Advancing genome editing through studying DNA repair mechanisms

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Since the discovery of CRISPR/Cas9, also known as molecular scissors, scientists around the world have been working to improve the revolutionary technique for altering DNA that earned Emmanuelle Charpentier and Jennifer Doudna the Nobel Prize in 2020. The method enables deep exploration of the human genome and shows enormous

potential for curing genetic diseases. While the precise alterations made by CRISPR/Cas9 were initially less predictable, scientists around the world are now working on further developments that enable precise changes to be made within DNA. A recent study by the group of Joanna Loizou, Group Leader at the Center for Cancer Research at MedUni Vienna and CeMM Adjunct Principal Investigator, was devoted to understanding how prime editing, a technique that promises greater targeting accuracy and efficiency in introducing DNA changes, can be made more efficient and precise.

Prime editing is a powerful genome engineering tool that allows for replacement, insertions, and deletion of DNA into any given genomic locus. However, to date, the efficiency of prime editing has been highly variable and depends not only on the targeted genomic region but also on the genetic background of the edited cell. Leading authors Joana Ferreira da Silva, CeMM Ph.D. student, and Gonçalo Oliveira from the Center for Cancer Research of the MedUni Vienna, devoted their study to the question of which factors influence the success of prime editing, taking a close look at DNA repair processes. Since genome editing relies on the intrinsic DNA repair machinery within a cell, it is imperative to know which DNA repair pathways are engaged and how this impacts the outcome of editing. Yet the underlying DNA repair machinery involved in prime editing is largely unknown. The study authors explain that "depending on the type of DNA damage, a cell has different cellular repair mechanisms. To find out which of these are active in prime editing, we performed a targeted genetic screening for DNA repair factors covering all known repair pathways."

Study leader Joanna Loizou adds, "Our results show that the DNA repair pathway, known as mismatch repair, influences prime editing outcomes. This is the pathway that deals with base mismatches in the genome. Depending on the cell line, type, and site of edit we want to make, we can increase the efficiency of prime editing by two to 17-fold by

eliminating mismatch repair." Specifically, the study showed an accumulation of the proteins MLH1 and MSH2—proteins involved in the DNA mismatch repair process and each responsible for recognition and removal of the incorrect base—at the site of genome editing. The results show that the activity of the mismatch repair proteins inhibits the efficiency of prime editing. "By removing the activity of the mismatch repair pathway from a cell, we show that the efficiency of prime editing can be increased, and its accuracy improved," Loizou said. This fundamental understanding will ultimately bring this technology closer to the clinic.

The research was published in *Nature Communications*.

More information: Joanna Loizou et al, Prime editing efficiency and fidelity are enhanced in the absence of mismatch repair, *Nature Communications*, DOI: 10.1038/s41467-022-28442

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