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Transcriptional control of *vitamin C defective 2* and *tocopherol cyclase* genes by light and plastid-derived signals: The partial involvement of GENOMES UNCOUPLED 1

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ABSTRACT

Previous findings have suggested that light and plastid-derived signals are involved in the regulation of biosynthetic pathways for L-ascorbic acid (AsA) and tocopherols (Toc). Photosynthetic electron transport (PET) activity, plastid gene expression (PGE), and the tetrapyrrole metabolism have been identified as signals that regulate nuclear gene expression through the GENOMES UNCOUPLED 1 (GUN1) protein. Here, we examined the effects of disrupting *GUN1* on these pathways. The expression of *vitamin C defective 2* (*VTC2*) and *tocopherol cyclase* (*TC*) genes, which encode key enzymes in the AsA and Toc biosynthetic pathways, respectively, was affected by illumination and darkness in parallel with the levels of both these antioxidants. However, the *GUN1* disruption had no effect on these biosynthetic pathways under light-dark conditions. All treatments that inhibited PET, PGE, and the tetrapyrrole metabolism interrupted both biosynthetic pathways; however, this was partially mitigated by the *GUN1* disruption. The expression patterns of *VTC2* and *TC* reflected the levels of both antioxidants under most of the conditions examined. Our results suggest that the transcriptional control of *VTC2* and *TC* by light and plastid-derived signals is important for the regulation of the biosynthetic pathways, and that GUN1 is at least partially involved in the plastid-derived signals-dependent regulation.

1. Introduction

L-Ascorbic acid (vitamin C, AsA) and tocopherols (vitamin E; Toc) are vitamins, which are synthesized in plants and need to be taken up in the diet for human health [1]. AsA and Toc are important soluble and insoluble antioxidants, respectively, in higher plants. AsA has been identified as a substrate and cofactor for many enzymes, including ascorbate peroxidases (APXs), which are the main regulators of cellular H_2O_2 levels [2]. AsA also serves as an electron donor in the regeneration of oxidized Toc to its reduced form, which scavenges singlet oxygen and protect polyunsaturated fatty acids from lipid peroxidation [3–5]. Thus, these antioxidants play key roles in the regulation of cellular redox states, responses to stress and hormones, and growth and development in higher plants [2,6]. Considering their biochemical interaction, the pool size of AsA and Toc are regulated to maintain cellular redox states.

Although several pathways have been proposed for the biosynthesis of AsA, the D-mannose/L-galactose (D-Man/L-Gal)

Abbreviations: AsA, L-ascorbic acid; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; D-Fru-6P, D-fructose-6-phosphate; D-Glu-6P, D-glucose-6-phosphate; D-Man-1P, D-manose-1-phosphate; D-Man-6P, D-mannose-6-phosphate; DMPBQ, 2,3-dimethyl-5-phytyl-1,4-benzoquinone; GalDH, L-galactose dehydrogenase; GalLDH, L-galactono-1,4-lactone dehydrogenase; GDP-Gal, GDP-L-galactose-1-phosphate; GDP-Man, GDP-D-manose-1-phosphate; GGP, GDP-L-galactose phosphorylase; GME, GDP-D-mannose-3′,5′-epimerase; GMP, GDP-D-mannose pyrophosphorylase; GPP, L-galactose-1-phosphate phosphatase; GUN1, GENOMES UNCOUPLED 1; L-Gal, L-galactose; L-Gal-1P, L-galactose-1-phosphate; L-GalL, L-galactono-1,4-lactone; HGA, homogentisic acid; HPT, homogentisate phytyltransferase; LIN, lincomycin; MPBQ, 2-methyl-6-phytyl-1,4-benzoquinone; MPBQMT, 2-methyl-6-phytylbenzoquinone methyltransferase; NF, norflurazon; PhANGs, photosynthesis-associated nuclear genes; PDP, phytyl diphosphate; PET, photosynthetic electron transport; PGE, plastid gene expression; PGI, phosphoglucose isomerase; PMI, phosphomannose isomerase; PMM, phosphomannomutase; PPi, pyrophosphate; SAM, S-adenosyl methionine; Suc, sucrose; TC, tocopherol cyclase; γ-TMT, γ-tocopherol methyltransferase; Toc, tocopherol.

