GENE TARGETING

Human genomic repair at your fingertips

Using customized DNA-cleaving enzymes, researchers can dramatically improve their success rate for homologous recombination in human cells—with potentially powerful implications for gene therapy applications.

The ability to actively rewrite the genomic sequence of living cells—and thereby of entire animals—through targeted homologous recombination has revolutionized biology. Unfortunately, the conventional targeting strategies that have proven so effective in mouse embryonic stem cells yield disappointingly low rates of homologous recombination when applied to human cells. Alternatives such as viral delivery can be effective, but have the risk of transgene integration at an inappropriate site or disruption of an endogenous gene sequence—with potentially dire consequences.

Homologous recombination events occur with greater frequency at the site of a double-stranded DNA break; theoretically, if one can precisely select a genomic region for breakage, then more efficient gene targeting should become possible. One of the best-known and characterized DNA-binding protein motifs is the zinc finger, which recognizes and binds specific 3–4 nucleotide sequences via an α-helical domain, and studies by Johns Hopkins investigator Srinivasan Chandrasegaran have demonstrated that nuclease target preference can be altered by swapping in zinc-finger domains with different specificities. Dana Carroll at the University of Utah and Matthew Porteus, now at the University of Texas Southwestern Medical Center (Dallas, Texas, USA), took this work a step further, using chimeric zinc-finger nucleases (ZFNs) to stimulate homologous recombination in frog, fruit fly and human cells (Bibikova M. *et al.*, 2003; Porteus & Baltimore, 2003).

In collaboration with Porteus, Michael Holmes and his colleagues at Sangamo BioSciences, Inc. (Richmond, California, USA) began to develop this technology, with an eye toward new gene therapeutic strategies. "We can target a zinc-finger protein to virtually any sequence in the genome," says Holmes, "and we do this by using domains that have three, four or six zinc fingers, which allows us to basically specify a domain that will recognize nine, twelve or eighteen base pairs." Two ZFNs have to act in concert for cleavage to take place: the two engineered ZFNs bind on either side of the site of interest, introduce the cut, and then homologous recombination can occur between the chromosome and a 'donor' DNA molecule, introducing the desired sequence change (**Fig. 1**) (Urnov *et al.*, 2005).

In experiments with human erythroleukemia K562 cells, the group targeted the *IL2RG* gene, which is responsible for X-linked severe combined immunodeficiency (SCID). Using their optimized ZFNs to mediate the introduction of a silent point mutation, the researchers achieved efficiency of recombination as high as 21%. These changes were stable over time, and the relative proportion of gene-converted cells remained constant even a month later. A second round of experiments demonstrated that in 6.6% of the treated cells, both alleles of the endogenous locus were modified. This strategy was also used to introduce frameshift mutations into the same gene, with appropriate decreases in mRNA and protein levels clearly detectable for both heterozygous and homozygous mutants. The group has since demonstrated that their ZFNs can also mediate the introduction of larger sequence changes as well as point mutations.

Having demonstrated the feasibility of efficient, targeted, biallelic gene modification, Holmes indicates that the group is now looking to improve the practicality of their system. "I think our shorter-term goal is to develop better delivery systems that allow us to introduce our nucleases and our donor molecules into primary cells such as T cells and stem cells, and do it in such a way that it's compatible with doing animal studies and the work necessary to move it to a clinical trial." This technique appears to offer strong potential for gene therapeutic applications, and initial planning is already underway for animal studies. "We are in the planning stages of animal experiments for our X-linked SCID project, and another project that we think has the biggest potential to go into a clinical trial in the near future is actually targeting *CCR5*—to modify *CCR5* to provide resistance to cells against HIV infection."

Michael Eisenstein

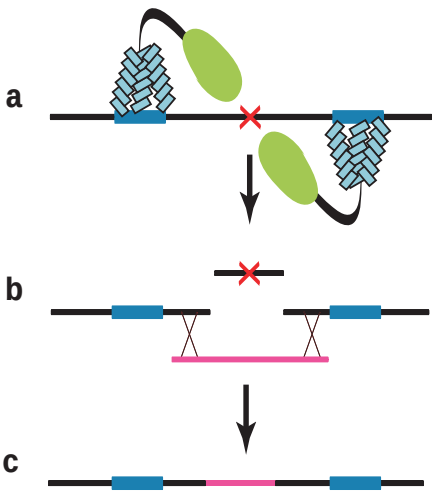

Figure 1 | ZFN-assisted homologous recombination. (**a**) Two ZFNs, each with a DNA-binding domain consisting of several zinc-finger motifs engineered to bind on either side of a genomic mutation (red), introduce a double-stranded DNA break, removing the mutation. (**b**) The homology-directed repair pathway mediates the efficient homologous recombination of a donor DNA fragment (purple) lacking the mutation. (**c**) The mutated gene has been repaired.

RESEARCH PAPERS

Urnov, F.D. *et al.* Highly efficient endogenous human gene correction using designed zinc-finger nucleases. *Nature*; published online 3 April 2005.

Porteus, M.H. & Baltimore, D. Chimeric nucleases stimulate gene targeting in human cells. *Science* **300**, 763 (2003).

Bibikova, M. *et al.* Enhancing gene targeting with designed zinc-finger nucleases. *Science* **300**, 764 (2003).