Gene *sll0033* from *Synechocystis* 6803 Encodes a Carotene Isomerase Involved in the Biosynthesis of all-*E* Lycopene

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The function of gene *sll0033* from *Synechocystis* 6803 which is homologous to the bacterial *crtI*-type phytoene desaturase genes was elucidated as a novel carotene isomerase. *Escherichia coli* transformed with all genes necessary for the formation of ζ-carotene and expressing a ζ-carotene desaturase synthesized the positional isomer prolycopene (7,9,7′,9′*Z* lycopene) which cannot be cyclized in the subsequent reactions to a- and β-carotene. Upon cotransformation with *sll0033,* the formation of all-*E* lycopene is mediated instead.

In cyanobacteria and plants, α-carotene, β-carotene and their derivatives dominate as all-*E* isomers. However, among the carotenogenic mutants, prolycopene (7,9,7′,9′Z lycopene) accumulating *Scenedesmus obliquus* (Ernst and Sandmann, 1988) and tangerine tomato fruit (Clough and Pattenden, 1983) can be found. Enzymological studies with plastids of *Narcissus pseudonarcisssus* indicated that the reaction product of the plant-type ζ-carotene desaturase (encoded by *crtQb* in cyanobacteria or *zds* in plants) is poly-*cis* prolycopene (Beyer *et al.*, 1991). Due to its steric configuration, this isomer cannot be cyclized to ionone end groups. Therefore in the carotenogenic pathway of cyanobacteria and eukaryotes with a similar ζ-carotene desaturase, a specific process may prevent the accumulation of prolycopene or convert it mainly to the all-*E* form which then can be further cyclized. This isomerization reaction may be catalyzed by a specific enzyme. It is the objective of this study to identify and demonstrate the existence of this carotene isomerase.

Complementation studies in *Escherichia coli* with different carotene background identified the function of the genes in the *Synechocystis* 6803 genome (Kaneko *et al.*, 1996) related to *pds*- and *crtI*-type carotene desaturases (Fernández-González *et al.*, 1997; Breitenbach *et al.*, 1998) with the exception of *sll0033* belonging to the latter type. The role of the corresponding protein in carotenogenesis remained open. Since CrtI and structurally-related CrtQa enzymes both produce all-*E* lycopene as their reaction products, the participation of *sll0033* in the formation all-*E* lycopene was investigated. For this purpose, *E. coli* was cotransformed with pACCRT-EBP which mediated the formation of ζ-carotene (Linden *et al.*, 1993), pBBR1MCS2zds carrying the ζ-carotene desaturase cDNA from *Capsicum annuum* (Breitenbach *et al.*, 1999) and with plasmid pTrc-0033 containing gene *sll0033* (Breitenbach *et al.,* 1998). In the control transformant, the latter plasmid was replaced by the cloning vector pTrc99A. Growth in the presence of ampicillin, kanamycin, chloramphenicol

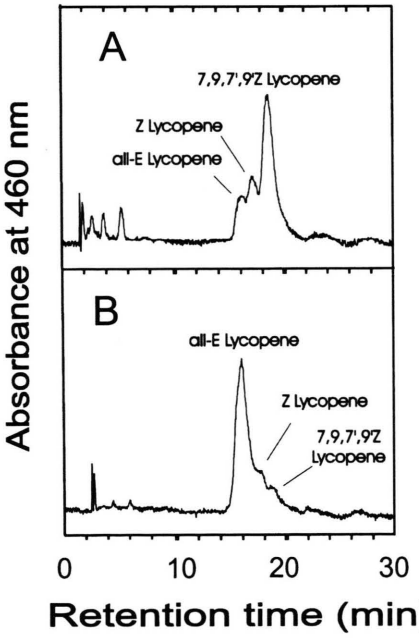

Fig. 1. HPLC separation of carotenoids from *E. coli*/pACCRT-EBP/pBBR1MCS2zds/pTrc99A (A) and *E. coli*/pACCRT-EBP/pBBR1MCS2zds/pTrc-0033 (B). Separation was carried out on a Nucleosil C_{18} 3μ column with acetonitrile/methanol/2-propanol (85:10:5).

col and isopropyl β-D-thiogalactopyranoside was for 20h at 26 °C. Details of the cultivation conditions, carotenoid extraction and HPLC analysis of the carotenoids are given in former publications (Breitenbach *et al.*, 1998 and 1999). Identification of the lycopene isomers was performed as recently described (Breitenbach *et al.*, 2001).

