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Carotenoid isomerase: a tale of light and isomers

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Carotenoids are terpenoid pigments containing systems of conjugated double bonds, each of which can exist in a *cis* or *trans* configuration. The existence of a carotenoid isomerase (CrtISO) mediating *cis*-to-*trans* isomerization has been suspected ever since the description of the tomato *tangerine* mutant in the early 1940s, whose fruits accumulate a poly-*cis*-isomer of lycopene. Recently, CrtISO has been cloned in *Synechocystis* and in plants, and has been shown to be related to bacterial carotene desaturases.

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Carotenoids colour the world around us, with hues ranging from the yellow of daffodils, to the orange of apricots, to the red of tomatoes. They are also present in leaves, where they play important roles in photosynthesis. The chromophore, a system of conjugated double bonds normally found in the centre of the carotenoid molecule, is responsible for the light absorption characteristics. Each of these double bonds can exist in a *cis* or *trans* configuration, influencing the wavelength and intensity of light absorption.

The tale starts with a tomato mutant, *tangerine*, whose fruits and flowers have an orange colour, instead of the red and yellow colour displayed by wild-type fruits and flowers, respectively [1]. NMR spectroscopy was used to shown that although wild-type tomatoes accumulate all-*trans*-lycopene, *tangerine* tomatoes accumulate 7,9,7′,9′-tetra-*cis*-lycopene (prolycopene) and traces of its poly-*cis* precursors, notably 9,9′-di-*cis*-ζ-carotene (pro-ζ-carotene) [2] (Fig. 1).

Cis and *trans* pathways for carotene desaturation

Naturally occurring all-*trans* lycopene derives from 15-*cis*-phytoene. Therefore, this pathway must involve four desaturations and one isomerization (Fig. 1). Bacterial desaturases, such as *Erwinia* CrtI, catalyse both types of reactions. However, plants and cyanobacteria contain two desaturases (Pds + Zds), each catalysing two desaturation steps, but no isomerization. Expression of CrtI in *E. coli* cells yields all-*trans*-lycopene, whereas expression of Pds + Zds yields prolycopene [3]. Considering the prolycopene structure, it must be concluded that Pds inserts *trans* double bonds at the 11,11′ positions, whereas Zds inserts *cis* double bonds at the 7,7′ positions. The mechanism of isomerization of the 15-*cis* double bond, as well as the origin of the 9,9′ *cis* double bonds, remain elusive.

Thus, two independent lines of evidence suggest that plants must contain an enzyme mediating the *cis*-to-*trans* isomerization of lycopene precursors: the existence of a plant mutant accumulating prolycopene, and the accumulation of the same compound in *E. coli* expressing the plant-type desaturases.

Molecular characterization of CrtISO

Two groups have recently reported the molecular cloning of the prolycopene isomerases of tomato and *Arabidopsis* [4,5]. The *tangerine* gene product of tomato has been cloned through a positional cloning approach taking advantage of the near-isogenic lines (NILs) developed by Dani Zamir. These are lines of cultivated tomato (*Lycopersicon esculentum*) carrying, in homozygous form, a chromosome fragment from a wild, cross-fertile species, such as *Lycopersicon pennellii*. Because the two species carry a base substitution every ~500 bases, it is easy to detect and follow molecular polymorphisms closely associated to a mutation in the progeny of a cross. These markers are then used for the map-based cloning of the gene of interest.

Two alleles of *tangerine* have been characterized: a less severe allele (*tangerine*^{3183}) carrying a deletion in the 5′ non-translated region and a more severe allele (*tangerine*^{mic}) carrying a deletion in the coding region. *tangerine*^{3183} accumulates equimolar amounts of prolycopene and pro-ζ-carotene in the fruit, whereas *tangerine*^{mic} accumulates predominantly pro-ζ-carotene. Expression in *E. coli* of the *Tangerine* gene product (CrtISO), in conjunction with Pds and Zds enabling the accumulation of all-*trans* lycopene, confirms that *tangerine* encodes a prolycopene isomerase.

