Characterization of dark-inducible genes involved in naphthoquinone biosynthesis in *Lithospermum erythrorhizon*

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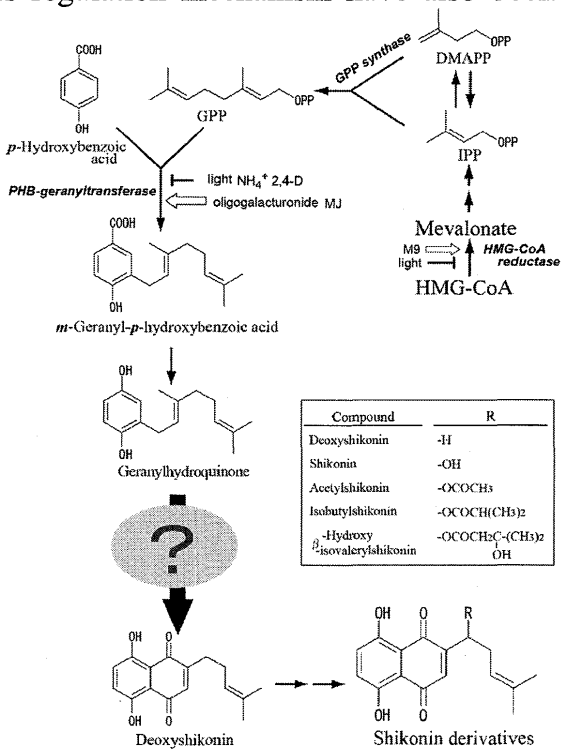
Lithospermum erythrorhizon Sieb. et Zucc. (Boraginaceae) is a perennial herbal plant rarely found in Japan, Korea, and China. Roots of this medicinal plant appear strong red-purple, as its root bark (cork layers) contains a large amount of red naphthoquinone pigments, shikonin derivatives. Many studies demonstrated that shikonin and its derivatives showed various pharmacological activities, i.e. antibacterial, anti-inflammatory, wound-healing, tumor-inhibiting, etc. From this plants, a cell culture system was established, which produced higher amount of shikonin derivatives than the intact plants. The production system of shikonin by the cell cultures was applied for its industrial production by Mitui Petrochemical Industries as the first example of the production of secondary metabolites by plant cell cultures. The biosynthetic route of shikonin and its regulation mechanism have also been intensively studied with cell cultures as a model of clear inducible system of secondary metabolites in plants.



Shikonin molecule is formed *in vivo* from geranyldiphosphate derived from mevalonate pathway and *p*-hydroxybenzoate (PHB) *via* shikimate pathway. The coupling of these pathways is achieved by a membrane-bound enzyme, PHB-geranyltransferase (LePGT), which functions as a regulatory enzyme for shikonin production. The geranylated PHB is then convertes to geranylhydroquinone, which forms a naphthalene ring leading to the production of shikonin. However, the biosynthetic reactions responsible for the formation of naphthalene ring are not clarified yet. In the present study, we have attempted to identify enzymes involved in the naphthalene formation as well as their substrates and reaction products using *L. erythrorhizon* cell cultures as a model system. Results obtained from this research will provide crucial information of the mechanism of polycyclic ring formation involved in secondary metabolites, such as anthraquinones.

First, PCR-selected subtraction was applied to *L. erythrorhizon* cultured cells, in which cDNA samples of dark-grown cultures (shikonin-producing) and of cell cultures under illumination (non-producing) were used as the tester and the driver DNAs, respectively. The dark-inducibility was evaluated by dot-blot analysis in each gene for ca. 1,000 clones, and we obtained 240 clones whose expression was promoted more than 5-times in the dark. In these clones, adding to preciously identified biosynthetic genes, e.g. LePGT, we have found some strong dark-inducible genes as candidates of the naphthalene-forming enzymes.