N-ACETYLGLUTAMATE SYNTHASE GENE SILENCING VIA DS-DNA NANOSWITCH AND ITS IMPACT ON LIPIDS METABOLISM AND GROWTH: A PROTEOMIC PERSPECTIVE

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Introduction

Our research uncovered that the arginine pathway regulates neutral lipid production in *Chlamydomonas reinhardtii*. Downregulation of the N-Acetylglutamate synthase (NAGS) gene alters L-arginine biosynthesis and the ornithine pathway, increasing metabolite accumulation and neutral lipids without inducing cell quiescence, a typical stress response in microalgae. Using gold nanoparticles functionalized with dsDNA, we achieved photothermal delivery of antisense DNA to silence NAGS, leading to lipid body formation within 1 to 4 hours without affecting cell growth. To understand the broader physiological effects and why cell growth remains unaffected, we conducted a proteomic analysis to identify the key metabolic changes linked to neutral lipid overproduction following NAGS silencing.

Materials and methods

Gold nanoparticles functionalized with disulfide-modified sense and Cy5-labeled antisense oligonucleotides targeted the NAGS gene in Chlamydomonas reinhardtii cells. The cells were chemically transfected and recovered for 8 hours before the antisense DNA was released into the cytoplasm using a photothermal process with a 50W green LED chip, reaching the NAGS gene\'s melting temperature of 46°C. For proteomic analysis, samples of 2x10^6 cells were collected at 1 and 4 hours post-antisense DNA release, with controls taken at 0 hours. Total proteins were extracted using a urea-thiourea buffer and sonication. Proteins (10 µg) were reduced, alkylated, and digested with trypsin. Peptides were desalted with StageTips and eluted in 0.1% formic acid. Mass spectrometry was conducted on an Orbitrap Fusion Lumos, with protein identification and quantification done using MaxQuant software with a 1% FDR. Data analysis was performed using Perseus software with label-free quantification (LFQ), and functional annotation was conducted with TopGO, applying an FDR of 0.05% and a P-value threshold of <0.05.

Results and Discussion

The statistical analysis revealed 238 and 300 proteins with differential abundancy between control (0h) and 1h and 4h, respectively. Common GO terms identified, such as GO:0044271, GO:1901566, GO:1901564, GO:0034641, and GO:0006807, are linked to nitrogen metabolism and amino acid biosynthesis. The term GO:0010467, associated with gene expression regulation, suggests that gene silencing affects transcriptional modulation or translation inhibition. GO terms GO:0003723, GO:0003729, and GO:0006412, related to mRNA stability and translation, indicate post-transcriptional regulation processes. Additionally, analysis of proteins at 1h and 4h post-treatment revealed 24 groups with GO terms GO:0009767, GO:0015979, GO:0019684, and GO:0009772, related to photosynthetic regulation and photosynthetic electron transport in photosystem II. The terms GO:0046168 and GO:0006072, associated with lipid synthesis, such as triacylglycerol, were also identified during NAGS gene silencing.

Conclusions

Proteomic analysis revealed that NAGS gene silencing significantly impacted molecular and metabolic processes, including reduced translation and decreased nitrogen compound biosynthesis, likely related to arginine metabolism. Changes in lipid synthesis pathways and alterations in light capture and photosynthesis adaptation were also observed. These findings suggest a connection between the arginine pathway and light-driven responses, potentially explaining the unaffected cell growth during NAGS silencing.

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