme CrtI has molecular mass approximately 60000 and contains 524 amino acids.

TABLE 1. Deductions about the carotenoid biosynthesis enzymes of Rhodobacter capsulatus. (Data from ref. 33). Crt A-K are the protein products of genes crt A-K, respectively.

Protein	Size (kDa)	Amino acids	Calculated mean hydropathy
Crt A	66	591	±0.01
B	38	341	-0.015
C	31	281	-0.04
D	55	494	+0.112
E	32	289	+0.151
F	44	393	+0.077
I	55	491	+0.009
K	18	160	+0.482

These results are in broad agreement with those of Giuliano et al (ref. 31) who used interposon mutagenesis to map the crt gene cluster of Rb. capsulatus. These workers concluded that, with the possible exception of crtI and crtB, all the crt genes are organized as single transcriptional units. By studying additional transposon Tn5.7 mutants, Armstrong (ref. 33) has now concluded that crtB and crtI also form separate operons, and has ascribed functions to the products of crtB, E and J; CrtB and CrtJ are proposed to be components of the prephytoene diphosphate synthase, and CrtE phytoene synthase.

Young et al. (ref. 34) have provided genetic evidence for superoperonal organization of the photosynthesis genes of Rb. capsulatus. The carotenoid genes crtE and crtF, together with the bacteriochlorophyll bch operon and the polypeptide genes, are co-transcribable. The significance of the regulatory interactions between the adjacent genes is not yet understood.

When Rb. capsulatus and other Rb. species are transferred from aerobic to anaerobic, photosynthetic growth conditions, a considerable increase in carotenoid content is seen; oxygen is the major regulatory factor. Giuliano et al. (ref. 31) have shown that mRNA levels for three genes, crtA, C and E, increase substantially following the transfer and that, with gene crtE, a transcript appears that is almost undetectable under aerobic conditions.

Rhodobacter sphaeroides. A cluster of six carotenoid biosynthesis genes (crtA, B, C, D, E, F) in Rb. sphaeroides was described in 1986 by Pemberton and Harding (ref. 35), but no detailed physical mapping nor rigorous characterization of the pigments and identification of the mutants was presented. We have collaborated with Dr C.N. Hunter (University of Sheffield) in a programme that used transposon mutagenesis to characterize the 45kb photosynthetic gene cluster of Rb. sphaeroides (ref. 36). This cluster is similar in size and composition to that in Rb. capsulatus (Fig. 4). Stable mutants showing crtF phenotype have not yet been identified, and no evidence has been obtained for genes equivalent to crtJ and crtK of Rb. capsulatus. Six genes were identified (crtA, I, B, C, D, E) and analysis by HPLC was used to prove the identification of the carotenoids that accumulated in the various mutants. Although most of these genes appear to be transcribed independently, the possibility of an operon transcribed in the order crtIB was indicated. Detailed work is in hand to extend these preliminary studies.

Deductions about the carotenogenic enzymes of Rhodobacter

From the number of genes that have been identified, it is clear that there is simply one enzyme for each reaction. For example there is only one gene, crtI, for the C-7,8, C-11,12 and C-11',12' desaturations, not different genes coding for the desaturation of phytoene, phytofluene and -carotene (or 7,8,11,12-tetrahydrolycopene). The enzyme molecules and their reaction mechanisms must, therefore, be the same. Similarly, there is only one gene, crtC, for C-1,2-hydration, and hence only one hydration enzyme, not one for each possible substrate, or different enzymes for the two ends of the molecule. This confirms the view (ref. 3) that the enzymes of carotenoid biosynthesis recognise a particular end-group or specific structural feature. The nature of the rest of the molecule is not important. Thus the enzyme that normally adds water across the C-1,2 double bond of neurosporene will also hydrate phytoene if this is present in place of neurosporene. The fact that 1-hydroxy-1,2-dihydrophytoene is present along with phytoene in desaturation (crtI) mutants confirms this, and shows that the genes for hydration (crtC) and desaturation (crtI) are transcribed independently. Although the earlier biosynthetic step, desaturation, is blocked by the mutation in crtI, the enzyme for the later reaction is present.

Further developments: extension to other organisms

The results obtained to date are exciting, but much more can be expected in the near future, e.g. the raising of specific antibodies to permit the localization and isolation of the individual enzymes, cloning of the enzymes, constructing enzyme systems for studies of organization and mechanism, detailed studies of the regulation of carotenoid biosynthesis, and modification of carotenogenic ability and versatility for the biotechnological production of carotenoids.

Antibodies. Some work in these directions is beginning to be reported. Antibodies have been raised against CrtI, phytoene desaturase from Rb. capsulatus (ref. 37). These were used for the immunodetection of phytoene desaturase in the cyanobacterium Aphanocapsa and also cross-reacted with phytoene desaturase from the alga Bumilleriopsis and from higher plants (rape and spinach). The apparent molecular masses of the immunoreactive phytoene desaturase proteins from all these sources were similar (55-64kDa).

