ENGINEERING SUBSTRATE SPECIFICITY INTO A PROMISCUOUS ANCESTRAL DITERPENE SYNTHASE

Karen Schriever, KTH Royal Institute of Technology, Science for Life Laboratory, Sweden
karensc@kth.se
Natalie M. Hendrikse, KTH Royal Institute of Technology, Science for Life Laboratory, Sweden
Patricia Saénz-Mendez, KTH Royal Institute of Technology, Science for Life Laboratory, Sweden
Antonino Biundo, KTH Royal Institute of Technology, Science for Life Laboratory, Sweden
Per-Olof Syrén, KTH Royal Institute of Technology, Science for Life Laboratory, Sweden

Key Words: Terpene biocatalysis, ancestral sequence reconstruction, enzyme specificity

Terpene synthases are a class of enzymes that catalyse the cyclisation of linear unsaturated hydrocarbons into a plethora of cyclic structures, of which many are industrially relevant as fragrances, flavours or due to their medical properties. Despite the structural and functional diversity of their products, members of this enzyme family show a high degree of structural and functional similarity, which is why the rules defining product and substrate specificity are not fully understood. Recently, we have used ancestral sequence reconstruction to design a hypothetical molecular ancestor of spiroviolene synthase - a diterpene synthase from *Streptomyces violens* that converts the linear C-20 precursor geranylgeranyl pyrophosphate (GGPP) to spiroviolene[1]. This ancestral enzyme shares 77 % sequence identity with the modern wild-type enzyme from *S. violens*. Compared to the modern enzyme, the ancestral enzyme showed increased thermostability and an additional new reactivity with the shorter C-15 substrate farnesyl pyrophosphate (FPP) that the modern enzyme did not display. To the best of our knowledge, this is the first time ancestral sequence reconstruction was used on a diterpene cyclase. Based on the ancestral enzyme, a library of enzyme-variants was designed with the aim to influence the substrate specificity of the promiscuous ancestral enzyme. We identified several variants that showed substantial preference for the native substrate GGPP (modern enzyme-like), which demonstrates that the subtle GGPP-preference of the ancestral enzyme could be “evolved” to the GGPP-specific modern enzyme phenotype. Most interestingly, we were also able to identify a few variants that showed reversed substrate preference for FPP over GGPP and thus demonstrated “evolvability” of the ancestor towards the unpreferred shorter substrate. Taken together these findings suggest that the hypothesis of promiscuous evolvable ancestral enzymes might be appropriate for this member of the diterpene synthase family. Moreover, it opens up the exciting prospect of using ancestral sequence reconstruction as a tool to engineer enzyme specificity – either by introducing new desired functionalities next to an already existing one or by reprogramming existing promiscuity towards a desired substrate specificity.

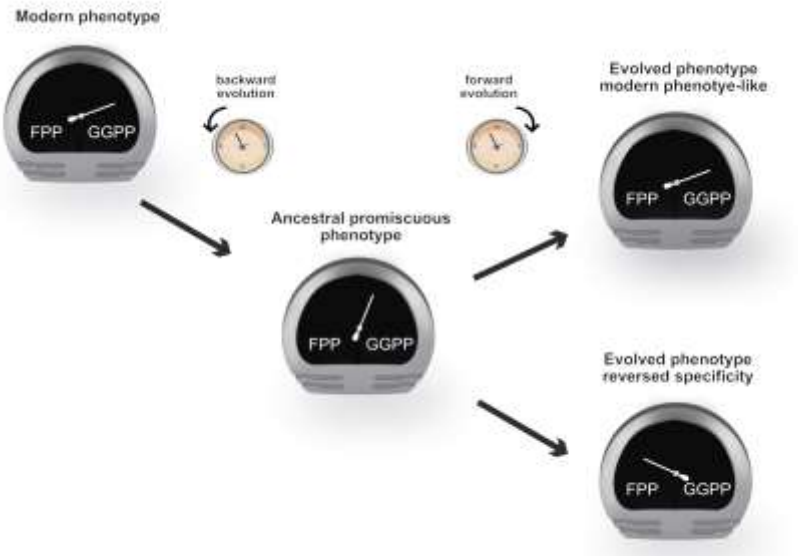

Figure 1 – Concept of redirecting substrate specificity by mimicking the process of evolution

1. Hendrikse, N.M., Charpentier, G., Nordling, E. & Syren, P.O., *FEBS J* 285, 4660-4673 (2018).