19. Gene editing, artificial chromosomes, and highly efficient conjugal gene transfer in diatoms

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Project Goals: Genome-scale metabolic models are fundamental for the analysis of cellular processes at a systems level and represent an ideal organizational framework for analyses of functional genomics, experimental work and computational studies. In recent years, there has been an increasing interest in high- quality metabolic reconstructions of phototrophic organisms and robust computational tools to integrate 'omic' data from these organisms within genome- scale models. The approach of the project is to combine cutting-edge genome manipulation and physiological characterization with metabolic modeling. The ultimate goal is the exploration of next-generation biofuels through a comprehensive understanding of light-driven lipid metabolism in the model marine diatom Phaeodactylum tricornutum.

The eukaryotic microalgae diatoms hold great promise for bioproduction of fuels and higher value chemicals. However, compared to model genetic organisms such as Escherichia coli and Saccharomyces cerevisiae, functional characterization of diatom genes and strain improvement has been hampered by the inefficient genetic tools. To date, diatoms are transformable only via particle bombardment, where the introduced DNA is integrated randomly into the nuclear genome in a (n=1 - many) copy number. The copy number and location effects introduce a wide range of efficiency in the resulting transformants, and the screening of transformants constitutes substantial labor. Similarly, the methods for gene silencing in diatoms depend upon the introduction of RNAi constructs via particle bombardment, with labor intensive screening of transformants using gene and protein expression, along with an a priori hypothesized phenotype. Here we describe improved methods for gene-specific genome editing using Transcription Activator-like Effector Nucleases (TALENs) and demonstrate its application in the diatom Phaeodactylum tricornutum by knocking out the Ni metalloenzyme urease and examining the phenotype by metabolomics. Further, we have engineered the first nuclear episomal vector for diatoms with a parallel delivery method via conjugation from Escherichia coli to the diatoms P. tricornutum and Thalassiosira pseudonana. We identify a yeast-derived sequence that enables stable episome replication in these diatoms even in the absence of antibiotic selection and show that episomes are maintained as closed circles at copy number equivalent to native chromosomes. This episome delivery system is several orders of magnitude more efficient than particle bombardment and lacks positional or copy number effects, thereby facilitating millions of experimental manipulations in reasonable time periods. The system also allows for efficient complementation of deletional mutants and examples of experimental cycles of forward and reverse genetics for functional gene identification in diatoms at the whole genome scale will be discussed.

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