CPP-PNA as an alternative approach for genetic engineering haptophytes

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Abstract

Haptophytes are microalgae with significant biotechnological potential due to their production of docosahexaenoic acid (DHA), biofuels and unsaturated fatty acids, as well as their synthesis of unique unsaturated methyl ketones called alkenones. However, their genetic engineering remains challenging due to low biomolecular permeability, uncharacterized genes, and limited transformation techniques. To address these challenges, we investigated the use of cell-penetrating peptide and peptide nucleic acid (CPP-PNA) conjugates as an alternative approach to genetically engineer haptophytes. First, we screened CPPs with high cell permeability in three haptophyte species, including *Tisochrysis lutea* (*T. lutea*), by modifying CPP sequences with various amino acids and synthesizing a CPP-fluorescein (CPP-FAM) library. Our findings revealed that CPP-FAM permeation varies across species, with specific CPPs exhibiting high permeation efficiencies. Next, we synthesized CPP-PNA conjugates, where PNA can bind to the mRNA of target genes encoding a light-harvesting protein (Lhcf) and alkenone desaturase (Akd1), and attempted to regulate protein translation in *T. lutea* strains. Permeation of these CPP-PNA conjugates led to observable changes in growth inhibition and compound production rates, suggesting successful intracellular delivery and mRNA specific translational regulation. In summary, our results demonstrate the feasibility of CPP-PNA mediated permeation for targeted protein translation in haptophytes, providing a promising tool for genetic engineering. We will continue to apply this method to elucidate the functions of various proteins, and explore its potential for industrial applications in haptophyte biotechnology.

Keywords: haptophytes, biomolecule delivery, translation regulation, Tisochrysis, cell penetrating peptides

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