Barry Pogson's group has used a similar approach to clone the *CrtISO* gene of *Arabidopsis*. A mutant, *ccr2*, containing reduced levels of the xanthophyll lutein in leaves was initially identified in a systematic high performance liquid chromatography screen [6]. Dark-grown leaves of this mutant accumulate significant amounts of pro-ζ-carotene and prolycopene, suggesting that *Ccr2*, like *Tangerine* and *Sll1003*, encodes CrtISO. Crossing *Columbia ccr2* plants to wild-type Landsberg *erecta* generated a mapping population of recombinant inbred lines, in which a series of molecular polymorphisms were closely linked to the gene, thus allowing map-based cloning of the gene.

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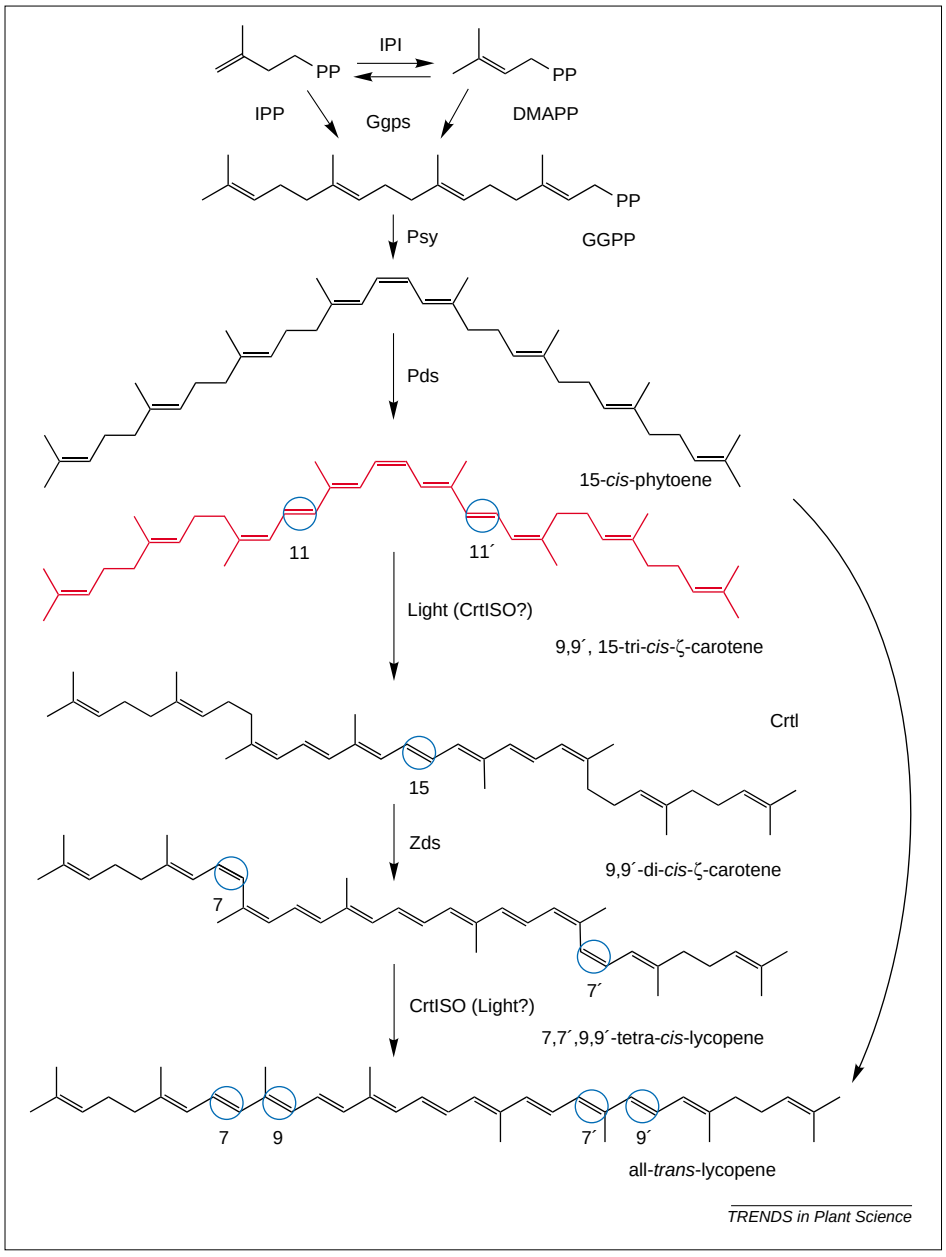

