Biosensors and high throughput engineering for the production of specialized plant metabolites in *E. coli*

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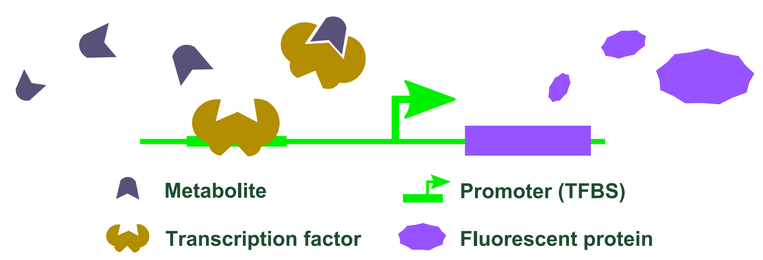
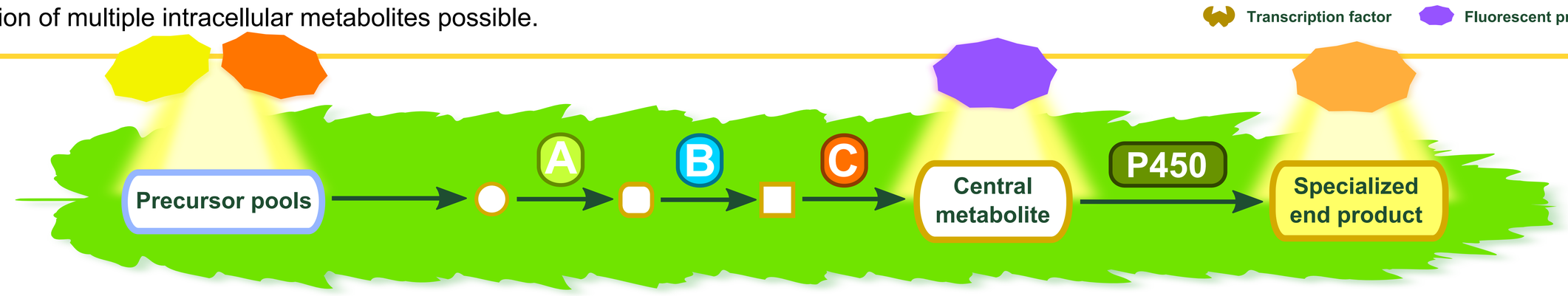
Introduction

Over the past few years, developments in the field of metabolic engineering, synthetic biology and systems biology made it possible to produce complex specialized plant metabolites with micro-organisms in a sustainable and efficient manner. However, the achieved yields, titers and productivities of these industrially important molecules are insufficient to compete with current production methods, like plant extraction. To make this biotechnology competitive, the heterologous biosynthetic pathway of interest and the native metabolism of the host strain have to be balanced and finetuned. This metabolic flux optimization process, however, through genetic modifications, is no sinecure due to the enormous complexity and strict regulation of the microbial metabolism.

Development and validation of metabolic biosensors

Transcription regulation-based metabolic biosensors are developed and used as high throughput screening method. These biosensors consist of three parts: the specific **transcription factor** which is derepressed when the specific metabolite is present, a promoter sequence with the complementary **transcription factor binding site** and a **fluorescent protein** which is being expressed in amounts relative to the amount of metabolite to be detected. These biosensors make simultaneous quantitative *in vivo* detection of multiple intracellular metabolites possible.

