Completing a pathway to plant vitamin C synthesis

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Vitamin C (L-ascorbic acid or L-ascorbate) is a metabolite with strong antioxidant activity and a cofactor for enzymes catalyzing numerous biochemical reactions, including those neutralizing the effects of reactive oxygen species. It is also a necessary nutrient for a limited number of animals, including humans, that are incapable of its synthesis and that must secure vitamin C by means of dietary uptake. Plants produce large amounts of L-ascorbic acid to facilitate resistance to the oxidative stresses associated with myriad biotic and abiotic challenges and inherent to photosynthesis. Plants also are the primary source of vitamin C intake with respect to human diets. Consequently, the study of plant ascorbate synthesis is highly relevant to both plant biology and human nutrition, with eventual engineering of plant ascorbate metabolism a logical and important target for crop quality improvement (1). Although the pathways responsible for L-ascorbate accumulation in plants are not completely understood, a major component of plant ascorbate synthesis is through the L-galactose pathway (2), in which GDP-D-mannose is converted to L-ascorbate by four successive intermediates, as summarized in Fig. 1. In this issue of PNAS, Laing *et al*. (3) identify homologous genes from *Arabidopsis* (*VTC2*) and kiwifruit encoding an L-galactose guanyltransferase that catalyzes the conversion of GDP-L-galactose to L-galactose-1-P. This activity represents the second committed step in plant vitamin C biosynthesis from GDP-D-mannose and the final gene to be cloned for the L-galactose ascorbate synthesis pathway.

The *Arabidopsis* L-galactose guanyltransferase (*VTC2*) gene sequence was previously isolated through positional cloning of the *vtc2-2* ozone-sensitive, low-vitamin-C-content mutant locus (4). Until now, the biochemical function encoded by this gene remained a mystery. *Arabidopsis* lines harboring mutant *vtc2* alleles have been shown to have reduced ascorbate levels (5), impaired stress response (5, 6), and altered levels of stress response proteins (7), all consistent with a role for the *vtc2* locus in ascorbate metabolism. Indeed, this original screen for ozone-sensitive mutants has proven extremely important to the discovery of plant vitamin C synthesis genes. The gene residing at the *vtc1* locus encodes GTP-mannose pyrophosphorolase, which catalyzes the synthesis of GDP-mannose from

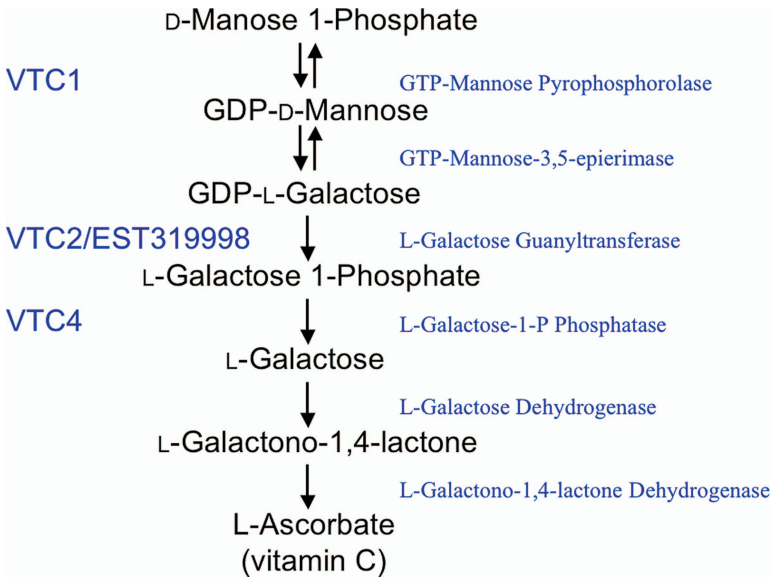

Fig. 1. The L-galactose pathway of ascorbate synthesis in plants. Pathway metabolites from D-mannose-1-P through L-ascorbate are shown with associated enzymes listed to the right of the pathway. Genes defined by *Arabidopsis vtc* mutations (5) are shown to the left of the pathway, where EST 319998 corresponds to the kiwifruit *VTC2* homolog (3).

mannose-1-P (8), whereas *vtc4* was recently shown to encode L-galactose-1-P phosphatase (9), the enzymatic step following that represented by *VTC2* (Fig. 1).

The *VTC2* sequence was used to identify a homologous gene from a kiwifruit EST collection (EST 319998), and both were expressed in *Escherichia coli* to produce recombinant proteins capable of catalyzing the conversion of GDP-L-galactose to L-galactose-1-P *in vitro*. To verify this *in vitro* activity *in planta*, the kiwifruit gene was expressed transiently in tobacco leaves and resulted in a 50-fold increase in L-galactose guanyltransferase activity and a 3-fold induction in leaf L-ascorbate levels (3). These results, combined with the low ascorbate levels of *vtc2* mutants, provide convincing evidence that the *Arabidopsis VTC2* and kiwifruit EST 319998 genes indeed encode the last remaining step in the plant L-galactose ascorbate synthesis pathway and further validate the importance of this route to vitamin C production in plants.

The ability to significantly elevate vitamin C levels by altering L-galactose guanyltransferase activity in transgenic tobacco leaves suggests that this activity is limiting for ascorbate synthesis in this particular species/tissue and thus points to a target for genetic manipulation of vitamin C content in food crops. Although additional pathways for ascorbate synthesis may contribute to the accumulation of this metabolite in plants (10, 11), the L-galactose pathway is clearly a major contributor. The availability of *VTC2* and those encoding other members of the L-galactose pathway for ascorbate synthesis will facilitate identification of optimal gene targets for genetic engineering through either biotechnology or targeted breeding approaches.

Worldwide vitamin C deficiency is considered a problem of limited scope primarily in the developing nations of Africa and southern Asia, where supply of fresh fruits and vegetables can be unreliable and availability of vitamin supplements is limited. Although the more severe effects of vitamin C deficiency (connective tissue defects, poor wound healing, and tooth loss) are rare in developed nations, significant proportions of these populations have intake levels below recommended levels. Strategies to manipulate crop plants for elevated vitamin C accumulation therefore will be important in both developing and developed nations, and the comple-

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tion of the gene repertoire for the plant L-galactose pathway to ascorbate should facilitate further studies toward understanding regulatory control of ascorbate accumulation and opportunities for increased vitamin C content of food crops.

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