Cofactor manipulation to drive biosynthesis of natural products

Qidou Gao, Mengyao Zhang, Xiaobing Yang*

College of Enology, Northwest A&F University, Yangling, Shaanxi, 712100, China

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(H2) and FAD (H2), 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(H2) 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 (H2) dependent biosynthetic pathway was constructed in the cytosol. FAD (H2) 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 (H2) supply by recruiting a de novo FAD (H2) biosynthetic pathway and a mitochondrial FAD exporter to avoid the perturbation of the mitochondrial FAD (H2) 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 (H2)-based biosynthesis route. The present research suggested that both the regeneration and relocation of FAD (H2) 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 (H2) and SAM to support the

* Corresponding author.
E-mail address: yangxb@nwafu.edu.cn (X. Yang).

https://doi.org/10.1016/j.synbio.2022.06.005
Received 28 April 2022; Received in revised form 22 May 2022; Accepted 15 June 2022
Available online 16 July 2022

2405-805X/© 2022 The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
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 conflicts of interest, and all the authors approved the submission.

Acknowledgments

This work was supported by supported by Natural Science Foundation of Shaanxi Province (2020JM-177), and Chinese Universities Scientific Fund (2452018314).

References

[1] Park D, Swayambhu G, Lyga T, Pfeifer BA. Complex natural product production methods and options. Synth Syst Biotechnol 2021;6:1–11.
[2] Chen R, Yang S, Zhang L, Zhou YJ. Advanced strategies for production of natural products in yeast. iScience 2020;23:100879.
[3] Liu Q, Yu T, Li X, Chen Y, Campbell K, Nielsen J, et al. Rewiring carbon metabolism in yeast for high level production of aromatic chemicals. Nat Commun 2019;10:4976.
[4] Chen R, Gao J, Yu W, Chen X, Zhai X, Chen Y, Zhang L, Zhou YJ. Engineering cofactor supply and recycling to drive phenolic acid biosynthesis in yeast. Nat Chem Biol 2022;18:520–9.
[5] Wang M, Chen B, Fang Y, Tan T. Cofactor engineering for more efficient production of chemicals and biofuels. Biotechnol Adv 2017;35:1032–9.
[6] Cao X, Yang S, Cao C, Zhou YJ. Harnessing sub-organelle metabolism for biosynthesis of isoprenoids in yeast. Synth Syst Biotechnol 2020;5:179–86.
[7] Moroños O, Andersén JN. Round,round we go-strategies for enzymatic cofactor regeneration. Nat Prod Rep 2020;37:1316–33.
[8] Cravens A, Payne J, Smolke CD. Synthetic biology strategies for microbial biosynthesis of plant natural products. Nat Commun 2019;10:2142.