Fig. 1. Proposed pathway for lycopene biosynthesis from IPP. Double bonds affected at each step are circled in blue. The existence of the red-coloured compound (9, 9′,15-cis-ζ-carotene) is hypothetical. Abbreviations: CrtI, bacterial-type phytoene desaturase; CrtISO, carotene isomerase, DMAPP, dimethylallyl diphosphate; GGPP, geranylgeranyl diphosphate; Ggps, geranylgeranyl diphosphate synthase; IPI, isopentenyl diphosphate isomerase; IPP, isopentenyl diphosphate; Pds, plant-type phytoene desaturase; Psy, phytoene synthase; Zds, ζ-carotene desaturase.

These data are in agreement with data obtained independently on the cyanobacterium *Synechocystis* sp., which has a (Pds + Zds)-type desaturation pathway. Analysis of its completely sequenced genome revealed several genes showing homology to known carotene desaturases but having an unknown function. The knockout of one of these genes (*sll0033*) results in the accumulation of *cis*-carotenoids (prolycopene and pro-ζ-carotene) in dark-grown cells, and its overexpression in *E. coli* mediates *cis*-to-*trans* isomerization [7,8].

Photoisomerization

Leaves of light-grown *ccr2 Arabidopsis* and *tangerine*^{mic} tomato display delayed greening, suggesting that, in the absence of CrtISO, carotenoid biosynthesis is rate limiting. Fully green leaves contain an almost normal complement of cyclic carotenoids, with only a slight change in the ratio of β to ε rings. By contrast, etiolated leaves of *ccr2 Arabidopsis* accumulate prolycopene and pro-ζ-carotene and their etioplasts lack a prolamellar body. These observations suggest that:

(i) Lycopene cyclases require all-*trans*-lycopene as a substrate.
(ii) Light is able to isomerize prolycopene to all-*trans*-lycopene in leaves.
(iii) Chlorophyll (or one of its precursors) seems to act as a sensitizer for such photoisomerization because photoisomerization occurs efficiently only in chlorophyll-synthesizing tissues.
(iv) The presence of cyclic xanthophylls (or of a functional CrtISO) is required for the formation of a prolamellar body.

In accordance with the suggestion that light is able to isomerize prolycopene to all-*trans*-lycopene in leaves, illumination of the *sll0033* mutant of *Synechocystis* is able to compensate for the isomerase deficiency, causing the accumulation of all-*trans* cyclic carotenoids [7].

Interestingly, all systems showing inefficient isomerization (dark-grown *ccr2 Arabidopsis*, dark-grown *E. coli* (Pds + Zds), dark-grown *sll0033 Synechocystis*, fruits of *tangerine*^{mic} tomato), accumulate pro-ζ-carotene in addition to prolycopene, suggesting that isomerization might also be required earlier in the pathway to allow Zds action. This needs clarification because Zds does not apparently show stereoisomeric preferences with respect to its substrate [9].

