Received: 28 October 2025 | Accepted: 13 March 2026

DOI: 10.1111/jpy.70165

RESEARCH ARTICLE

The interaction between PII and NAGK regulates arginine biosynthesis in the green microalga *Haematococcus pluvialis*

Ruijuan Ma[1] | **Ziyue Chen**[2,3,4] | **Junjie Liu**[2,3,4] | **Xing Meng**[2,3,4] | **Xinyi Tao**[2,3,4] | **Yucheng Chen**[2,3,4] | **Chunxiao Zhang**[1] | **Ling Wang**[1] | **Kangle Lu**[1] | **Xueshan Li**[1] | **Kai Song**[1] | **Jianfeng Chen**[2,3,4] | **Youping Xie**[2,3,4]

[1]State Key Laboratory of Mariculture Breeding, Fisheries College, Jimei University, Xiamen, China

[2]Marine Biological Manufacturing Center of Fuzhou Institute of Oceanography, Fuzhou University, Fuzhou, China

[3]Fujian Engineering and Technology Research Center for Comprehensive Utilization of Marine Products Waste, Fuzhou University, Fuzhou, China

[4]Fuzhou Industrial Technology Innovation Center for High-Value Utilization of Marine Products, Fuzhou University, Fuzhou, China

Correspondence
Youping Xie, Marine Biological Manufacturing Center of Fuzhou Institute of Oceanography, Fuzhou University, Fuzhou 350108, China.
Email: ypxie@fzu.edu.cn

Funding information
National Natural Science Foundation of China, Grant/Award Number: 32202960; Natural Science Foundation of Fujian Province, Grant/Award Number: 2024J09039; Initial Fund for Scientific Research of Jimei University-Natural Science, Grant/Award Number: ZQ2024067

Editor: N. Poulsen

Abstract

PII protein is widely acknowledged to regulate intracellular nitrogen and carbon metabolism by interacting with several crucial proteins. N-acetyl-L-glutamate kinase (NAGK), a rate-limiting enzyme for arginine biosynthesis, is regarded as a potential target of PII protein. Nevertheless, the regulatory function remains ambiguous in green algae and has not been investigated in *Haematococcus pluvialis*. In this study, the NAGK enzyme and PII protein of *H. pluvialis* (designated as HpNAGK and HpPII, respectively) and their interaction relationships were characterized. The results indicated that HpNAGK showed high similarity with the same enzyme in the green algae. A subcellular localization assay indicated that both HpPII and HpNAGK were located in the chloroplasts. Yeast two-hybrid, pull-down, and bimolecular fluorescence complementation assays distinctly verified the interaction between HpPII and HpNAGK, which occurs in the chloroplasts. The structure of the HpPII-HpNAGK complex was predicted through docking analysis. Moreover, the HpNAGK activity was significantly enhanced by HpPII in the presence of glutamine in vitro. Under nitrogen starvation, HpNAGK activity declined in vivo, concomitant with a reduction in arginine accumulation. The regulatory function of HpPII on HpNAGK activity aligned with that in *Chlamydomonas reinhardtii* but differed from that in *Dunaliella salina*, suggesting species specificity among green algae. These findings provide insights into the regulatory function of PII protein in green algae and help to unveil the response mechanisms of *H. pluvialis* to different nitrogen statuses.

KEYWORDS
arginine biosynthesis, *Haematococcus pluvialis*, NAGK activity, nitrogen starvation, PII protein

INTRODUCTION

Haematococcus pluvialis, a unicellular green alga, holds significant commercial potential due to its ability to accumulate substantial quantities of astaxanthin, a secondary carotenoid known for its diverse health-promoting properties (Mazumdar et al., 2019; Ramarui et al., 2024; Ren et al., 2021). The life cycle of *H. pluvialis* is mainly divided into two phases: a green vegetative phase and a red nonmotile phase (Nogami et al., 2014;

Abbreviations: 2-OG, 2-oxoglutarate; Arg, arginine; ATP, adenosine triphosphate; BiFC, bimolecular fluorescence complementation; EGFP, enhanced green fluorescent protein; Gln, glutamine; GUS, β-glucuronidase gene; IPTG, β-D-thiogalactoside; LB, Luria-Bertani; NAG, N-acetyl-L-glutamate; NAGK, N-acetyl-L-glutamate kinase; NJ, neighbor-joining; PCR, polymerase chain reaction; Y2H, yeast two-hybrid; YFP, yellow fluorescence protein.