Cloning of carotenogenic enzymes. Several attempts to express the carotenoid biosynthesis genes of Rb. capsulatus in Escherichia coli have been unsuccessful. Pemberton and Harding, however, (ref. 38) have succeeded in obtaining carotenoid production by transferring a piece of DNA containing all the known Rb. sphaeroides carotenoid biosynthesis genes into several closely related species of non-phototrophic and normally non-carotenogenic bacteria, e.g. Agrobacterium tumifaciens and Paracoccus denitrificans. No detailed characterization of the carotenoids produced was reported.

The carotenoid gene cluster from the yellow non-phototrophic bacterium Erwinia herbicola has been expressed in E. coli (ref. 39). A closely similar system has now been explored in more detail. Misawa et al. (ref. 40) cloned the carotenoid biosynthesis genes from Erwinia uredovora as a 6913 base pair fragment in E. coli. The main carotenoid produced was zeaxanthin di-ß-glucoside, and six independently transcribed genes, designated zexA-F, were identified (Fig. 6). E. coli carrying zexE,A,D and C produced ß-carotene at 2mg/g dry weight. Similar work is reported at this meeting by Ausich et al. (ref. 41) who have expressed the genes for GGDP synthase, phytoene synthase and phytoene desaturase from Erwinia herbicola in E. coli. The introduction of only these three genes was sufficient for lycopene to be produced smoothly and in good yield.

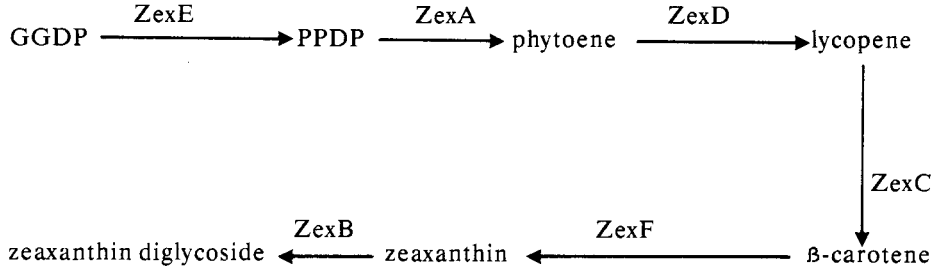

Fig. 6 The pathway for the biosynthesis of carotenoids in Erwinia uredovora and identification of the products (Zex A-F) of the carotenoid biosynthesis genes in a 6913 base pair gene fragment. The genes are designated zexA-F, from left to right along the cluster, with zexF being read in the opposite direction to all the others. [Data from ref. 40].

This reveals a number of points about the organization of the carotenogenic enzymes. These enzymes are generally believed to be located, as an organized multi-enzyme complex, in or bound to membranes, in carotenogenic organisms, yet this work has shown that these enzymes can be expressed and correctly organized in E. coli, an organism that is normally incapable of carotenoid biosynthesis. A model is therefore indicated in which phytoene synthase is able to recognize and attach to a protein-binding site on the E. coli membrane. The required four molecules of the desaturase enzyme must be able to associate with the membrane-bound synthase, in the correct orientation. It seems likely that there are binding sites on the synthase that are recognized. This suggests, therefore, that the bacterial carotenogenic enzyme system is self-assembling, needs no additional binding or regulatory proteins, and involves no separate isomerase enzymes. The implications of this work for further investigations of carotenoid biosynthesis may be far-reaching.

Although such a simple model may be applicable to the bacteria, it is likely that, in higher organisms, the arrangement and organization of the carotenogenic enzymes may be much more complicated. This would obviously be the case with the daffodil chromoplast system discussed earlier. A comparative study of the enzymes and their organization in systems of increasing complexity should prove fascinating.

REFERENCES

1. G. Britton, in Carotenoids: Chemistry and Biology (N.I. Krinsky, M.M. Mathews-Roth and R.F. Taylor, eds.), pp. 167-184. Plenum, New York (1990).
2. B.H. Davies and R.F. Taylor, Pure Appl. Chem., 47, 211-221 (1976).
3. G. Britton, Pure Appl. Chem., 47, 223-236 (1976).
4. J. Avalos and E.R. Schrott, FEMS Microbiol. Letters, 66, 295-298 (1990).
5. M. El-Jack, A. Mackenzie and P.M. Bramley, Planta, 174, 59-66 (1988).
6. K. Humbeck, Photochem. Photobiol., 51, 113-118 (1990).
7. A. Martinez-Laborda, M. Elias, R. Ruiz-Vazquez and F.J. Murillo, Mol. Gen. Genet., 205, 107-114 (1986).

