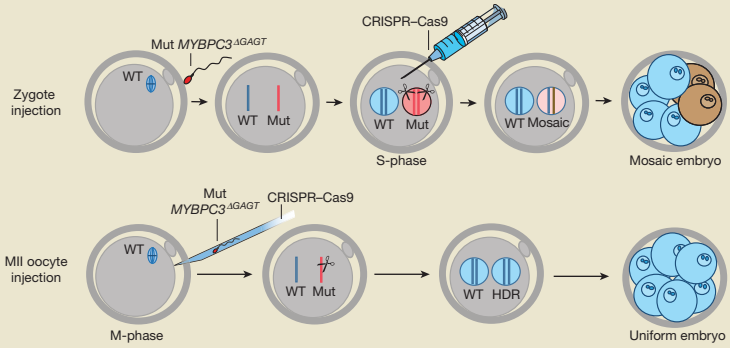
Precision editing in the human embryo

A new study from Shoukhrat Mitalipov and collaborators in the United States, South Korea and China[1] marks a major advance in genome editing of the human germline. Injecting the programmable endonuclease CRISPR–Cas9 into human one-cell embryos, the team reports high-efficiency correction of a heterozygous, disease-associated mutation by homology-directed repair, with no detectable genetic changes at any off-target site.

Previous papers on targeted genome editing in human embryos were marred by technical shortcomings that limit insights for research and would rule out applications in human reproductive medicine. Correction efficiency was low; Cas9 cuts were repaired by non-homologous end joining, which often introduces indels, rather than by homology-directed repair; embryos were mosaic (containing genetically non-identical blastomeres); and Cas9 activity at off-target sites—often seen in other edited cell types—was not ruled out. The new work by Ma *et al.*[1] describes substantial progress on these issues.

The authors focused on a heterozygous, four-base-pair deletion in the *MYBPC3* gene, a mutation associated with cardiomyopathy. Following standard methods, they generated single-guide RNA (sgRNA)–Cas9 constructs targeted to the mutation and single-stranded DNA templates carrying the wild-type sequence (with synonymous single-nucleotide mutations for identification) flanked by homology arms. When these editing agents were applied to induced pluripotent stem cells bearing the mutation, the overall targeting efficiency was low (27.9%), and 58.8% of the mutations were repaired by non-homologous end joining rather than by homology-directed repair.

For their studies in human embryos, Ma *et al.*[1] used a more efficient delivery method: microinjection of sgRNA and Cas9 protein rather than plasmid transfection. Wild-type



oocytes were fertilized with *MYBPC3*-mutated sperm. In a first set of experiments, the editing agents were injected into S-phase zygotes (18 hours after fertilization), and the embryos were analyzed three days later by single-cell sequencing. The overall targeting efficiency was much higher than in induced pluripotent stem cells (72.2% vs. 27.9%), and 66.7% of the embryos were non-mutant, compared with 47.4% of control embryos that did not receive the editing agents. However, many of the embryos were mosaic, which would be impermissible in reproductive medicine.

The authors reasoned that the mosaicism arose either because editing occurred after the zygote had replicated the mutant allele during S phase, with only one allele undergoing editing, or because Cas9 remained active in some blastomeres after zygote division. Aiming to prevent both possibilities, they injected the editing agents earlier, during metaphase II, at the same time as oocyte fertilization by intracytoplasmic sperm injection, similar to work in mouse embryos by Tony Perry and colleagues[2]. Now, targeting efficiency reached 100%. 72.4% of the embryos were non-mutant, and nearly all embryos (57/58) were not mosaic. A search for off-target effects using Digenome-seq, whole genome sequencing and whole exome sequencing turned up no mutations at off-target sites.

The relatively high rate of homology-directed repair vs. non-homologous end-joining (estimated to be 22.4% vs. 27.6% in M-phase-injected embryos) represents a striking improvement over previous results on Cas9 genome editing in mouse, monkey and human embryos. Investigating the underlying mechanism will be an important next step.

Ma *et al.*[1] found that the template for homology-directed repair was almost always the wild-type allele present in the maternal genome rather than the single-stranded donor DNA. "It's not clear to me how recombination can occur between paternal and maternal chromosomes across tens of microns—the distance between paternal and maternal genomes for several hours in newly formed one-cell embryos," Perry said, "or once pronuclei have formed, effectively partitioning parental genomes until after S-phase." The authors propose that human germ cells may have a uniquely stringent DNA repair system that has yet to be discovered—"a different DNA damage response system, perhaps reflecting the evolutionary importance of maintaining germline genome integrity."

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1. Ma, H. *et al. Nature* http://dx.doi.org/10.1038/nature23305 (2017).
2. Suzuki, T. *et al. Sci. Rep.* **4**, 7621 (2014).