

game of chess? Not likely. More complex problems are far more amenable to solution via traditional computation. However, the true power of DNA computation was never to mimic *in silico* accomplishments that have for many years outstripped biology (even savants manipulate large numbers much more slowly than the lowliest hand-held electronic calculator). Rather, molecular computers can be used to directly mimic and even solve problems that occur within the provenance of biology: that is, inside of a cell (Fig. 2). This application will lead to a more profound understanding of extant biological networks and will aid in the implementation of new cellular functions. What makes the automaton most interesting is not that schoolchildren the world over can now try their hand at outwitting DNA, but rather that

it serves as the first use of programmable enzymes in a complex network.

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## Corn with enhanced antioxidant potential

Peter Dörmann

**Characterization and manipulation of a novel prenyltransferase from monocot plant seeds reveal its capacity to produce tocotrienols and increase this form of vitamin E in transgenic plants by 10- to 15-fold.**

Vitamin E is a generic term describing a group of eight lipophilic compounds in the tocopherol and tocotrienol families. On the basis of the number and position of methyl groups on the chromanol ring, four different forms of both tocopherols and tocotrienols can be distinguished ( $\alpha$ ,  $\beta$ ,  $\gamma$  or  $\delta$ )<sup>1</sup>. Whereas tocopherols carry a saturated long-chain phytol group, the side chain of tocotrienols includes three *trans* double bonds. Vitamin E is synthesized in plants, but cannot be produced in animals and thus represents an essential component of the human diet. It is a strong antioxidant, which protects polyunsaturated fatty acids in membranes against degradation by reactive oxygen species such as ozone, singlet oxygen, peroxides and hyperoxides. In this issue, Cahoon *et al.*<sup>2</sup> report the sequence of an enzyme specific for tocotrienol synthesis in monocots and over-

express it in transgenic plants achieving an increase in tocotrienols of 10- to 15-fold and 6-fold in *Arabidopsis thaliana* leaves and in corn seeds, respectively. This large increase in tocopherol/tocotrienol content is unprecedented, and emphasizes our capacity to alter metabolic pathways in transgenic plants.

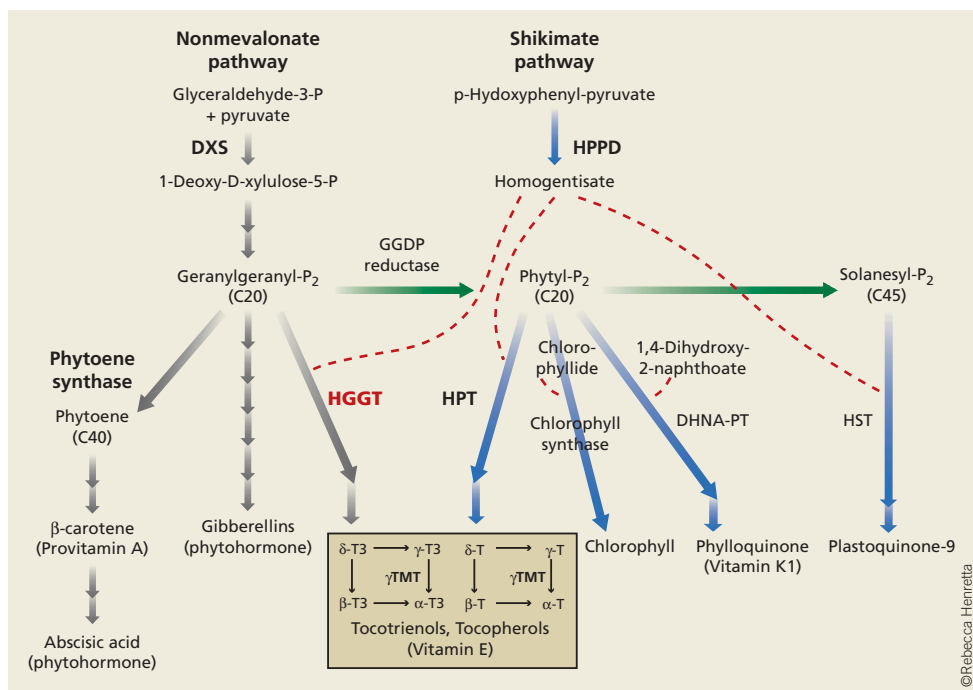
In plants, biosynthesis of tocopherols and tocotrienols is localized to the plastids of seeds and the chloroplasts of leaves. Despite its protective characteristics, total loss of tocopherol in mutants of cyanobacteria or higher plants has no obvious effect on physiology and growth. This might be a result of overlapping activities of tocopherol with other antioxidants<sup>3,4</sup>. The gene encoding homogentisic acid phytyltransferase (HPT), the first step unique to tocopherol synthesis, has recently been isolated<sup>3</sup>. It was assumed that tocotrienols are produced from homogentisic acid and geranylgeranyl diphosphate, the unsaturated precursor of phytol diphosphate, in an analogous reaction (see Fig. 1). On the basis of sequence similarities among the different prenyltransferases from plants, Cahoon and coworkers have now isolated a novel gene from monocot

