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Cofactor manipulation to drive biosynthesis of natural products

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Microbial production of value-added products is a promising alternative to plant- and chemical-based routes [1]. However, only few of interested chemicals are producing at bulk scale with microbial cell factories (eg. artemisinic acid) while most are staying at bench level. To enable the microbes toward efficient bioproduction, extensive endeavors have been expended on enzyme exploration and pathway decoration, leaving the role of cofactors, especially the rare cofactors like SAM, FMN(H_2) and FAD (H_2), largely unconsidered [2,3].

Cofactors are highly demanded organic compounds in propelling various biochemical processes, where particularly the knot-controlling enzymes may suffer from inadequate cofactor supply. Unveiling novel cofactor manipulation strategies would benefit microbial production of target products, especially the complex ones. Nevertheless, challenges including the complexity of metabolism network and the lack of necessary information always render the efforts. In the recent work published in Nature Chemical Biology [4], Yongjin J. Zhou and co-workers systematically engineered the supply and recycling of three cofactors (NADPH, FAD(H_2) and SAM) for the production of caffeic acid and ferulic acid in *Saccharomyces cerevisiae* (Fig. 1).

Phenolic acids are essential precursors for complex lignan chemicals, whose *de novo* biosynthesis involves multiple oxidation and esterification steps that are fueled by cofactor circulating. The authors first enhanced the upper metabolic flux of shikimate pathway, and then reconstructed a plant-derived, and NADPH dependent pathway for caffeic acid production [4]. To boost the caffeic acid titer, they sought to enhance NADPH generation by streamlining the pentose phosphate pathway (PPP). Pulling the non-oxidative PPP downstream steps improved caffeic acid production from 286.3 mg/L to 385.2 mg/L with an elevated level of NADPH/$NADP^+$. NADPH is generally taken in priority due to its higher cellular concentration, and the easiness in rational rewiring. However, introducing multiple NADPH-dependent steps would disturb significantly the intrinsic redox equilibrium, and abate the host cell viability. To further enhance the caffeic acid production, a FAD (H_2) dependent biosynthetic pathway was constructed in the cytosol. FAD (H_2) mainly localizes in mitochondrion for maintaining redox homeostasis, and is at least 20 times less than NADPH in the cytosol. Upon this, they enhanced the cytosolic FAD (H_2) supply by recruiting a *de novo* FAD (H_2) biosynthetic pathway and a mitochondrial FAD exporter to avoid the perturbation of the mitochondrial FAD (H_2) homeostasis, which significantly improved the caffeic acid production. Interesting, they found that enhancing the biosynthesis of riboflavin (the FAD precursor), and expressing its importer MCH5 successfully elevated the caffeic acid production by 93%, indicating that the availability of the FAD precursor might be a limiting step for efficient FAD (H_2)-based biosynthesis route. The present research suggested that both the regeneration and relocation of FAD (H_2) played critical roles in driving natural product biosynthesis when an elevated metabolic flux established, and the synergy between metabolic flux and cofactors supply should be finely handled [5]. Though pathway compartmentalization has been extensively developed for enhancing the biosynthesis efficiency [6], this study showed that engineering the cofactor metabolism among sub-organelles could further drive the bioproduction of natural products in yeast and even other eukaryotes.

The most innovative part of their work is expediting the SAM recycling to drive the SAM-dependent methylation during the ferulic acid biosynthesis from caffeic acid that catalyzed by the *O*-methyl-transferase (Omt). Boosting the SAM supply failed in increasing the ferulic acid titer through the strategies that were previously documented successfully in full-filling the SAM pool [5], which included (1) expressing the rate-limiting methionine adenosyl-transferase (Mat), (2) increasing the supply of 5-methyl-tetrahydrofolate in methionine biosynthesis, and (3) feeding methionine during yeast cultivation. Alternatively, the authors constructed the drainage system for the degradation of S-adenosyl-L-homocysteine (SAH), a byproduct of transmethylation and potent inhibitor of the Omt, which lead to an accelerated methyl cycle, and a significantly increased ferulic acid production from caffeic acid (64% (w/w) conversion). This study is a typical example for recycling the cofactor SAM to support *de novo* biosynthesis of complex compounds, and should expand our in-depth understanding of the fundamentals for fine-tuning cofactors to drive cellular metabolism [7,8].

In summary, this study has developed tailored strategies for manipulating the cofactors such as NADPH, FAD (H_2) and SAM to support the

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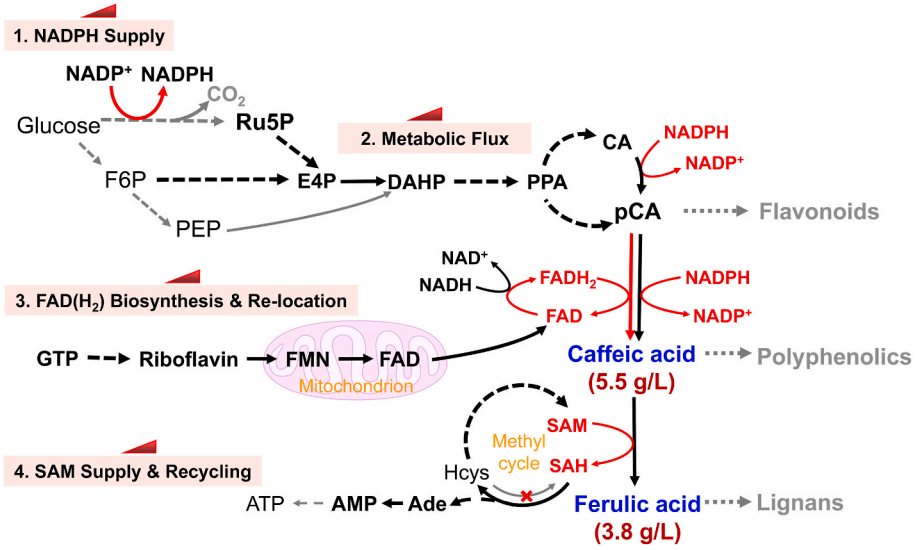

Fig. 1. Cofactor engineering strategies applied for biosynthesis of caffeic acid and ferulic acid. The production of caffeic acid from p-coumaric acid is heavily driven by both NADPH and FAD(H_2), while the ferulic acid biosynthesis from caffeic acid invovles an essential methylation step with SAM as the methyl donor. F6P: fructose 6-phosphate; PEP: ribulose 5-phosphate; RU5P: ribulose 5-phosphate; E4P: erythrose-4-phosphate; DAHP: 3-deoxy-D-arabino-heptulosonate-7-phosphate; NADPH: reduced nicotinamide adenine dinucleotide phosphate; PPA: prephenate; CA: cinnamic acid; pCA: p-coumaric acid; GTP: guanosine triphosphate; FMN: flavin mononucleotide; FAD(H_2): (reduced) flavin adenine dinucleotide; Ade: adenosine; SAM: S-adenosylmethionine; SAH: S-adenosy-L-homocysteine. The dotted lines indicate multiple biosynthetic steps.

high-level production of caffeic acid (5.5 g/L) and ferulic acid (3.8 g/L) in yeast (Fig. 1). These results demonstrate that cofactors supply and recycling play an essential role in driving natural product biosynthesis.

CRediT authorship contribution statement

Qidou Gao: Writing – original draft, wrote the manuscript and drew the graph abstract. **Mengyao Zhang:** Writing – review & editing, edited the manuscript. **Xiaobing Yang:** Writing – review & editing, manuscript edition, Project administration, and, Funding acquisition.

Declaration of competing interest

The authors declare no conficts of interest, and all the authors approved the submission.

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