METABOLIC ENGINEERING

Biosensor keeps DOPA on track

Biosensors are emerging as an important tool to evolutionarily engineer metabolic pathway enzymes for the microbial production of chemicals. A colorimetric biosensor used to increase dopamine levels in yeast now enables the production of benzylisoquinoline alkaloids from glucose.

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Alkaloids are nitrogen-containing natural products that include analgesic, anticancer and antimalarial agents. Complex alkaloids are challenging synthetic targets, and it is often more cost effective to isolate commercial quantities from the native plants than to produce them using organic synthesis; such is the case with the anticancer agent vincristine and the analgesic morphine. Nevertheless, plants' long maturation times and low yields of secondary metabolites, as well as the difficulty of separating a specific alkaloid from other metabolites, often result in high prices and limited availability of alkaloid-derived therapeutics. Metabolic engineering offers an alternative approach to the production of complex alkaloids in which the expression of plant pathways in microbes turns these microorganisms into chemical factories. However, for engineered biosynthetic pathways to operate successfully, all of the necessary enzymatic machinery must work efficiently. Research from DeLoache *et al.*[1] now describes a new strategy to search for improved enzymes and its successful application to a key enzyme in an alkaloid pathway.

Benzylisoquinoline alkaloids (BIAs), derived from the amino acid tyrosine, have been extensively engineered in microbes, most likely because there is extensive knowledge of their biosynthetic pathways. BIA pathway engineering in bacteria has focused on the upper portion of the pathway[2], from glucose to reticuline, whereas engineering in yeast has focused on the lower BIA pathway steps[3–5], from norcoclaurine to codeine and morphine, albeit not sequentially and in different strains. A longstanding problem in the production of BIAs in yeast has been the inability to produce dopamine at sufficient levels to support alkaloid production. Dopamine is produced by decarboxylation of L-DOPA, which in turn is obtained from the monooxidation of tyrosine (**Fig. 1a**). Tyrosine monooxidation is nontrivial, as tyrosine hydroxylases carry out multiple oxidations resulting not only in L-DOPA but also in the byproduct dopaquinone, a melanin precursor. A tyrosine hydroxylase selective for monooxidation of tyrosine would go a long way toward solving the dopamine production problem.

Actually, selective tyrosine hydroxylases are known, but none of them are able to function in yeast. Indeed, a common problem when transferring plant enzymes into microbes is their unpredictable activity in the new host; heterologous enzymes do not often express well, their *in vivo* activity is insufficient to achieve the desired chemical yield, or necessary cofactors are not produced by the host organism. Enzyme function can be improved through directed evolution, but high-throughput assays for enzymatic activity typically rely on colorimetric or fluorescent readouts; if no such readout is available, improving enzyme activity is challenging, as only low-throughput chromatography-based screens testing ~100 samples per day can be used. Chemical biosensors can enable the application of evolutionary strategies to the engineering of metabolic pathways and have recently been used to improve the microbial synthesis of chemicals[6,7]. However, they have yet to reach wide application, primarily because a new biosensor must be engineered for every chemical of interest.

To tackle this issue in the context of alkaloid biosynthesis, DeLoache *et al.*[1] engineered a L-DOPA biosensor by leveraging a DOPA dioxygenase to route L-DOPA to a yellow pigment, betaxanthin, and dopaquinone to a purple pigment, betanidin[8] (**Fig. 1b**). The authors used their sensor to first identify a tyrosine hydroxylase that is functional in yeast and then evolve it for improved L-DOPA production via random mutagenesis and by combining beneficial mutations, resulting in a tyrosine hydroxylase with a threefold improvement in L-DOPA production. Coupling the evolved tyrosine hydroxylase with a high-specificity DOPA decarboxylase in a tyrosine-overproducing strain improved dopamine production 16-fold, resulting in 24 mg l^{-1} of dopamine. The authors showed that improvements in dopamine yield came from improved tyrosine hydroxylation, reduced L-DOPA oxidation to dopaquinone and improved enzyme expression. The evolved tyrosine hydroxylase enables sufficient dopamine production to allow the microbial synthesis of the BIA reticuline directly from glucose when it is expressed with six other genes in the pathway, including a newly characterized norcoclaurine synthase.