Fig. 1 shows the HPLC separation of the carotenoids from *E. coli*/pACCRT-EBP/pBBR1MCS2-zds/pTrc-99A without *sll0033* as a control (A) and the transformant expressing *sll0033* additionally (B). ζ-Carotene desaturase alone mediates the formation of mainly prolycopene (7,9,7′,9′*Z*) and small amounts of all-*E* lycopene (Fig. 1A). The latter may result from photoisomerization to which prolycopene is very susceptible. The *Z*-lycopene peak running between prolycopene and all-*E* lycopene consists mainly of 7Z and other lycopene isomers. The quantitative distribution of the lycopene isomers is x15% all-*E*, 19% *Z* and 66% prolycopene. Dominance of prolycopene as the reaction product was also observed for the z-carotene desaturase CrtQb from *Synechocystis* (Breitenbach *et al.*, 2001). In the presence of pTrc-0033 (Fig. 1B), all-*E* is the major product of ζ-carotene desaturation representing 79% of the lycopene isomers together with 11% all-*E* and 10% prolycopene. From this result it can be concluded that *sll0033* encodes a protein which either isomerizes prolycopene to the all-*E* form or shifts the isomer composition to all-*E* during ζ-carotene desaturation. *In vitro* studies with the isolated Sll0033 should tell whether this protein is acting either simultaneously with ζ-carotene desaturase to prevent the formation of prolycopene instead of all-*E* lycopene or sequentially as an independent prolycopene isomerase converting this poly-*cis* isomer to all-*E* lycopene.

Formation of all-*E* lycopene in the carotenogenic pathway can be catalyzed by combination of different gene products or by CrtI alone (Fig. 2). The 4-step desaturation by CrtI is the ancient bacterial/archebacterial desaturation reaction. During evolution, this enzyme was replaced by a newly acquired 2-step phytoene desaturase Pds at the stage of cyanobacteria. As a consequence, a second z-carotene desaturase is required to catalyze the two remaining desaturation steps. In *Anabaena* 7120, this function is exerted by CrtQa which evolved from CrtI and like CrtI produces all-*E* lycopene. In other cyanobacteria like *Synechococcus* and *Synechocystis*, ζ-carotene desaturation is cata-

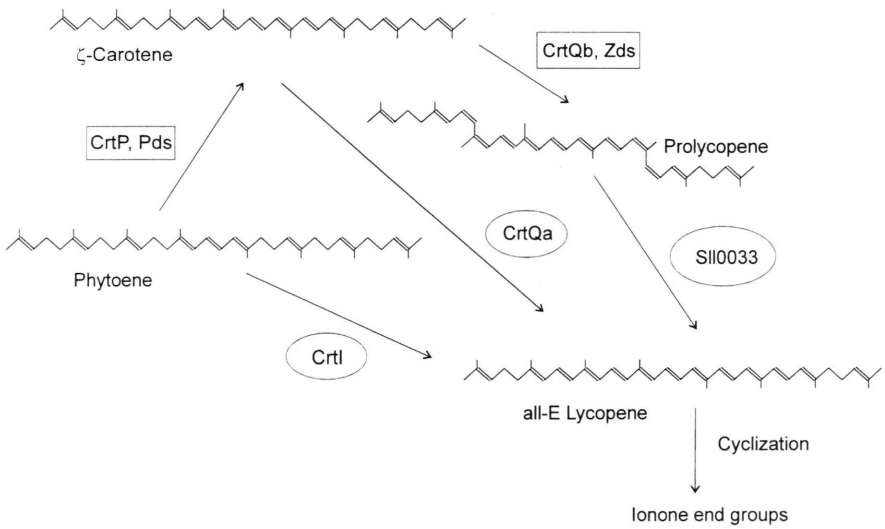

Fig. 2. Reaction sequences from phytoene to all-*E* lycopene involving different gene products. CrtI, 4-step phytoene desaturase from bacteria or archebacteria; CrtP and Pds, 2-step phytoene desaturases from cyanobacteria and plants; CrtQb and Zds, ζ-carotene desaturase from cyanobacterial and plants; CrtQa, ζ-carotene desaturase from *Anabaena* 7120; Sll0033 carotene isomerase from *Synechocystis* 6803. Boxes and cicles indicate the structual relationship to the *pds*- or *crtI*-type of carotene desaturases, respectively.

lyzed by CrtQb (formerly named CrtQ-2) from which the plant ζ-carotene desaturase evolved (Sandmann and Vioque, 1999).

Obviously, in the course of evolution of carotenogenesis from bacteria via cyanobacteria to plants, the simple situation of one enzyme for the entire reaction sequence from phytoene to all-*E* lycopene changed to a more complex process involving three individual enzymes, a novel phytoene and ζ-carotene desaturase as well as a carotene isomerase which is phylogenetically related to CrtI. Only the CrtI-type enzymes seem to have the property to catalyze the formation of all-*E* lycopene, the most important isomer for the formation of cyclic carotenoids. In addition to the isomerase gene *sll0033* from *Synechocystis*, a highly similar sequence AAF631149 (Blast score of 592, E-value of 10^{-168}) is present in the data base of the complete *Arabidopsis thaliana* genome sequence. This gene is the best candidate for a corresponding plant carotene isomerase gene.

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