Tangerine Dreams: Cloning of Carotenoid Isomerase from Arabidopsis and Tomato

Carotenoids are vital components of photosynthetic cells, where they play important accessory roles in light harvesting and energy transfer to the chlorophylls, maintain structural integrity of the photosynthetic apparatus, and provide photoprotection against the damaging effects of reactive oxygen species that are by-products of photosynthesis. Violaxanthin and neoxanthin also serve as precursors for biosynthesis of the hormone abscisic acid. In higher plants, carotenoids provide the bright yellow, orange, and red colors of flowers and fruits. Carotenoids also are important in human nutrition, serving as antioxidants in protecting against certain degenerative diseases such as macular degeneration of the eye, a leading cause of age-related blindness in the United States. Additionally, \( \beta \)-carotene is the precursor of vitamin A.

**BIOSYNTHESIS**

Carotenoids are long (mainly 40-carbon) isoprenoid chains that may be linear or cyclized at one or both ends of the molecule. The first committed step in carotenoid biosynthesis in plants and cyanobacteria is the production of the colorless compound phytoene via the condensation of two molecules of geranylgeranyl diphosphate by phytoene synthase. In photosynthetic organisms, phytoene desaturase (PDS) and \( \zeta \)-carotene desaturase (ZDS) catalyze successive dehydrogenation reactions of phytoene to yield the colored molecules \( \zeta \)-carotene and lycopene, respectively. In nonphotosynthetic (anoxygenic) bacteria, the desaturation of phytoene to lycopene is catalyzed by a single enzyme, bacterial phytoene desaturase (CrtI). The sequence of CrtI is unrelated to that of PDS or ZDS, suggesting that some of the steps in carotenoid biosynthesis arose independently in photosynthetic organisms (higher plants and cyanobacteria) and nonphotosynthetic bacteria (Hirschberg et al., 1997).

Phytoene, \( \zeta \)-carotene, and lycopene are linear molecules. Cyclization of lycopene marks a branch point of the pathway to either \( \alpha \)- or \( \beta \)-carotene. Hydroxylation of \( \alpha \)-carotene yields lutein, the major xanthophyll in the light-harvesting apparatus of most higher plants, whereas hydroxylation of \( \beta \)-carotene leads to the formation of the xanthophylls zeaxanthin, violaxanthin, and neoxanthin.

**ISOMERIZATION**

The isoprenoid chains of carotenoids contain numerous double bonds that allow the formation of a variety of cis or trans geometric isomers. The cis-trans configurations are critical features of carotenoids, because they determine the spectral quality and resulting photochemical properties and colors of these pigments. Carotenoid isomerization in plants has been something of a mystery for more than 60 years since the tangerine (f) mutant of tomato, with its orange-tinted flowers and characteristic tangerine-orange fruit, was described by Zechmeister et al. (1941) and Tomes et al. (1953). L. Zechmeister, a noted chemist who went on to publish numerous articles on the stereochemistry of carotenoids in the 1940s and 1950s, collaborated with Fritz Went and Linus Pauling to determine the carotenoid composition of the tangerine tomato. They determined that the fruit of tangerine accumulate a poly-cis-isomer of the carotenoid lycopene, named prolycopene, whereas the wild-type red tomato varieties produce mainly trans-isomers of lycopene and other carotenoids (Zechmeister et al., 1941). Like tomato, most plants appear to produce mainly trans forms of lycopene and other carotenoids (Britton, 1988). Isomerization could occur at any number of steps, and this aspect of carotenoid biosynthesis is not well understood in higher plants.

In this issue of *The Plant Cell*, Isaacson et al. (pages 333–342) and Park et al. (pages 321–332) present the identification of carotenoid isomerase (CRTISO) from tomato and Arabidopsis, respectively. In both species, the mutants lacking CRTISO accumulate prolycopene and/or \( \zeta \)-carotene and very little trans-lycopene, and the CRTISO enzymes are shown to be capable of converting poly-cis-lycopene to all-trans-lycopene when expressed in *Escherichia coli*. Interestingly, although the plant CRTISO lacks desaturase activity, the sequences were found to be homologous with those of the bacterial CrtI phytoene desaturase and are not related to the plant desaturases PDS and ZDS. Thus, in photosynthetic organisms, three enzymes (PDS, ZDS, and CRTISO) catalyze the desaturation and isomerization reactions from phytoene to trans-lycopene (Figure 1) that are performed by a single enzyme, CrtI, in nonphotosynthetic bacteria.

