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Base editors: past, present, future

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The breakthrough technology of genome editing has dramatically reshaped the field of molecular and cellular biology over the past ten years and is set to enter the healthcare industry, with the first therapy, exagamglogene autotemcel (Casgevy), approved in 2023. Based on the natural bacterial anti-phage CRISPR system and built on earlier technologies that used designer zinc finger nucleases and TAL effector nucleases, the first generation of genome editing tools relied on introducing double-stranded breaks by RNA-targeted Cas9 nuclease followed by error-prone repair via non-homologous end-joining or error-free replacement via homologous recombination. The technology was soon improved by the development of more precise engineered Cas9 versions, introduction of Cas12 targeting modules and, more recently, the appearance of base editors, in which a catalytically dead Cas9 targeting part (dCas9) is fused to an effector module, such as cytosine deaminase, which selectively modifies nucleobases within a structure-defined editing window.

The first base editors were based on natural cytosine deaminases of the AID/APOBEC family and converted cytosine to uracil, with the intent to cause dAMP misincorporation and ultimately, a C>T (G>A) transition mutation. It became apparent immediately that cellular DNA repair systems counteract this type of targeted DNA damage, so the next addition to the system was a specific viral protein (Ugi) that inhibits uracil repair. Engineered versions of tRNA adenosine deaminase were used to obtain base editors converting A to hypoxanthine with wider mutation spectra. The later generation of base editors additionally fused specific DNA glycosylases to dCas9 to facilitate the appearance of a non-instructive abasic site and further increase the mutation range. Finally, engineered DNA glycosylases were used that can excise normal nucleobases and do not need the deamination module at all. To narrow the editing window and decrease off-target effects, “split” architectures were developed, in which the functional module is inserted between Cas9 domains.

In addition to editing the sequence of genomic DNA *in situ*, fusion of functional modules to Cas9 allow installing and erasing epigenetic modifications such as 5-methylcytosine (mC) and 5-hydroxymethylcytosine (hmC) to regulate gene activity. The functional modules used for this purpose include DNA–cytosine-C5-methyltransferases, TET family dioxygenases, and DNA glycosylases specific either to oxidized/deaminated mC derivatives (TDG) or to mC/hmC themselves (plant DML/ROS proteins).

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