species and present compelling evidence that it encodes homogentisic acid geranylgeranyltransferase (HGGT), the only known enzyme specific for tocotrienol synthesis<sup>2</sup>.

The precursors for tocopherol and tocotrienol synthesis in plants originate from two different pathways. Phytol diphosphate and geranylgeranyl diphosphate are synthesized via the nonmevalonate pathway of plastid isoprenoid synthesis (Fig. 1). Homogentisic acid, the precursor of the vitamin E head group, is derived from shikimate. Biotechnological approaches to modify vitamin E content have to take into account the complexity of plastid prenyl lipid synthesis, because geranylgeranyl diphosphate, phytol diphosphate and homogentisic acid are not only the substrates for vitamin E synthesis, but are also critical for the production of many other compounds important for plant development. Two additional substances with vitamin activity,  $\beta$ -carotene (provitamin A) and phyloquinone (vitamin K1), are synthesized from geranylgeranyl diphosphate and phytol diphosphate, respectively. Furthermore, the synthesis of photosynthetic pigments and electron acceptors (chlorophyll, carotenoids, plastoquinone-9) and of two phytohormones (gibberellins, abscisic acid) depends on the plastid isoprenoid pathway (Fig. 1).

Previous approaches to alter vitamin E amounts in plants include overexpression of *p*-hydroxyphenyl-pyruvate dioxygenase and homogentisic acid phytyltransferase (HPT)<sup>5,6</sup>. However, the increases in total tocopherol/tocotrienol achieved in these studies were only 1.5- to 4-fold. Possible explanations for these observations are the restricted availability of the vitamin E precursors phytol diphosphate, *p*-hydroxyphenyl-pyruvate or homogentisic acid, or their rapid consumption by competing pathways. Overexpression of 1-deoxy-D-xylulose-5-phosphate synthase with the aim to stimulate carbon flux into the plastid nonmevalonate pathway results in a moderate increase in tocopherol (about 1.4-fold) and a concomitant increase in carotenoids, chlorophyll, abscisic acid and gibberellins<sup>7</sup>. The fact that overexpression of phytoene synthase in transgenic rape seeds and in rice ('golden rice') results in a drastic increase in carotenoid synthesis (*e.g.*,  $\beta$ -carotene/provitamin A) suggests that geranylgeranyl diphosphate is not limiting for prenyl lipid synthesis in seeds<sup>8,9</sup>. In agreement with this, Cahoon *et al.* found that overexpression of HGGT in transgenic *Arabidopsis* and corn leads to a much stronger increase in tocotrienol synthesis than the increase

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**Figure 1** Synthesis of prenyl lipids in plants. Activated isoprenoids (phytyl diphosphate, geranylgeranyl diphosphate, solanesyldiphosphate) derived from the plastid nonmevalonate pathway are the precursors for the synthesis of prenyl lipids in plants, some of which have an important function for human nutrition (vitamin E, vitamin K1, provitamin A). Enzymatic steps that have been manipulated in transgenic plants to boost prenyl lipid synthesis are depicted in bold. The HGGT pathway described by Cahoon *et al.*<sup>2</sup> is shown in red. Abbreviations: DHNA-PT, 1,4-dihydroxy-2-naphthoate phytyltransferase; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; GGDP reductase, geranylgeranyl diphosphate reductase; HGGT, homogentisic acid geranylgeranyltransferase; HPPD, *p*-hydroxyphenyl-pyruvate dioxygenase; HPT, homogentisic acid phytyltransferase; HST, homogentisic acid solanesyltransferase; P, phosphate; T, tocopherol; T3, tocotrienol;  $\gamma$ -TMT,  $\gamma$ -tocopherol methyltransferase.

observed for tocopherol synthesis in plants overexpressing HPT<sup>2,6</sup>.

