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博 士 学 位 论 文

海洋浮游植物藻华宏蛋白质组学研究

Metaproteomic study of marine phytoplankton blooms

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摘 要

浮游植物是海洋初级生产力的主要贡献者，也是海洋食物链的重要组成，在海洋碳循环、生态系统维持和全球气候调节中起着重要作用。浮游植物藻华，特别是高频发生的甲藻和硅藻藻华，深刻地影响着海洋生态系统、全球气候以及人类健康。目前在浮游植物藻华形成的生态学和海洋学机制方面开展了大量工作，但我们对藻华形成的分子机制知之甚少。

本论文将宏蛋白质组学理念和方法应用于海洋浮游植物藻华研究，结合生物信息学分析手段，建立了海洋浮游植物藻华宏蛋白质组学研究方法，比较研究了藻华爆发区与非藻华区、藻华爆发期不同阶段以及现场藻华和实验室培养的浮游植物蛋白质表达谱，鉴定、确认了参与藻华形成和爆发的重要功能蛋白及其生物学过程，探讨浮游植物藻华形成的分子机制。主要研究结果如下：

（1）比较研究了东海藻华爆发区和非藻华区浮游植物蛋白质表达谱。在藻华爆发区和非藻华区浮游植物样品中分别鉴定到 3,912 和 2,762 个高可信度蛋白质。蛋白质物种来源表明，爆发区甲藻的贡献量达到 92.0%，非藻华区的贡献量为 60.6%，硅藻、蓝藻、异鞭藻和定鞭藻的贡献量在 5.5%-8.3%之间。藻华爆发区参与核糖体结构、翻译、转录、光合作用、复制重组修复和蛋白修饰等过程的蛋白质高表达，表明爆发区浮游植物细胞的蛋白质合成活跃，光能需求旺盛。此外，鉴定到参与甲藻尿素循环的重要催化酶和尿素降解酶，表明甲藻存在胞内有机氮利用机制。非藻华区参与能量代谢合成、核糖体翻译和光合作用等过程的蛋白质高表达。光能利用和色素蛋白质的差异表达揭示不同浮游植物类群对光能利用存在差异性和偏好性。在非藻华区硅藻、蓝藻和异鞭藻中鉴定到较高丰度的高亲和 ABC 转运蛋白，且结合底物多为有机物，表明它们启动了有机物利用途径以适应无机盐竞争激烈的现场环境。

（2）比较研究了东海原甲藻藻华爆发期早期和中期浮游植物蛋白质表达谱。在爆发期中期和早期样品中分别鉴定到 4,720 和 3,300 个高可信度蛋白质。蛋白质物种来源表明两个样品中甲藻贡献量均达到 90%，爆发期中期比重略高于爆发期早期。爆发期早期样品中参与转录、翻译、核糖体结构蛋白质和蛋白质反转修饰等过程的蛋白质表达量远高于爆发期中期，表明藻华爆发期早期的细胞蛋白质合成活跃；爆发期中期样品中参与光合作用、碳水化合物、脂质、氨基酸、辅酶代

谢等过程的蛋白质高表达，表明藻华爆发中期细胞内物质和能量代谢活跃，以维持快速的细胞分裂和旺盛的细胞活力。藻华样品中鉴定到表达丰度相对较高的甲藻尿素降解酶、参与尿素循环的几乎全部催化酶以及无机磷转运蛋白，但未鉴定到参与有机磷转运、利用的蛋白质，表明东海原甲藻细胞存在内部尿素循环利用机制和无机磷的高效转运体系，这可能是低无机氮、磷条件下东海原甲藻藻华常发的一个重要原因。此外，细胞周期分裂调控蛋白和细胞程序性死亡蛋白在爆发期中期的表达量高于爆发期早期，表明它们在甲藻藻华维持和衰亡过程中起着重要的调节作用。非藻华浮游植物类群中，参与光合作用、能量代谢、碳代谢、核糖体结构和翻译等过程的蛋白质表达丰度较高，主要用于维持细胞基本的生命活动。

（3）比较研究了现场藻华爆发期和实验室培养指数期中肋骨条藻蛋白质表达谱。在现场和实验室样品中分别鉴定到 1,150 和 1,061 个蛋白质，其中 839 个为共有蛋白质。参与光合作用、能量代谢、蛋白翻译和核小体组装等生物学过程的蛋白质在海区和实验室藻华样品中均为高丰度表达蛋白，如参与光能利用的岩藻黄素叶绿素 a/c 蛋白、光能捕捉蛋白、光合固碳蛋白 RuBisCO、能量代谢蛋白 ATP 合成酶等，表明藻华爆发期间细胞对能量和碳的需求高。现场细胞中参与光能捕获、光合色素合成、光保护和细胞分裂的蛋白质表达丰度明显高于实验室样品，而实验室培养细胞中参与翻译、蛋白质和氨基酸代谢、CO_2 和氮吸收的蛋白质表达丰度明显高于海区现场样品。现场藻华及实验室培养样品蛋白质表达谱的差异及特异性蛋白质的鉴定，表明中肋骨条藻已进化了对不同环境条件变化的适应性响应能力，这可能是导致不同海区复杂环境中硅藻藻华易发的一个重要原因。

关键词：浮游植物藻华；甲藻；硅藻；东海原甲藻；中肋骨条藻；宏蛋白质组学；蛋白质组学

Abstract

Phytoplankton are the major contributors to marine primary production and also important component of marine food chain (web), they play important roles in marine carbon cycling, ecosystem maintenance and global climate regulation. Phytoplankton blooms, especially high frequent occurrence of dinoflagellates and diatoms, significantly influence marine ecosystem, global climate and human health. Much effort has been devoted to the ecological and oceanographic mechanisms of phytoplankton bloom formation. However, little is known about the molecular mechanism of bloom formation.

