Promoter analyses of light responsive prenyltransferases involved in naphthoquinone

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Shikonin, a red naphthoquinone derivative, is a secondary metabolite that specifically occurs in boraginaceous plants, and is the active principle of the medicinal plant *Lithospermum erythrorhizon*. Since this compound and its derivatives exhibit anti-bacterial activity, their functions as phytoalexins have also been reported. The biosynthesis of shikonin includes a key prenylation step catalyzed by geranyl diphosphate (GPP): 4-hydroxybenzoate (4HB) 3-geranyltransferase; i. e., coupling of the shikimate and mevalonate pathways. This enzyme plays a critical role in the regulation of shikonin biosynthesis in cell cultures of *L. erythrorhizon*, i. e., up- and down-regulation of this enzyme activity directly affects the production of shikonin. The enzyme activity is strongly suppressed by light irradiation, ammonium ion, and the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D), whereas this activity is enhanced with the addition of oligogalacturonide (OG) and methyl jasmonate (MJ) to the medium. It has also been reported using a partially purified enzyme that this protein is ER membrane-bound and shows strict substrate specificity for geranyl (C_{10}) diphosphate for the chain-length of the prenyl donor, which is in clear contrast to the mitochondrial polyprenyltransferase for ubiquinone biosynthesis.

In our previous study, two cDNAs of geranyltransferase from *L. erythrorhizon* were isolated, which were designated LePGT-1 and LePGT-2 [1]. Their gene expressions were regulated in the same manner as observed on the enzyme activity level, suggesting that this biosynthetic step was regulated at gene expression level. In expression analyses of LePGT-1 and LePGT-2 in *Lithospermum* cell cultures, no clear difference between LePGT-1 and LePGT-2 was observed. However, they showed different enzymatic properties, e.g. Km value, which suggested that they have different physiological roles in plant.

In this study, I have isolated the promoter regions of LePGT-1 and -2 by TAIL-PCR and prepared GUS reporter constructs using these promoters, with which model plants, such as tobacco and Arabidopsis, were transformed to analyze their promoter activities. The detailed analyses revealed that both promoters are very active in root tissues of both heterologous host plants, but the regulation on promoter activity strongly depends on the host plant species. I also introduced the promoter-GUS construct into cultured *Lithospermum* cells, the homologous host, by particle bombardment method to find the *cis*-element responsible for the negative regulation on the gene expression by light.

REFERENCES

[1] Yazaki, K., Kunihisa, M., Fujisaki, T., Sato, F. (2002) J. Biol. Chem. 277(8): 6240-6246.