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Genome editing in microalga *Chlamydomonas reinhardtii* via CRISPR/Cas9

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Statement of the Problem: It was found that the genome of a popular model organism, single-celled microalga Chlamydomonas reinhardtii encodes at least 18 sensory photoreceptors and functions of many of them are not completely characterized or unknown. We developed the efficient CRISPR/Cas9 based gene inactivation protocol (Greiner et al.2017) and disrupted 11 non-selectable photoreceptor genes. By sequencing of mutated genes, we found that precise repair of Cas9 induced double-stranded breaks (DBS) through homologous recombination with supplied donor DNA was a rare event and mutated clones contained various DNA modifications of the target site. For further structure-functional investigation of photoreceptors and other proteins, it is required to generate predefined amino acid substitutions and insertions of fluorescent tags at their native genomic locus. The purpose of this study is to better understand DNA repair pathways and increase the efficiency, predictability, and fidelity of Cas9-induced site-directed mutagenesis *in Chlamydomonas*.

Methodology & Theoretical Orientation: We generated and characterized mutants on DNA repair pathways containing different ku80, ku70, polQ null alleles and used them as recipients for targeted mutagenesis with CRISPR endonucleases, including SpCas9, SaCas9 or LbCpf1, and various donor DNA substrates.

Findings: Inactivation of the POLQ gene resulted in a dramatic decrease of the rate of occasional and targeted DNA insertions into the nuclear DNA and the high sensitivity to the DNA damaging agents zeocin and MMS. Oppositely, all ku80 and ku70 mutants demonstrated the rate of occasional and targeted DNA insertions, spectra of targeted mutations and zeocin and MMS sensitivity similar to the wild-type cells.

Conclusion & Significance: POLQ could be responsible for the most of occasional and targeted insertions of DNA fragments into Chlamydomonas nuclear genome. Inactivation of KU80, Ku70 or POLQ does not increase predictability and fidelity of Cas9-induced mutagenesis *in Chlamydomonas*.

Biography

Irina Sizova has her expertise in evaluation and passion in genome editing *in Chlamydomonas*. Earlier, together with colleagues, she designed a resistance marker for paromomycin, which is now one of the most popular markers for the transformation of Chlamydomonas. Together with colleagues of Humboldt University of Berlin she has created or adapted a number of methods for site-directed modification of Chlamydomonas nuclear genome, including the use of single-stranded DNA vectors, zinc-finger nuclease and the system CRISPR/Cas9.

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