Nature Reviews Molecular Cell Biology | Published online 16 Dec 2015

IN BRIEF

GENOME ENGINEERING

Structure-guided improvement of Cas9 specificity

The Cas9 endonuclease is guided to its targets by single guide RNAs (sgRNAs). Looking to improve the DNA targeting-specificity of Cas9, Slaymaker *et al.* hypothesized that DNA strand separation enhances Cas9 nuclease activity, and therefore that inhibiting Cas9 helicase activity would reduce the strength of sgRNA–off-target-DNA interactions and thus decrease off-target cleavage. To test this, they generated *Streptococcus pyogenes* Cas9 (SpCas9) mutants at 32 positively charged residues within a SpCas9 groove, which is likely to support strand separation, and found 3 mutation combinations that retained SpCas9 wild-type on-target activity with undetectable off-target cleavage at selected genes. These SpCas9 mutants exhibited increased sensitivity to mismatches between sgRNA and target DNA, and genome-wide reduction in off-target cleavage levels.

ORIGINAL ARTICLE Slaymaker, I. M. *et al.* Rationally engineered Cas9 nucleases with improved specificity. *Science* http://dx.doi.org/10.1126/science.aad5227 (2015)

LIPID METABOLISM

Lipid droplet growth in brown fat

Unlike white fat cells, which store lipids as a single large lipid droplet, brown adipocytes feature multiple smaller lipid droplets in their cytoplasm. This facilitates the high rates of lipid metabolism that are necessary for the heat-generating function of these cells. Barneda *et al.* studied how a brown fat-specific protein, CIDEA, mediates the fusion, and thus the growth of lipid droplets. They demonstrate that an amphipathic helix within the CIDEA carboxyl terminus is first inserted into the membranes of droplets, followed by the formation of intermolecular CIDEA complexes on adjacent droplets to facilitate their aggregation. Finally, lipids are transferred between droplets in a process that depends on the interaction of the CIDEA amphipathic helix with phosphatidic acid on lipid droplet membranes. These data shed light on the mechanisms of lipid droplet fusion and size regulation in brown fat. To better understand the physiological differences in lipid droplet growth between brown and white fat, it would be interesting to study how CIDEC — the equivalent of CIDEA in white adipocytes — mediates lipid droplet fusion.

ORIGINAL ARTICLE Barneda, D. *et al.* The brown adipocyte protein CIDEA promotes lipid droplet fusion via a phosphatidic acid-binding amphipathic helix. *eLife* http://dx.doi.org/10.7554/eLife.07485 (2015)

RNA

Transcriptional mutagenesis by R-loops

During transcription, the nascent RNA can anneal to its DNA template and form RNA–DNA hybrids (R-loops), thereby exposing the other DNA strand to transcription-associated mutagenesis (TAM). Chen *et al.* examined whether a greater propensity of transcripts to fold into stable RNA structures, which are less able to form R-loops, would decrease TAM. They manipulated the yeast *CAN1* gene to change the folding properties of its transcript and found that the stably folding version formed significantly fewer R-loops than the weakly folding equivalent. Furthermore, expression of the weakly folding version increased TAM, whereas R-loop degradation by RNase H1 reduced TAM. Along with additional, genome-wide analyses in yeast and humans, the data indicate that strong nascent RNA folding reduces TAM.

ORIGINAL ARTICLE Chen, X. *et al.* Nascent RNA folding mitigates transcription-associated mutagenesis. *Genome Res.* http://dx.doi.org/10.1101/gr.195164.115 (2015)