Regulation and physiological function of C5 isoprenoid metabolites in plastids.

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Isoprenoids, a diverse group of compounds derived from the five-carbon building units, isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP), are essential for survival in all organisms. In higher plants, IPP and DMAPP are biosynthesized via two pathways, mevalonate pathway localized in cytosol and MEP pathway in plastid. Various plant secondary metabolites such as isoprene, monoterpene, carotenoid, prenylated compounds, are derived from MEP pathway. I have first demonstrated why many plant species emit isoprene, and then performed the molecular cloning and characterization of prenyltransferases involved in biosynthesis of prenylated flavonoids.

Isoprene emission – its physiological role in plants

Isoprene is a volatile C5 isoprenoid emitted from leaves of many plants [1]. Despite its massive amount, the physiological function of isoprene emission from plants is not well understood. In this study, I have cloned isoprene synthase cDNA from *Populus alba* (*PaIspS*) to analyze the gene expression to environmental changes. *PaIspS* was strongly induced by heat treatment, and was substantially decreased in the dark, suggesting that isoprene emission was regulated by light at the transcriptional level [2]. Then, I prepared transgenic *Arabidopsis thaliana* overexpressing *PaIspS* gene, which is thought to be a non-isoprene emitter. A striking difference was observed when both transgenic and wild type plants were treated with heat at 60˚C for 2.5 hr, i.e. transformants revealed clear heat tolerance compared to the wild type (Figure 1). High isoprene emission and a decrease in the leaf surface temperature were observed in transgenic plants during the heat stress treatment [3]. Contrary, neither strong light nor drought treatments gave an apparent difference. These data suggest that the isoprene emission plays a crucial role as a heat protection mechanism in plants.

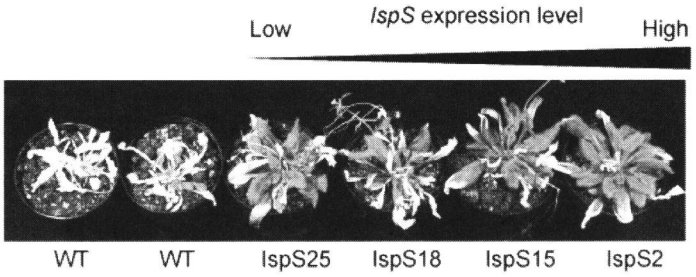

Figure 1. Heat treatment (60˚C for 2.5 hr) of transgenic and wild-type plants (4-week-old).

Discovery of the first gene for flavonoid-specific prenyltransferase

The prenylation of aromatic compounds is a major contributor to the diversification of plant secondary metabolites due to differences in prenylation position on the aromatic ring, various length of prenyl side chain, and further modifications of the prenyl moiety, e.g. cyclization and hydroxylation, resulting in the occurrence of more than 1,000 prenylated compounds in plants. In particular, prenylated flavonoids in higher plants protect them by exhibiting strong antibacterial and antifungal activities [4]. Also, many prenylated flavonoids have been identified as active components in medicinal plants with biological activities, such as anti-tumor, anti-androgen, anti-leishmania, and anti-NO production. Due to the beneficial effects for human health, prenylated flavonoids are of particular interest as lead compounds for producing new drugs and functional foods. The prenylation of the flavonoid core increases the

lipophilicity and the membrane permeability, which is one of the proposed reasons for the enhanced biological activities of prenylated flavonoids [5]. However, none of genes responsible for the prenylation reactions have been identified for more than 30 years in this research field.

I have isolated a prenyltransferase gene from *Sophora flavescens*, *SfN8DT-1*, responsible for the prenylation of the flavonoid naringenin at the 8-position, which is specific for flavanones and dimethylallyl diphosphate as substrates (Figure 2) [6]. Phylogenetic analysis shows that SfN8DT-1 has the same evolutionary origin as prenyltransferases for vitamin E and plastoquinone. The gene expression of *SfN8DT-1* is strictly limited to the root bark where prenylated flavonoids are solely accumulated *in planta*. The ectopic expression of *SfN8DT-1* in *Arabidopsis thaliana* resulted in the formation of prenylated apigenin, quercetin, and kaempferol, as well as 8-dimethylallylnaringenin. SfN8DT-1 represents the first flavonoid-specific prenyltransferase identified in plants and paves the way for the identification and characterization of further genes responsible for the production of this large and important class of secondary metabolites.

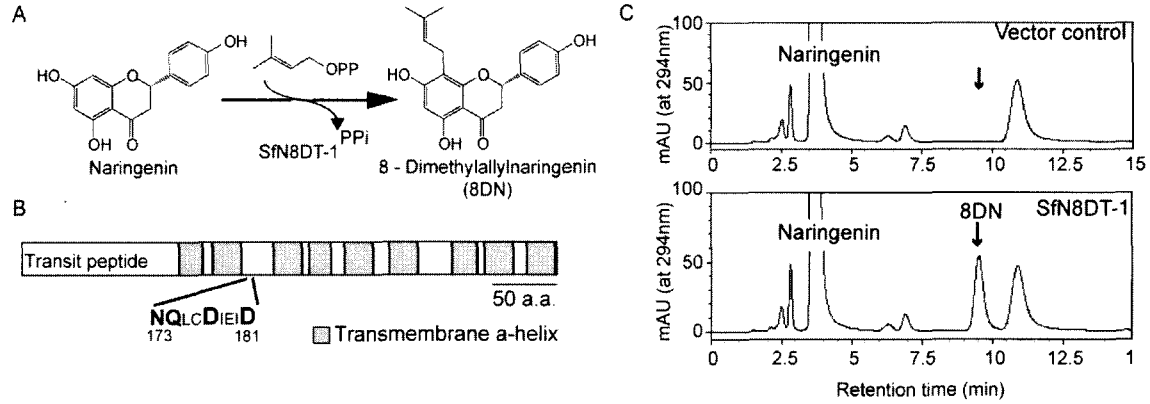

Figure 2. Enzymatic characterization of recombinant SfN8DT-1 expressed in yeast.

(A) Prenylation reaction of naringenin by SfN8DT-1.

(B) Structural features of the SfN8DT-1 polypeptide.

(C) HPLC chromatograms of ethyl acetate extract of incubation mixture of naringenin and DMAPP with recombinant protein.

References

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