Improving gene-editing nucleases

A flurry of recent papers report refinements to the activity and the assembly of engineered nucleases.

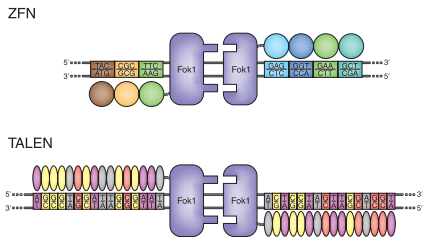
It is now possible to make targeted changes to the genomes of numerous species using engineered nucleases. But the potential of these tools will not be fully realized until they can be simply and efficiently designed (or inexpensively purchased) by any laboratory and unless they can achieve the desired editing outcome at essentially any target sequence. Methods development in this area thus continues at a fast pace.

In one set of recent studies, three independent groups engineer 'nickase' zinc-finger nucleases (ZFNs) that cleave DNA on only one strand. Repair of the typical ZFN-induced double-strand break (DSB) occurs by one of two pathways: homology-directed repair (HDR), which requires a donor template, or nonhomologous end joining (NHEJ), an error-prone process that frequently results in small insertions or deletions at the DSB site. The latter has been exploited by many groups to mutate genes of interest. However, if the goal of genome editing is gene correction or addition by HDR, NHEJ represents a competing mutagenic pathway that reduces the number of correctly modified clones. It is therefore desirable to be able to bias the outcome of repair of a genomic break to one versus the other pathway.

The groups of Keith Joung, Jin-Soo Kim and Michael Holmes independently approached this problem by engineering ZFN nickases—a strategy that works because single-strand DNA breaks are not expected to be resolved via NHEJ. Building on a recent report that a D450A mutation renders the Fok1 nuclease (the source of the nuclease component of ZFNs) catalytically inactive, the researchers showed that ZFN pairs consisting of one wild-type and one D450A mutant monomer function as nickases *in vitro*.

Working in cultured human cells, all three groups observe that repair after nickase activity is indeed strongly biased toward HDR over NHEJ. Joung and colleagues tested four nickases with two reporter systems; the other two groups also used the nickases for sequence addition to endogenous genes and were essentially unable to detect NHEJ at these sites even using deep sequencing.

All three groups observed that gene-editing activity is lower in the engineered nickases than in the ZFNs from which they are derived, although it appears sufficient for the isolation of modified clonal lines. Nickases are also predicted to be less likely to mutagenize off-target sites; indeed, Holmes and colleagues observed fewer genome-wide DSBs with nickases than with their parent ZFNs, and Kim and colleagues could not detect mutations at known off-target sites of a CCR5-targeted nicking enzyme.



Schematic (not to scale) of ZFN and TALEN pairs.

In a separate study, Joung and colleagues report high-throughput assembly of transcription activator–like effector nucleases (TALENs), the new kids on the engineered nuclease block. Much of the excitement around TALENs rests on the fact that they appear to have a simple code for DNA recognition. The researchers now exploit this in an iterative, bead-based procedure for TALEN assembly that is amenable to automation with a lipid-handling robot. Not only did this allow the rapid generation of many functional TALENs, but it also enabled systematic testing of the consequence of varying particular design features (such as number of repeats, spacer length and binding-site choice) on TALEN performance.

Continuing progress on these and other fronts should hopefully put flexible and effective genome-editing tools into the hands of many scientists in the near future.

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RESEARCH PAPERS

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Reyon, D. *et al.* FLASH assembly of TALENs for high-throughput genome editing. *Nat. Biotechnol.* **30**, 460–465 (2012).

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