8. J.M. Balsalobre, R.M. Ruiz-Vazquez and F.J. Murillo, Proc. Natl. Acad. Sci., USA, 84, 2359-2362 (1987).
9. A. Martinez-Laborda and F.J. Murillo, Genetics, 122, 481-490 (1989).
10. E.R. Bejarano, F. Parra, F.J. Murillo and E. Cerda-Olmedo, Arch. Microbiol., 150, 209-214 (1988).
11. E.R. Bejarano and E. Cerda-Olmedo, Phytochemistry, 28, 1623-2626, (1989).
12. G. Britton, in Plant Pigments (T.W. Goodwin, ed.) pp. 133-182. Academic Press, London (1988).
13. P.M. Bramley and A. Mackenzie, Curr. Topics Cell Regulation, 29, 291-343 (1988).
14. O. Dogbo and B. Camara, Biochim. Biophys. Acta, 920, 140-148 (1987).
15. O. Dogbo, A. Laferriere, A. D'Harlingue and B. Camara, Proc. Natl. Acad. Sci. USA, 85, 7054-7058 (1988).
16. M. Lutzow and P. Beyer, Biochim. Biophys. Acta, 959, 118-126 (1988).
17. P. Beyer, M. Mayer and H. Kleinig, Eur. J. Biochem., 184, 141-150 (1989).
18. G. Britton, Z. Naturforsch., 34C, 979-985 (1979).
19. S. Ernst and G. Sandmann, Arch. Microbiol., 150, 590-594 (1988).
20. J.M. Clough and G. Pattenden, J. Chem. Soc. Perkin Trans. I, 3011-3018 (1983).
21. A. Ben-Amotz, A. Lers and M. Avron, Plant Physiol., 86, 1286-1291 (1988).
22. G. Sandmann and S. Kowalczyk, Biochem. Biophys. Res. Commun., 163, 916-921 (1989).
23. G. Sandmann, H. Linden and P. Boger, Z. Naturforsch., 44C, 787-790 (1989).
24. E. Cerda-Olmedo and S. Torres-Martinez, Pure Appl. Chem., 51, 631-637 (1979).
25. B. Marrs, in Carotenoid Chemistry and Biochemistry (G. Britton and T.W. Goodwin, eds.) pp. 273-277, Pergamon, Oxford (1982).
26. M.A. Nelson, G. Morrelli, A. Carattoli, N. Romano and G. Macino, Mol. Cell Biol., 9, 1271-1276 (1989).
27. L.M. Salgado, E.R. Bejarano and E. Cerda-Olmedo, Exp. Mycol., 13, 332-336 (1989).
28. H.A. Frank, C.A. Violette, J.K. Trautman, A.P. Shreve, T.G. Owens and A.C. Albrecht, this volume.
29. B. Marrs, J. Bacteriol., 146, 1003-1012 (1981).
30. G.A. Armstrong, M. Alberti, F. Leach and J.E. Hearst, Mol. Gen. Genet., 216, 254-268 (1989).
31. G. Giuliano, D. Pollock, H. Stapp and P.A. Scolnik, Mol. Gen. Genet., 213, 78-83 (1988).
32. G.E. Bartley and P.A. Scolnik, J. Biol. Chem., 264, 13109-13114 (1989).
33. G.A. Armstrong, Ph.D. Thesis, University of California, Berkeley (1989).
34. D.A. Young, C.A. Bauer, J.C. Williams and B.L. Marrs, Mol. Gen. Genet., 218, 1-12 (1989).
35. J.M. Pemberton and C.M. Harding, Curr. Microbiol., 14, 25-29 (1986).
36. S.A. Coomber, M. Chaudhri, A. Connor, G. Britton and C.N. Hunter, Molec. Microbiol., 4, 977-989 (1990).
37. A. Schmidt, G. Sandmann, G.A. Armstrong, J.E. Hearst and P. Boger, Eur. J. Biochem., 184, 375-378 (1989).
38. J.M. Pemberton and C.M. Harding, Curr. Microbiol., 15, 67-71 (1987).
39. K.L. Perry, T.A. Simonitch, K.J. Harrison-Lavoie and S.T. Liu, J. Bacteriol., 168, 607-612 (1986).
40. N. Misawa, K. Kobayashi, M. Nakagawa, S. Yamano, Y. Izawa, K. Nakamura and K. Harashima, Abstr. 6th Int. Symp. Genetics Indust. Microorganisms, Strasbourg (1990).
41. R. Ausich, F. Brinkhaus, I. Mukharji, J. Proffitt, J. Yarger, H.C.B. Yen, M. Fink, L. Hardin, E. Hatch, W. Hunsaker, K. Wilber and L. Wohlfahrt, Abstr. 9th Int. Symp. Carotenoids, Kyoto (1990).