Overexpression and functional analysis of a membrane-bound aromatic prenyltransferase LePGT-1 in *Lithospermum erythrorhizon*

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Aromatic substrate prenyltransferases widely occur from bacteria to human as a subfamily of the prenyltransferase family. They play important roles in the biosysthesis of quinones molecule, such as ubiquinone and vitamine E. One of the most intensively studied members is *p*-hydroxybenzoate : polyprenyltransferase involved in ubiquinone biosynthesis. Thus far, yeast gene *Coq2* and *Arabidopsis* gene *AtPPT1* were characterized in plants and they showed broad substrate specificity for prenyl chain length, whereas a member isolated from *Lithospermum erythrorhizon*, LePGT-1, showed strict substrate specificity for geranyl diphosphate. These members have 8 and 9 transmembrane domains, and the ubiquinone biosynthetic protein members are located at mitochondria, while LePGT-1 involved in shikonin biosynthesis in localized at ER membrane.

To understand the molecular mechanism of this unique enzymatic reaction, i,e. aromatic protein substitution with prenyl chain by C-C bound formation, we tried to establish an expression system of LePGT-1 aiming at the X-ray crystallographic analysis of this protein subfamily. In this study, we have established a heterologous expression system of LePGT-1 with baculovirus in insect cultured cells (Sf9), and a purification protocol of LePGT-1 retaining its catalytic function. With purified enzyme, kinetic parameters were determined.

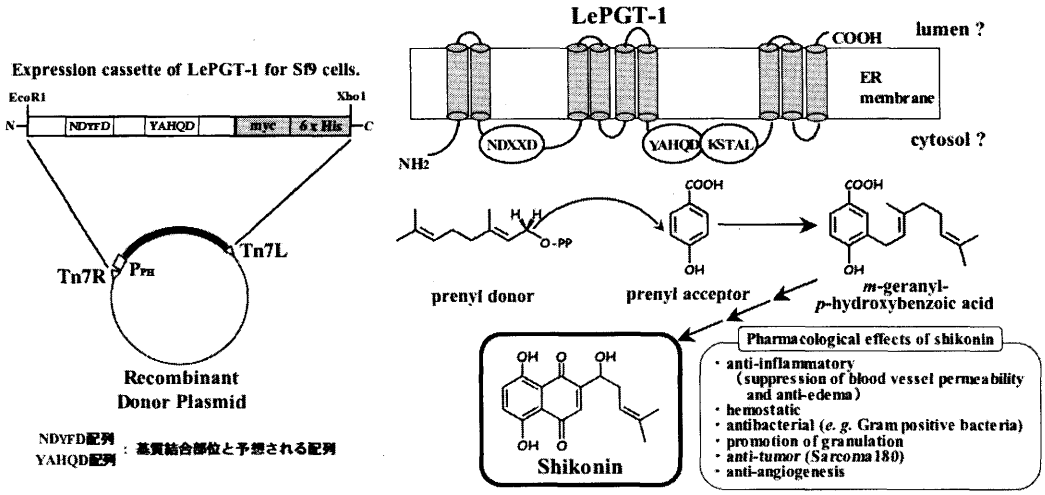

Fig. 1 Putative structure and reaction mechanism of LePGT-1