Given that downstream BIA pathway enzymes have already been shown to express in yeast, this work opens the door to the production of complex BIAs directly from glucose. Although the amount of reticuline produced in yeast is substantially lower than that produced in bacteria, yeast is far more amenable than bacteria to expressing the transmembrane P450 enzymes found in the late steps of plant alkaloid pathways. Yeast is thus the preferred microbial host for plant alkaloid production in the long term. Further, yeast's robustness in industrial conditions, including low pH and high sugar concentration[9], brings commercial microbial production of plant BIAs closer to reality. Additional steps toward that goal can be readily imagined; for example, screening simultaneously for dopamine and against dopaquinone production, an extremely powerful directed evolution strategy[10], would enable further improvements of tyrosine hydroxylase activity and increase BIA microbial production. Similarly, moving from a medium-throughput chemical biosensor, such as that reported by DeLoache *et al.* [1], to improved chemical sensors capable of testing >10^7 samples per day, could be used to carry out pathway evolution to rapidly identify enzyme

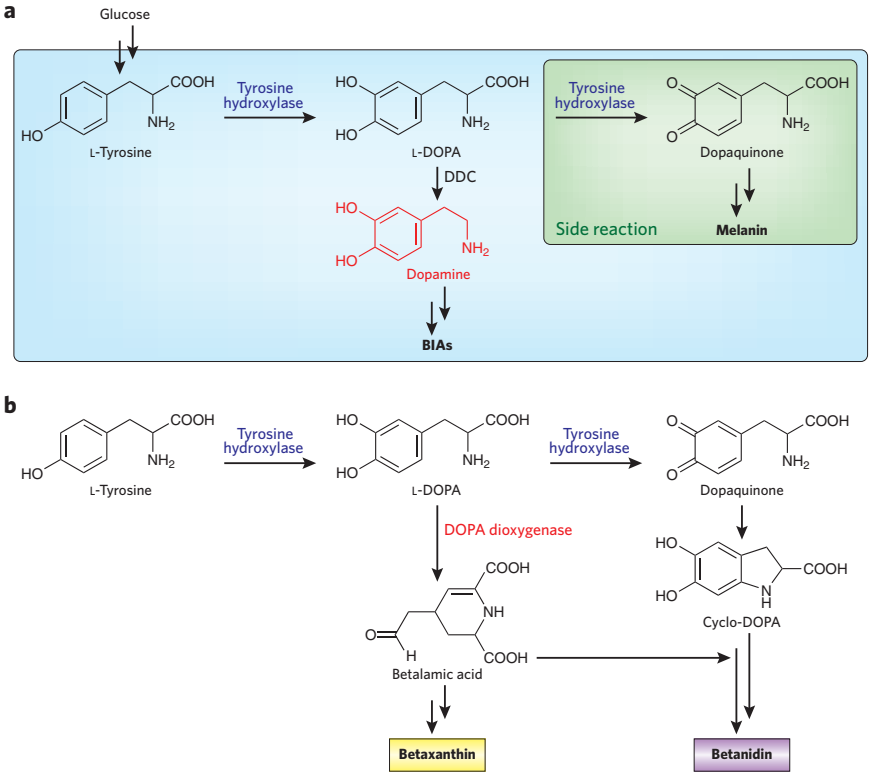

Figure 1 | Biosensor-aided development of a tyrosine hydroxylase for the microbial production of BIAs from glucose. (**a**) Production of BIAs from glucose in yeast has been long limited by low levels of dopamine owing to the poor activity of tyrosine hydroxylase, which not only oxidizes tyrosine to L-DOPA but also diverts L-DOPA to dopaquinone in a side reaction. DDC, L-DOPA decarboxylase. (**b**) DeLoache *et al.*[1] developed an L-DOPA biosensor by using the enzyme DOPA dioxygenase to ultimately convert L-DOPA into the yellow compound betaxanthin and the side product dopaquinone into the purple pigment betanidin. The authors were able to use this assay to search for reduced L-DOPA oxidation activity.

combinations that result in improved microbial production. In addition to practical advances, the sensor could be used in conjunction with genome engineering to identify new network regulators for dopamine production.

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Competing financial interests

The author declares no competing financial interests.