HGGT belongs to a family of plant prenyltransferases that includes HPT and chlorophyll synthase<sup>2</sup>. Tocotrienols are highly abundant in seeds of monocot plants, such as cereals, but mostly absent from dicots. Furthermore, HGGT sequences from rice, barley and wheat are more closely related to HPT sequences from monocots than from dicots<sup>2</sup>, suggesting that the monocot prenyltransferases might have a common evolutionary ancestor. Interestingly, the plant HPT enzymes have been shown to be specific for phytyl diphosphate, but to discriminate against geranylgeranyl diphosphate, whereas the cyanobacterial HPT enzyme is active with both substrates<sup>3</sup>. On the basis of data obtained in transgenic plants, it is likely that HGGT is specific for geranylgeranyl diphosphate<sup>2</sup>. However, direct evidence for this awaits characterization of the *in vitro* substrate specificity of HGGT.

The 'activity of vitamin E' historically has been defined by the rat fetal reabsorption assay. Because of different affinities to the  $\alpha$ -tocopherol transport protein in the blood plasma, which determine the bio-availability of vitamin E molecules, the forms of tocopherols and tocotrienols significantly differ in vitamin E activity, with  $\alpha$ -tocopherol showing the highest activity set to 'one  $\alpha$ -tocopherol equivalent' (1  $\alpha$ -TE)<sup>1</sup>. The  $\alpha$ -TE values for  $\gamma$ -tocopherol,  $\alpha$ -tocotrienol and  $\gamma$ -tocotrienol are 0.1, 0.3 and 'below detection,'

respectively. Because overexpression of HGGT in plants mostly results in an increase in  $\gamma$ -tocotrienol with the other vitamin E forms remaining constant<sup>2</sup>, an elevation in the total amount of tocopherols/tocotrienols by a factor of 10 translates into a much smaller increase in vitamin E activity, as measured in  $\alpha$ -TE units. However, the relevance of the traditional rat fetal reabsorption assay for the diverse biological functions of tocopherols and tocotrienols in human nutrition is questionable. Furthermore, *in vitro* studies have demonstrated that tocotrienols exert a much stronger antioxidant activity<sup>10</sup>, which might be the reason for their superior therapeutic effects in certain clinical contexts, such as hypercholesterolemia, thrombosis and cancer<sup>11</sup>.

Not surprisingly, an entire industry has developed around the production and commercialization of tocotrienols as 'nutraceuticals.' Because of their high antioxidant activity, an increase in tocotrienols is expected to extend the shelf life of vegetable oils used for human nutrition. Furthermore, transgenic plants accumulating tocotrienols in their leaves might show a higher resistance to oxidative stresses originating from drought and heat. The conversion of a large fraction of  $\gamma$ -tocotrienol accumulating in HGGT transgenic plants into  $\alpha$ -tocotrienol (which has a higher vitamin E activity; see above) might be achieved via introduction of a second transgene,  $\gamma$ -tocopherol methyltransferase ( $\gamma$ -TMT).  $\gamma$ -TMT has been

shown to be critical for the last methylation step in the production of  $\alpha$ -tocopherol and  $\alpha$ -tocotrienol<sup>12</sup>.

Genetic engineering of crop plants for the production of high levels of tocotrienols raises the issue of whether carbon flux into other important nutraceuticals produced via the plastid prenyl lipid pathway (e.g., provitamin A and vitamin K) might be affected. It has already been shown that an increase in carotenoids by overexpression of phytoene synthase results in a reduction of tocopherol and chlorophyll in seeds of rape<sup>8</sup>. Finally, the health benefits proposed for the individual forms of tocotrienols and tocopherols are still under debate, and further research is needed to assign specific roles to each vitamin E form in human nutrition.

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