Influence of DNA Delivery on Mechanism of CRISPR-Cas Genome Editing in Marine Diatoms

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Diatoms, a major phylogenetic group of phytoplankton, play a significant role in shaping marine systems in a wide range of aquatic environments. They greatly contribute to the pool of organic carbon fixed via photosynthesis in contemporary oceans, producing between 25% and 40% of the 40-50 billion tons of organic matter generated annually. They also flourish in conditions where nutrient availability is variable, such as coastal upwelling and polar regions. Along with their ecological significance, diatoms have unique metabolic properties that hold promising biotechnology potential in sustainable biofuels and nano-materials. Sequencing data from diatoms, most notably *Phaeodactylum tricornutum (Pt)* and *Thalassiosira pseudonana (Tp)*, greatly aids in determining the genetic and genomic basis for diatoms' ecological and biotechnological importance, yet genetic manipulation tools essential to explore such areas are not well developed in diatoms. To date, tools such as RNAi and TALENs have been employed in the laboratory strains *Pt* and *Tp*, but the more powerful, sophisticated, and flexible genome engineering tool, CRISPR-Cas, has yet to be implemented. Here, CRISPR-Cas genome engineering was utilized to functionally investigate and disrupt the urease and nitrate reductase genes. Two DNA repair mechanisms, non-homologous end joining (NHEJ) and homology directed repair (HDR), were used to induce mutations randomly and introduce scar-less mutations, respectively, at the CRISPR-Cas target locus. Also, two genetic transformation techniques available, micro-particle bombardment and bacterial conjugation, where harnessed to deliver the CRISPR-Cas machinery to diatom *Pt*. This study demonstrates that precise targeted mutagenesis via HDR can be accomplished when employing micro-particle bombardment delivery, and NHEJ-mediated mutations can be induced using either transformation avenues. The development of the CRIPSR-Cas technology using both micro-particle bombardment and bacterial-conjugation vastly widens the possibilities in the exploration diatom genetics.