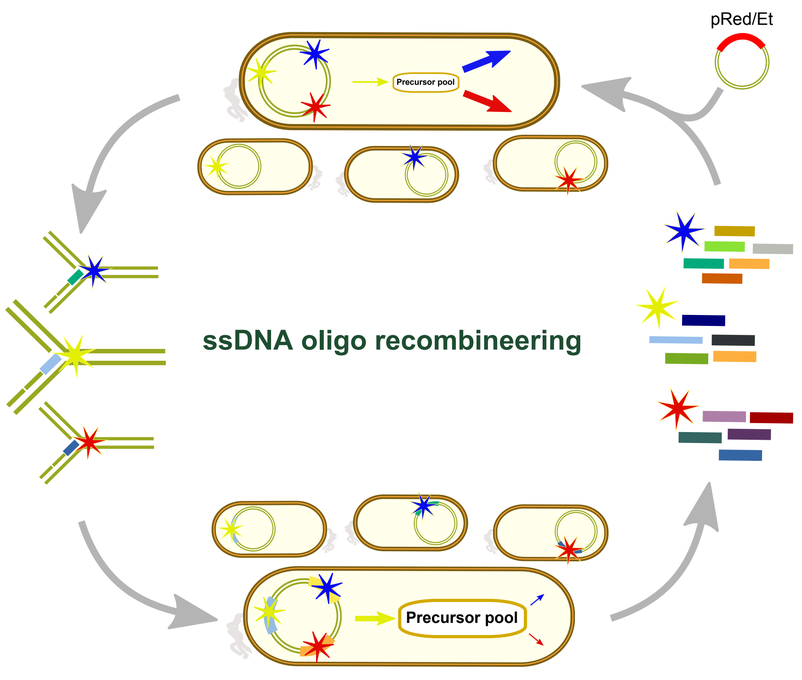




Native metabolism

Precursor pools

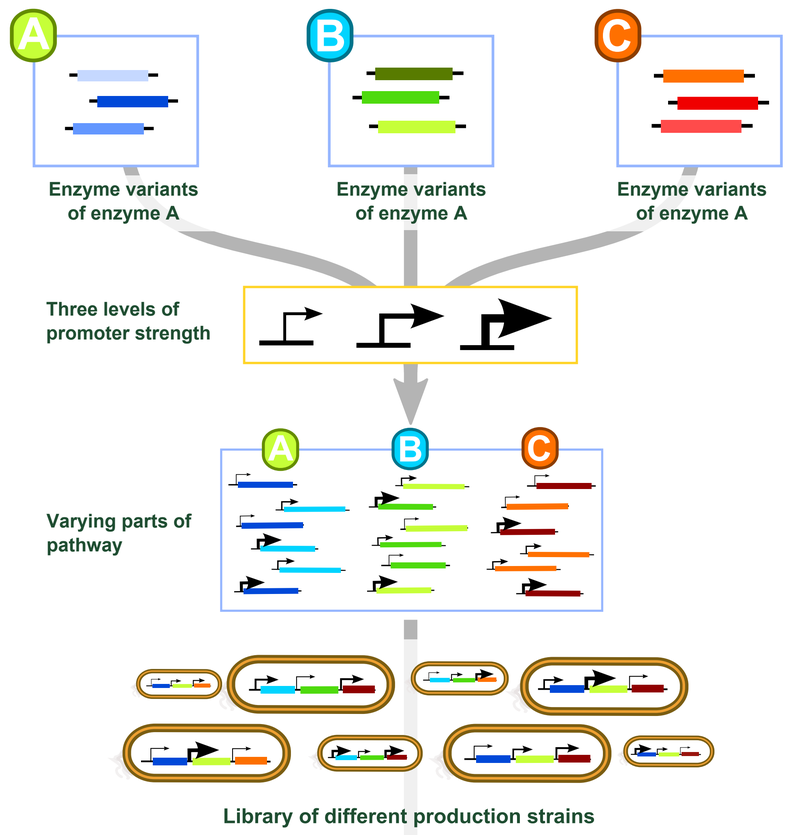
Synthetic single-strand DNA oligonucleotides act as artificial Okazaki fragments that create insertions, deletions or mutations on different targeted sites of the genome, simultaneously. Multiple cycles of this fast **ssDNA oligo recombineering**, targeting multiple sites relevant to alleviating precursor pools, creates a library of strains with different precursor levels. The strains with the highest levels of precursors are selected with the developed biosensors.



Plant pathway part 1:

Central plant metabolite

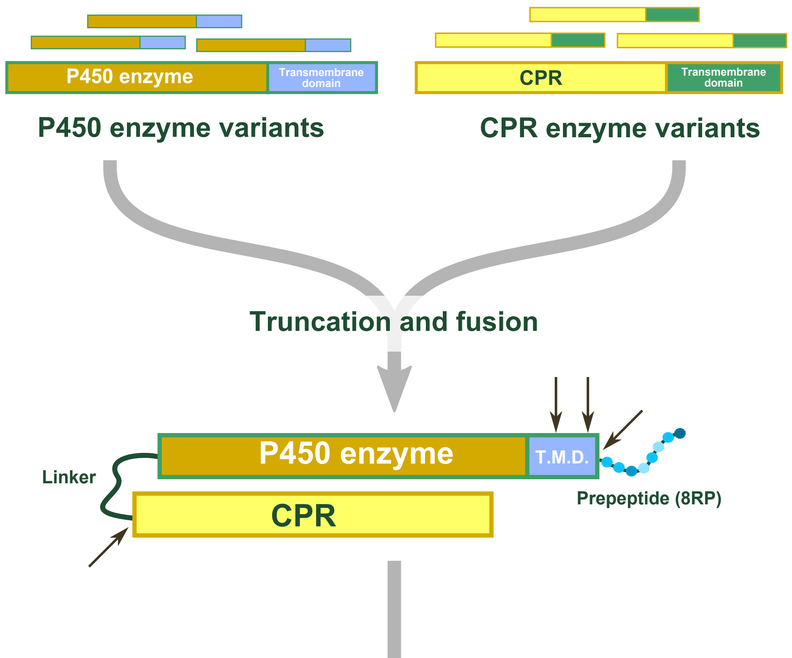
Combinatorial assembly of different enzyme variants in the pathway and three different promoters with different levels of strength creates a library of central metabolite producing strains with different production characteristics. The best producing strains are selected with the developed biosensor.



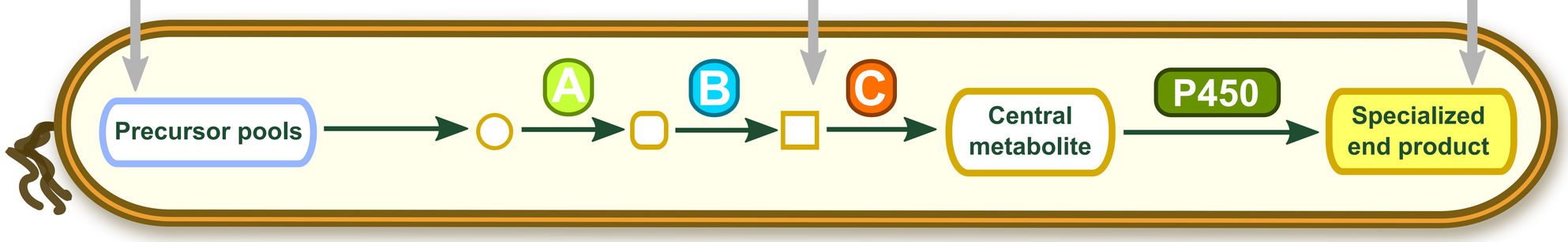
Plant pathway part 2:

Cytochrome P450 enzymes

The plant pathway, responsible for the chemical decoration of this central metabolite, uses unique cytochrome P450 monooxygenases (P450s). These **P450s** are linked to a cytochrome P450 reductase partner (**CPR**) and the transmembrane domain of the resulting fusion protein is truncated to enhance the enzyme activity in the prokaryotic host. These P450s will be linked to the CPRs in a combinatorial manner and with different truncation strategies.



Biosensors



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