

Development of multi-functional photolyase as an ultraviolet protectant using CRISPR-Cas9 genome editing

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Ultraviolet (UV) exposure of DNA causes formation of harmful DNA damage, such as cyclobutane pyrimidine dimers (CPDs) and pyrimidine(6–4)pyrimidone photoproducts (6–4PPs), which block DNA replication and induce mutagenesis, leading to carcinogenesis. Repair of these lesions is a vital pathway to maintain genetic integrity, and thus loss of the genes responsible for DNA repair causes cell death or cancer. Among the DNA repair systems, photoreactivation is the simplest pathway, in which a sole photo-responsive enzyme, photolyase (PL), can repair UV-induced DNA lesion in a light-dependent manner. Therefore, PLs are molecules applicable to the UV protectant under sunlight.

PLs are classified into two major classes, CPD photolyase and (6–4) photolyase, depending on their target substrates (CPD and 6–4PP, respectively). If a hybrid type of PL that can recognize and repair both CPDs and 6–4PPs is developed, such a molecule excellently inhibits formation of UV-induced DNA damage and functions as a UV protectant. In this study, I intended to develop the hybrid photolyase by combination of CRISPR-Cas9 genome editing technology and directed evolution of photolyase. Plasmids were designed to knock the modified PL genes in human cells via homology-independent targeted integrity, with a promoter and antibiotic-resistant gene. After transfection of the plasmid with Cas9 and gRNA-producing plasmids, however, antibiotics-resistant strains were not obtained, although the target genes were integrated into the AAVS1 site. Thus, the plasmid was used for functional screening of the hybrid PL in bacteria, the results of which are shown in this report.