CRISPR/Cas9-directed mutagenesis in *Volvox carteri*

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Abstract:

Cas9 can be used to make targeted indels by non-homologous end-joining (NHEJ) or precise changes in the genome by homology-directed recombination (HDR), resulting in knockout mutations and precise genome edits, respectively. To expedite genetic analysis in *V. carteri*, we set out to develop a transgene-based CRISPR/Cas9 system that can be used to mutate candidate genes and to make precise edits. We replaced the plant-specific regulatory sequences present in an Arabidopsis/rice Cas9 vector with regulatory sequences from *V. carteri* genes and targeted test genes with known mutant phenotypes, including *glsA* and *regA*. The *V. carteri*-specific Cas9 gene is carried on one plasmid and the guide RNA gene on a second plasmid containing a hygromycin resistance marker. Biolistic co-transformation of the Cas9 plasmid and a guide RNA gene plasmid targeting *glsA* into Reg mutant 153-68 generated hygromycin-resistant transformants that produced Gls mutant progeny, and co-bombardment of the Cas9 vector with a guide RNA gene plasmid targeting *regA* into wild type strain EVE generated hygromycin-resistant Reg transformants. Mutation rates varied from <0.1% to 100%. We cloned and sequenced the targeted *glsA* and *regA* regions in those mutants and found they contained frameshift-causing indel mutations, indicating that these were Cas9-generated NHEJ mutations. Efforts are underway to test HDR via Cas9 and to mutate genes of unknown function in *V. carteri*.