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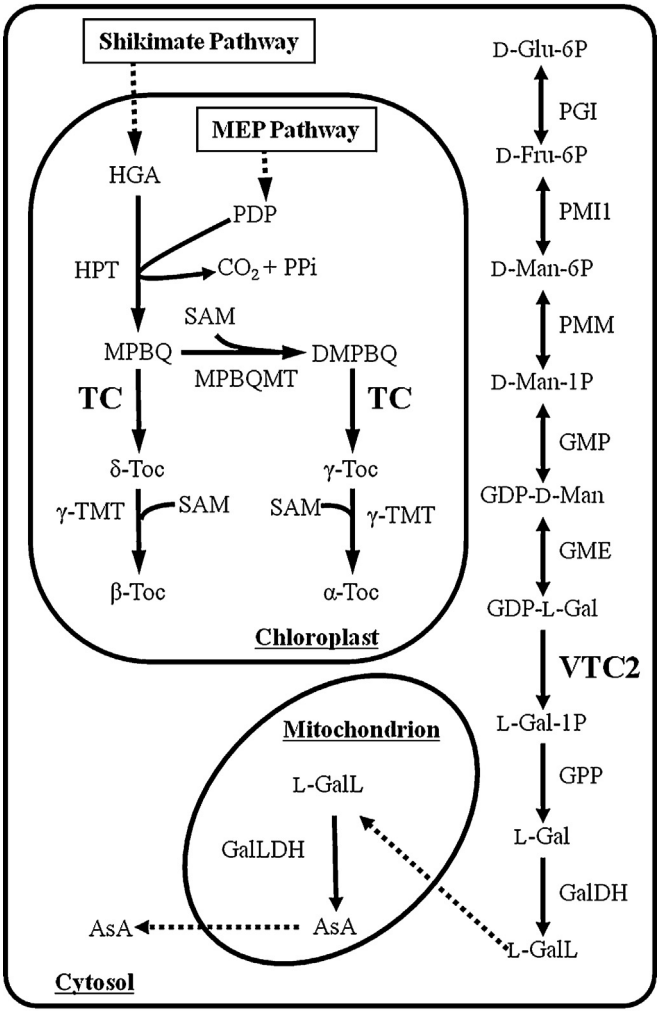

Fig. 1. Biosynthetic pathways for AsA and Toc in higher plants. *Compound abbreviations*: AsA, L-ascorbic acid; D-Fru-6P, D-fructose-6-phosphate; D-Glu-6P, D-glucose-6-phosphate; D-Man-1P, D-manose-1-phosphate; D-Man-6P, D-mannose-6-phosphate; DMPBQ, 2,3-dimethyl-5-phytyl-1,4-benzoquinone; GDP-Gal, GDP-Man, GDP-D-manose-1-phosphate; GDP-L-galactose-1-phosphate; HGA, homogentisic acid; L-Gal, L-galactose; L-Gal-1P, L-galactose-1-phosphate; L-GalL, L-galactono-1,4-lactone; MPBQ, 2-methyl-6-phytyl-1,4-benzoquinone; PDP, phytyl diphosphate; PPi, pyrophosphate; SAM, S-adenosyl methionine; Toc, tocopherol. *Enzyme abbreviations*: GalDH, L-galactose dehydrogenase; GalLDH, L-galactono-1,4-lactone dehydrogenase; GGP, GDP-L-galactose phosphorylase; GME, GDP-D-mannose-3′,5′-epimerase; GMP, GDP-D-mannose pyrophosphorylase; GPP, L-galactose-1-phosphate phosphatase; HPT, homogentisate phytyltransferase; MPBQMT, 2-methyl-6-phytylbenzoquinone methyltransferase; PGI, phosphoglucose isomerase, PMI, phosphomannose isomerase; PMM, phosphomannomutase; *TC*, tocopherol cyclase; γ-TMT, γ-tocopherol methyltransferase.

pathway has been shown to play a predominant role in the biosynthesis of AsA in photosynthetic cells [2]. As shown in Fig. 1, AsA is sequentially synthesized by D-fructose-6P, D-Man-6P, D-Man-1P, GDP-D-Man, GDP-L-Gal, L-Gal-1P, L-Gal, and the final precursor, L-galactono-1,4-lactone (L-GalL), with catalytic reactions through phosphomannose isomerase (PMI), phosphomannomutase (PMM), GDP-D-Man pyrophosphorylase (GMP), GDP-D-Man-3,5-epimerase (GME), GDP-L-Gal phosphorylase (GGP), L-Gal phosphatase (GPP), L-Gal dehydrogenase (GalDH), and L-GalL dehydrogenase (GalLDH) enzymes, respectively. L-GalL is synthesized in the cytosol, and the conversion of this compound to AsA is catalyzed in mitochondria [2,7]. Although *Arabidopsis* has two PMI isoforms, PMI1 and PMI2, only PMI1 was found to be involved in this pathway [8]. Among the five *vitamin C defective* mutants identified, four mutants, *vtc1*, *vtc2*, *vtc4*, and *vtc5* were shown to be defective in the D-Man/L-Gal pathway: *VTC1*, *VTC2/5*, and *VTC4* encode GMP, GGP, and GPP, respectively [9–12]. A double mutant lacking both *VTC2* and *VTC5* exhibited a seedling-lethal phenotype in the absence of a treatment with AsA or L-Gal, which suggested that AsA produced by the D-Man/L-Gal pathway was required for plant viability [12].