Evolutionary origin of CrtISO

The presence of CrtISO seems to be restricted to the cyanobacterial and plant lineage, which possesses a poly-*cis* desaturation pathway mediated by Pds + Zds. Its protein sequence is related to both CrtI-type and plant-type carotene desaturases (Fig. 2a). Thus, although in purple photosynthetic bacteria and non-photosynthetic organisms, the desaturation and isomerization functions are thought to be combined in a single, CrtI-type enzyme, in cyanobacteria and plants, they are separated on different enzymes: Pds + Zds for the desaturation and CrtISO for the isomerization. Mutants whose carotene isomerization is affected in the dark or in non-photosynthetic tissues have been described in *Scenedesmus*, a green unicellular alga, and in maize [10,11]. It still needs to be proved that the corresponding genes encode CrtISO. The evolutionary relatedness of carotenoid

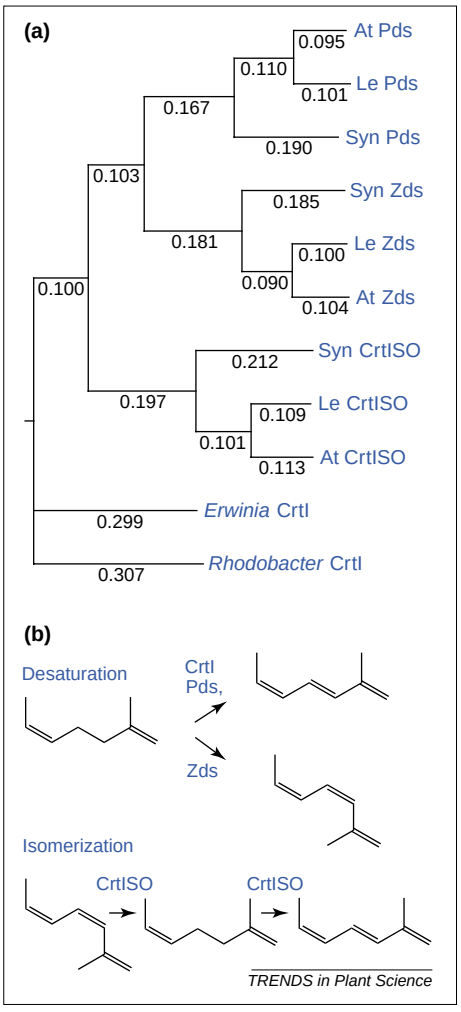

Fig. 2. (a) ClustalW dendrogram of CrtISO, CrtI, Pds and Zds enzymes. (b) Proposed mechanisms of action of carotene desaturases and CrtISO. Abbreviations: At, *Arabidopsis thaliana*; CrtI, bacterial-type phytoene desaturase; CrtISO, carotene isomerase; Le, *Lycopersicon esculentum*; Pds, plant-type phytoene desaturase; Syn, *Synechocystis* sp.; Zds, ζ-carotene desaturase.

desaturases and CrtISO suggests that the two classes of enzymes probably have similar mechanisms of action. *cis*-to-*trans* isomerization can be regarded as a reversible saturation–desaturation reaction, coupled to rotation around the transiently generated single bond (Fig. 2b).

Concluding remarks

Although the cloning of CrtISO furthers our understanding of carotenoid isomerization, several questions remain:

(i) Is enzymatic isomerization restricted to the early steps in carotenoid biosynthesis? 9′ and 13′ *cis* isomers of lutein, violaxanthin and β-carotene are present in photosynthetic antennas and reaction centres, where they presumably derive from isomerization of their all-*trans* counterparts [12].

(ii) What is the exact isomeric composition of some biosynthetic intermediates (pro-ζ-carotene) and what is the origin of the 9,9′-cis bonds in prolycopene?

(iii) Is CrtISO function restricted to double bond isomerization, or it does it have other functions as well (e.g. desaturation of ζ-carotene or prolamellar body formation)?

Note added in proof

Sequencing of the *Chlorobium tepidum* genome revealed an open reading frame with high levels of homology to CrtISO [13].

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