This thesis applied proteomic theory and methodology to the study of marine phytoplankton bloom combining with bioinformatics analysis, established metaproteomic studying method of phytoplankton blooms, compared protein expression profiles of blooming and non-blooming samples, different blooming stages, and field bloming and laboratory culture phytoplankton, identified and confirmed important proteins and their biological processes involved in formation and blooming of phytoplankton blooms, and discussed molecular mechanism of phytoplankton bloom formation. The main results were as follows:

（1）The global protein expression profiles of the blooming and non-blooming samples were compared. The result showed that 3,912 and 2,762 proteins were confidently identified in blooming and non-blooming samples respectively. The taxonomic origin of proteins indicated that Dinophyta contributed about 92% and 60.6% to the proteins in blooming and non-blooming samples, respectively, followed by Bacillariophyta, Cyanophyta, Ochrophyta and Haptophyta species with the proportion ranging from 5.5% to 8.3%. In blooming sample, proteins involved in translation, transcription, protein turnover and modification, and photosynthesis presented higher expressions, indicating active protein synthesis and high requirement of light energy. Moreover, nearly all urea cycle enzymes and one urea degradation eyzyme, urea carboxylate, were detected in two samples, indicating utilizing mechanism of organic nitrogen, such as urea in dinoflagellates. In non-blooming sample, proteins participating in energy production and conversion, translation and photosynthesis were more abundant. However, the expressions of light utilization and pigment proteins varied significantly among those key phytoplankton groups in non-blooming sample, indicating differences and preference of different

phytoplankton groups on light utilization. High affinity ABC transporters binding organic compounds were identified more abundant in Bacillariophyta, Cyanophyta, and Ochrophyta in non-blooming sample, especially in Bacillariophyta, indicating that they initiated organic compound utilization mechanisms in order to adapt high competition of inorganic nutrient in field environment.

（2）The global protein expression profiles of *Prorocentrum donghaiense* samples collected at early and middle blooming stages were compared. The result showed that 3,300 and 4,720 proteins were confidently identified with two or more peptides in early and middle blooming *P. donghaiense* samples, respevtively. Taxonomic origin of proteins indicated that more than 90% of identified proteins were matched to dinoflalgellates, and the proportion in the middle blooming sample was higher than that in the early blooming sample. Proteins involved in translation, transcription, protein turnover and modification were more abundant in early blooming sample, indicating more active protein biosynthesis in early blooming sample. While proteins participating in carbohydrate, lipid, anmino acid and coenzyme compound metabolic process presented higher expressions in the middle blooming sample, indicating more active substance and energy metabolism in the middle blooming sample in order to maintain rapid cell division and vigorous cell activity. More abundant urea degradating enzyme, almost all catalytic enzymes involved in urea cycle, and inorganic phosphate transport protein were identified in the blooming samples, but no organic phosphorus transport and utilization proteins were identified, indicating utilization mechanism of intracellular urea and high efficient transport system of inorganic phosphate in *P. donghaiense*, which might be an important reason resuling in the frequent occurrence of *P. donghaiense* in the East China Sea. Moreover, higher abundances of some cell cycle and growth regulation proteins and programmed cell death protein were detected in the middle blooming sample, indicating their importance in maintainging dinoflagellate blooms and decay process. For those non-blooming phytoplankton groups, proteins involoved in photosynthesis, energy metabolism, carbohodrate metabolism, and translaton were more abundant which maintained cell basic life activities.

（3）The global protein expression profiles of both field-collected (FC) and laboratory-cultured (LC) blooming cells of *Skeletonema costatum* were compared. 1150 and 1061 proteins were identified in the FC and LC samples, respectively. Of

which, 839 were shared proteins between two samples. Proteins involved in photosynthesis, energy metabolism, protein translation and nucleosome assembly were high abundant in both samples, such as fucoxanthin chlorophyll a protein, light capture protein, photosynthetic carbon fixing protein Rubisco, energy metabolism protein ATP synthase, indicating high requirements of energy and carbon in the blooming cells. In the FC blooming sample, protein involved in light harvesting, photopigment synthesis, light protection and cell division were more abundant than those in the LC sample, while proteins participating in translation, protein and amino acid metabolism, CO_2 and nitrogen uptake presented higher expressions than those in the FC sample. The differences of protein expression profiles and the identification of specific proteins in the FC and LC samples suggested that *S. costatum* had evolved adaptive mechanisms to the changing environment, which might explain their dominant status in taking the niche in the harsh and variable marine environment.

Key words: Phytoplankton bloom; Dinoflagellate; Diatom; *Prorocentrum donghaiense*; *Skeletonema costatum*; Metaproteomics; Proteomics