**TOMATO CRTISO**

Isaacson et al. identified the tomato CRTISO gene at the tangerine locus via map-based cloning using introgression
IN THIS ISSUE

The tangerine tomato (top left) and ccr2 mutant of Arabidopsis (top right) lack a functional CRTISO enzyme and accumulate the tangerine-orange pigment poly-cis-lycopene. PDS, phytoene desaturase; ZDS, \( \beta \)-carotene desaturase. (Figure courtesy of Hyoungshin Park.)

Park et al. (2002) isolated the CRTISO mutant in Arabidopsis as a carotenoid and chloroplast regulation mutant named ccr2. The ccr class of mutants and a similar class of lutein-deficient (lut) mutants were identified in a screen of an ethyl methane sulfonate–mutagenized population that was based on HPLC profiles of carotenoid pigments (Pogson et
al., 1996), and additional alleles of \( ccr2 \) were identified in a delayed greening screen (Park et al., 2002). Analysis of the \( ccr2 \) mutations revealed that dark-grown tissue lacked all xanthophylls and produced a tangerine-like carotenoid profile; lutein deficiency was a secondary effect. The phenotype of the \( ccr \) mutants, which have altered plastid development and pigment composition, suggests that carotenoid composition is important in early plastid development.

The Arabidopsis \( CRTISO \) sequence also was identified by map-based cloning and is related closely to the sequence from tomato and to an open reading frame from the cyanobacterium \( Synechocystis \), suggesting that the enzyme may be present in all photosynthetic organisms in which phytoene desaturation is performed by the desaturases PDS and ZDS. The tomato genome appears to have a single copy of the gene (Isaacson et al., 2002). The Arabidopsis genome contains an open reading frame that is related to \( CRTISO \) (Park et al., 2002). Expression characteristics and the possible function of the \( CRTISO \)-like sequence, located on the opposite end of chromosome I from \( CRTISO \), are unknown.

**FUNCTIONAL ANALYSIS**

The functional importance of \( CRTISO \) activity is unclear; \( crtISO \) mutant plants are late greening compared with the wild type but otherwise are completely viable and appear “normal.” Moreover, photoisomerization of carotenoids has been observed in vitro and is believed to occur in vivo (Cunningham and Schiff, 1985; Sandmann, 1991). Thus, \( CRTISO \) activity could be partially redundant in the light.

Analysis of plastids using transmission electron microscopy by Park et al. (2002) showed that etiolated seedlings of the Arabidopsis \( crtISO \) mutant \( ccr2 \) lacked prolamellar bodies (PLBs), which are lattices of paracrystalline membrane tubules that are a defining characteristic of etioplasts (Figure 2). The formation of etioplasts is characteristic of skotomorphogenic (dark-grown) development of angiosperm seedlings. PLBs have a lipid composition similar to thylakoid membranes, so the etioplasts are poised to develop rapidly into functional chloroplasts in the light. The Arabidopsis \( ccr2 \) mutant seedlings turned green at about half the rate of wild-type seedlings when transferred from darkness to light. Park et al. (2002) show convincingly that the slow rate of greening is attributable to the lack of PLB formation in the mutant. They hypothesize that the mutant seedlings are incapable of PLB formation because the shape of the \( cis \)-isomer of prolycopene destabilizes the peculiar PLB membrane structure.

Of course, an obvious function of CrtISO in tomato involves its influence on fruit and flower color. In fruit and flowers, carotenoids are synthesized...
IN THIS ISSUE

in nonphotosynthetic chromoplasts. Isaacson et al. show that CRTISO expression is upregulated in flowers and during fruit ripening. Thus, CRTISO appears to have a unique function in dark-grown and nonphotosynthetic tissues. Because cyanobacteria also appear to synthesize trans-lycopene via PDS, ZDS, and CRTISO and yet lack both etioplasts and chromoplasts, there may be other important functions of CRTISO in photosynthetic organisms. Park et al. (2002) and Isaacson et al. (2002) discuss the possibility that the cis configuration of prolycopene is not cyclized easily by lycopene cyclases because of steric hindrance. It may be that greater steric hindrance of the cis-cyclase by certain lycopene isomers explains the reduced lutein in mature chloroplasts. This interesting possibility deserves further investigation.

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