Lycopene cyclase paralog CruP protects against reactive oxygen species in oxygenic photosynthetic organisms

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AUTHOR SUMMARY

Carotenoids with cyclic end groups play essential roles in photosynthesis and photoprotection. Understanding processes governing the formation and maintenance of cyclic carotenoids is essential for developing plants and algae that are capable of supplying food and fuels in the face of climate change-related stresses. CruP was previously suggested to be an accessory lycopene cyclase involved in catalyzing the formation of the cyclic carotenoid β-carotene (1). Here, we report that CruP instead confers increased fitness to oxygenic photosynthetic organisms under stress conditions by preventing accumulation of reactive oxygen species (ROS), thereby inhibiting the degradation of β-carotene. We find no evidence to support lycopene cyclase activity for CruP.

The formation of cyclic carotenoids from lycopene is catalyzed by various lycopene cyclases as follows: CrtL found in plants and some cyanobacteria, CrtY and heterodimeric types found in diverse bacteria, and a Cru type found in chlorobi and in some cyanobacteria (1). The Cru type is composed of two groups of enzymes, CruA (found in chlorobi and cyanobacteria lacking CrtL) and a paralog CruP, present in plants and other organisms that have another, more active lycopene cyclase. An alternative function for CruP is indicated by its codistribution with a second, more active lycopene cyclase. Further, we could not detect lycopene cyclase activity of CruP with a widely used genetic test; therefore, we analyzed CruP gene regulation, gene coexpression, gene neighborhood, and phylogenetic distribution. We also analyzed WT, KO, and cruP-overexpressing plants. We examined transcriptional regulation of cruP (using Genevestigator, Arabidopsis Coexpression Data Mining Tools, and the Rice Oligonucleotide Array Database) and the phylogenetic distribution of cruP and bacterial gene clustering around cruP (using the SEED database). Arabidopsis cruP is highly expressed during cold stress and under dark anoxic conditions, which lead to increased production of ROS. Green tissues, especially cotyledons and pedicels, also express cruP at high levels. These tissues are sensitive to photoinhibition and generate higher levels of ROS compared with true leaves (2, 3).

In Arabidopsis and rice, cruP is coexpressed with genes involved in protection or repair of photosystem II (PSII) from oxidative damage, such as the PSII reaction center D1 photoproteins DEG5, DEG8, FtsH5, and PPL1 (PPL1), which are required for efficient repair of PSII (4). Arabidopsis cruP was also coexpressed with genes encoding the dicarboxylate transporters DIT1 and DIT2 (5), as well as ribose 5-phosphate isomerase, which are involved in CO2 fixation. Analysis of genes in close proximity to cruP in cyanobacterial genomes revealed that they encode proteins with functions similar to those that were coexpressed with plant cruP. Examples include genes encoding proteins with roles related to PSII D1 degradation and repair; an FtsH5 homolog in Synechococcus sp. PCC 7002; and the ClpC and ClpB proteases in Gloeobacter violaceus PCC 7421, Synechococcus sp., JA-2-3b (2-13), and Synechococcus sp. JA-3-3Ab. The genes encoding the Synechococcus elongatus PCC 7942 PSII reaction center proteins D2 and CP43 are also clustered near cruP. Genes relating to carbon fixation were also clustered with cruP, such as those encoding a RuBisCO small subunit protein used in carbon fixation in Nostoc punctiforme PCC 73102 and a carbon dioxide-concentrating protein in Nostoc azollae 0708.

The above-mentioned coexpressed and clustered genes reflect the conditions and locations of expression of cruP in plants, such as those that lead to increased ROS production, photoinhibition, or lipid peroxidation. Therefore, we analyzed the pigment profile and ROS levels of cruP KO, cruP-overexpressing, and WT Arabidopsis. The cruP KO or cruP-overexpressing plants do not exhibit reduced or increased production of cyclized carotenoids, which would be expected if CruP were a lycopene cyclase. Instead, green tissues of plants with a nonfunctional cruP
accumulated substantially higher levels of ROS and a degradation product of β-carotene catalyzed by ROS, β-carotene-5,6-epoxide. Plants overexpressing cruP showed reduced levels of ROS and β-carotene-5,6-epoxide compared with KO and WT plants. Under cold stress, KO and WT plants produced high levels of anthocyanin pigments in contrast to three overexpressing lines that were virtually free of anthocyanins, and appeared healthy and green.

Phylogenetic analysis showed that cruP sequences are confined to oxygenic photosynthetic organisms, in contrast to other lycopene cyclase genes. Further analysis showed cruP is only present in cyanobacteria from habitats characterized by increased ROS production caused by large variations in temperature and inorganic carbon availability. For example, cruP sequences were undetectable in the genomes of open-ocean cyanobacteria living in an environment characterized by steady temperatures and consistent inorganic carbon supply, suggesting that CruP affords increased fitness to oxygenic photosynthetic organisms that inhabit environments characterized by fluctuating conditions.

The pigment profile of Arabidopsis cruP KOs and overexpressors strongly suggests that CruP possesses a function other than lycopene cyclization. Considering the consistently observed up-regulation of cruP transcripts in response to ROS production, the limited phylogenetic distribution of cruP, the inverse association between β-carotene-5,6-epoxide and cruP transcript levels, and the extreme reduction of anthocyanin production in cruP-overexpressing plants under cold stress, we are compelled to conclude that CruP appears to play a role in preventing ROS accumulation (Fig. P1). In fact, the evidence presented here suggests that CruP is not a lycopene cyclase and, instead, represents a unique target for developing plants and algae with increased tolerance to temperature and anoxia. The availability of such organisms will help address the escalating demands for food and fuels brought about by global warming.