

Engineering a novel pathway for isoprenoid synthesis

by

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Isoprenoids comprise a large class of chemicals, of significant interest due to their diverse properties. Most isoprenoids are plant secondary metabolites and are of commercial importance due to their varied applications in fields spanning medicine, agriculture, flavors, fragrances, cosmetics and nutrition. Biological production of isoprenoids in microbes is considered to be the most efficient and commercially viable way for their large-scale production. Thus far, isoprenoid biosynthesis has been performed through pathways inextricably linked to glycolysis. Furthermore, these pathways are inherently limited due to their extensive cofactor requirements, complex regulation and large number of steps. In this thesis we present a novel pathway for isoprenoid synthesis, the Isopentenol Utilization Pathway (IUP), which aims to overcome these limitations. This pathway functions through the double phosphorylation of an isopentenol, either isoprenol or prenol, to produce the main precursors to isoprenoid synthesis, isopentenyl diphosphate (IPP) or dimethylallyl diphosphate (DMAPP). This pathway is radically different from naturally-occurring pathways or their engineered variants because it is only two steps long, uses an externally-provided isoprenol as its substrate instead of a glucose-derived catabolite, and uses only a single co-factor, ATP.

We identify suitable enzymes, construct the pathway and proceed to demonstrate an *in vivo* proof of concept. After optimizing the pathway feedstock, we proceed to show that IUP is decoupled from central carbon metabolism. We demonstrate that the IUP can quickly produce copious amounts of IPP & DMAPP and can be used for the production of a variety of isoprenoids. The IUP flux exceeded the capacity of almost all downstream pathways tested, was competitive with the highest isoprenoid fluxes reported as well as against state-of-the art isoprenoid pathways. Furthermore, we elaborate on our progress towards improving the capacity of a downstream farnesene synthesis pathway, to catch up with and fully utilize IUP's production capacity. Finally, we propose a new scheme for the use of the IUP to produce functionalized isoprenoids using functionalized isopentenols to introduce functionalizations in isoprenoid backbones, and we show preliminary results of this application.

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