The biosynthetic pathway for Toc has also been completely characterized [13–16]. In the biosynthesis of Toc, the shikimate and non-mevalonate (MEP) pathways synthesize the head group and hydrophobic tail, respectively (Fig. 1). Homogentisic acid (HGA), which is produced by the shikimate pathway, is prenylated with phytyldiphosphate (PDP) to yield the first committed intermediates in the synthesis of Toc, 2-methyl-6-phytylplastoquinone (MPBQ). MPBQ methyltransferase (MPBQMT) then adds a second methyl group to MPBQ to form 2,3-dimethyl-5-phytyl-1,4-benzoquinone (DMPBQ). *Tocopherol cyclase* (*TC*) converts MPBQ and DMPBQ to δ- and γ-Toc, respectively. Finally, γ-Toc methyltransferase (γ-TMT) converts δ- and γ-Toc to β- and α-Toc, respectively. All enzymes involved in the biosynthesis of Toc were shown to be localized in chloroplasts (Fig. 1).

Light is the most important environmental cue for the regulation of AsA biosynthesis. We previously reported that AsA levels were enhanced by illumination and suppressed by darkness in *Arabidopsis* [8,17]. Consistent with these findings, the transcript levels of *PMI1*, *GMP*, *VTC2*, and *GPP* were up-and down-regulated by illumination and darkness, respectively [8,17]. The biosynthetic capacity of AsA is also known to be regulated by light intensity. High light irradiation has been shown to increase the AsA pool size accompanied by upregulation of *VTC2* [12], and similar findings were observed in the tomato and kiwifruit [18,19]. The transient and constitutive overexpression of *VTC2* has been shown to enhance AsA levels in *Arabidopsis*, tobacco, tomato, strawberry, and potato plants [20–23]. α-Toc levels as well as the expression of *TC* were shown to be markedly increased under high light irradiation [24,25]. The overexpression of *TC* has also been shown to increase Toc levels of *Arabidopsis* [24], tobacco [26], and lettuce plants [27]. These findings suggest that *VTC2* and *TC* are rate-limiting enzymes in the biosynthesis of AsA and Toc, respectively, and the light-dependent transcriptional control of *VTC2* and *TC* are crucial for regulating AsA and Toc levels in plants.

Previous studies demonstrated that the treatment of cells with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), an inhibitor of photosynthetic electron transport (PET) chain, suppressed the light-dependent activation of AsA biosynthesis, which suggesting a role for PET in the regulation of AsA biosynthesis [8,17]. The redox states of PET are known to regulate the expression of nuclear-encoded genes by acting as a retrograde signal from plastids to the nucleus. For example, the expression of photosynthesis-associated nuclear genes (*PhANGs*) and cytosolic *APX2* under illumination was diminished when plants were treated with DCMU [28,29]. Furthermore, the inhibition of plastid gene expression (PGE) and carotenoid biosynthesis by lincomycin (LIN) and norflurazon (NF), respectively, the latter of which affects the metabolism of tetrapyrroles, decreased the expression of *PhANGs* [30,31]. Thus, PGE and the metabolism of tetrapyrroles as well as the redox states of PET were confirmed to be involved in retrograde signaling from plastids to the nucleus [30,31]. Previous studies reported that a nuclear-encoded plastidic protein, GENOMES UNCOUPLED 1 (GUN1), integrated PET-, PGE-, and tetrapyrrole metabolism-evoked signals within plastids, and transmitted these signals to the nucleus in order to regulate the expression of *PhANGs*, though it remains unclear how GUN1 is involved in the retrograde pathways [32,33]. GUN1 has been found to affect cotyledon opening and expansion, anthocyanin biosynthesis, and hypocotyl elongation, and therefore, to be involved in stress response and photomorphogenesis in plants [34,35]. Based on these findings, we hypothesized