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The type III polyketide synthase involved in plant polyphenol biosynthesis

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The type III polyketide synthase(PKS)s belong to the plant chalcone synthase (CHS) superfamily of condensing enzymes and have been exclusively studied from the plant kingdom for several decades. The discovery of plantlike PKS proteins in bacterial systems, which utilize acetate as a polyketide chain elongation primer, has provided a new dimension to our understanding of the importance and hence the ubiquitous distribution of these enzymes. Whereas prototype plant CHSs predominantly use phenylpropanoid substrates to synthesize flavonoid precursors, the type III PKSs produce diverse metabolites that are important in secondary metabolic pathways likewise curcuminoids, bitteracids in hop, cannabinoids.

The three unprecedented type III PKSs were cloned from medicinal plants; Rhubarb and Aloe, which catalyze formation of an aromatic penta-, hepta- and octa-ketides selecting acetyl-CoA as a starter. The function of these three type III PKSs was shown to be swapped each other by steric modulation of the chemically inert single to triple residues lining the active-site cavity accompanied by conservation of the Cys-His-Asn catalytic triad. For example, the site-directed mutagenesis of octaketide synthase revealed that small-to-large substitutions of a single residue Gly207 (G207A, G207T, G207M, G207L, G207F, and G207W) resulted in loss of the octaketide-forming activity and concomitant formation of shorter chain length polyketides including a pentaketide chromone, and a hexaketide pyrone, depending on the size of the side chain. In contrast, the three active site residues conserved in CHSs (T197, G256, S338) were replaced in the heptaketide synthase from Rhuberb.

On the otherhand the S338V mutant of *Scutellaria baicalensis* CHS, the prototype CHS, produced octaketides (SEK4/SEK4B) that were shunt products of the minimal type II PKS . The octaketides-forming activity was dramatically increased in the triple mutant (T197G/G256L/S338T). In this striking example, changing chemically inert amino acid residues of the CHS resulted in the enzyme producing SEK4/SEK4B that was not previously produced ever by prototype CHSs, effectively turned the enzyme into a related type III PKS. With the knowledge of the detailed structures and functions of those enzymes, we can judiciously engineer "artificial" versions of the biosynthetic pathways linking to an artificial gene or a set of genes.

The genes for two type III PKSs of Common morning glory, which generate chromone derivatives, as well as one for benzal acetone synthase from Rhubarb, are introduced to *Arabidopsis* to investigate whether these transgenic plants could be a promosing resource for plant polyphenols.