URLs

GENE THERAPY

Permanent correction without selection

Rapid and stable modification of the human genome at a specific location has been hindered for a long time by technical limitations. Urnov *et al.* now describe a method that uses engineered zinc-finger nucleases (ZFNs) to stimulate homologous recombination, both in transformed and primary human cells. Although this approach has been used previously in model systems, the new study establishes the usefulness of ZFN-driven genome editing for human genetics, and highlights the potential for "gene modification" therapy of inherited diseases.

The approach takes advantage of two fundamental biological processes: DNA recognition and DNA repair. Targeted cleavage of DNA is achieved by zinc-finger proteins that have been designed to recognize unique chromosomal sites and are fused to the non-specific DNA cleavage domain of a restriction enzyme. A double-strand break that is induced by the resulting ZFNs can create specific alterations in the genome by stimulating homology-directed repair between the locus of interest and an extrachromosomal donor molecule.

The authors designed a large collection of selected zinc-finger modules that would improve ZFN specificity and efficiency. They created a donor plasmid that carries a wild-type *GFP* sequence, which was introduced into cells along with a mutated GFP-encoding gene. Action by the ZFNs restored *GFP* function in 10% of cells — this represents a substantial increase in homologous recombination frequency over that obtained in other selection-free settings. But can we achieve permanent and precise modification of an endogenous gene?

Indeed, the authors succeeded in changing the sequence of the interleukin 2 receptor-γ (*IL2RG*) gene in a targeted fashion by transfecting ZFNs and a mutated donor DNA, both into transformed and primary T cells, and showed that ZFNs can induce modification of the endogenous locus in 20% of cells within 4 days of treatment and without drug-based selection. Taking this result a step further, cells that were homozygous for the knockout allele were isolated and rapidly corrected to the wild-type phenotype by using the same ZFNs and a donor DNA molecule that repairs the mutation.

Although this approach was efficiently used to correct a mutation, it still requires optimization in appropriate whole-organism systems. Delivery of ZFN-encoding and donor DNA molecules to cells and the potential immunogenicity of ZFNs impose certain limitations, but these recent advances are promising for therapeutic strategies that involve *ex vivo* cell manipulation, especially for correcting monogenic disorders of the haematopoietic system and disrupting genes that are involved in infectious disease.

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References and links

ORIGINAL RESEARCH PAPER Urnov, F. D. *et al.* Highly efficient endogenous human gene correction using designed zinc-finger nucleases. *Nature* 3 April 2005 (doi:10.1038/nature03556)

WEB SITE

Sangamo Biosciences: http://www.sangamo.com/tech/tech.html