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Genome editing gets a makeover with CRISPR 2.0

Applications of the genome editing system CRISPR are appearing at a furious pace, and gathering momentum toward therapeutic use in human cells. Indeed, Chinese scientists recently began a human clinical trial using CRISPR-edited cells to fight lung cancer, and U.S. clinical trials are slated to begin in 2017. But leading up to this exciting milestone, researchers performed some editing on the CRISPR system itself. Here’s a look at some recent CRISPR upgrades that are helping to move it closer toward use in clinics.

By Caitlin Smith

Genome editing using CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 made an impressive splash onto the scientific scene only a few years ago, empowering researchers to edit a specific gene with greater precision and ease than ever before. Yet a few warts emerged in the new technology, such as cutting DNA at the wrong site, and even unintentional DNA editing.

But scientists quickly began tweaking CRISPR in the right places, and now innovative molecular features are making it work even better and for more cell types. The rapid emergence of CRISPR applications means that clinical trials related to HIV, cancer, sickle cell disease, and other diseases are on the horizon.

Today CRISPR is a cutting-edge tool for many more researchers, and more suitable for future therapeutic use than other gene-modulating methods. “Back when RNA interference [RNAi] hit, it went into hyperdrive,” says Mark Behlke, senior vice president and chief scientific officer at Integrated DNA Technologies (IDT), which supplies RNAi and CRISPR reagents. “But now CRISPR makes that look like a child’s game—it’s just mind-blowing.”

CRISPR reagents get a makeover

Faster, cheaper, and easier to use than gene editing methods such as TALENs (transcription activator-like effector nucleases) or zinc-finger nucleases, CRISPR was quickly seized upon by researchers in many fields. For example, cancer researchers transformed cell lines with plasmids containing DNA that encoded CRISPR guide RNA (gRNA) and Cas9 (CRISPR-associated protein 9) to create different cancer cell lines for study.

But Matt Porteus, a physician and associate professor of pediatrics at Stanford University School of Medicine, had a different initial experience with CRISPR. “Everyone was saying that CRISPR would solve all the problems of the world, but when we tried to use CRISPR DNA plasmids in cells that we thought were important for therapeutic applications, like hematopoietic cell lines or other primary human cell types, the system didn’t work at all,” he says. So the Porteus lab developed a different delivery method for CRISPR/Cas9 editing in human primary cells, one that doesn’t require DNA plasmids (1) (see “Aiming CRISPR at human diseases” on page 209).

Variations of this method exist that introduce CRISPR/Cas9 reagents into cells in the form of ribonucleoproteins (RNPs). “The researcher combines these reagents [gRNA and Cas9 protein] and allows them to form a complex for five to ten minutes” to create RNPs, says Jon Chesnut, senior director of synthetic biology R&D at Thermo Fisher Scientific. “The CRISPR RNPs can then be delivered to the cell by lipid nanoparticles or electroporation.” cont.>

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The Cas9 protein uses a guide RNA (gRNA) sequence to cut DNA at a complementary site. RNA is in red, DNA is in yellow.
“For C. elegans, CRISPR has been a real game changer.”

— Brian Kraemer

Even though RNP reagents are widely available, it’s only in the past year that researchers have been showing more interest in them as a CRISPR method. “But there’s still an awareness level that’s not there yet, because so many DNA tools remain broadly available from the earlier plasmid-based approaches,” says Louise Baskin, senior product manager at Dharmacon (part of GE Healthcare). The benefits of DNA-free, RNP-based CRISPR over plasmid delivery include no danger of unintended DNA insertion, reduced toxicity, better on-target efficiency, and improved specificity.

These advantages make DNA-free CRISPR tools more amenable for developing therapeutic applications. “For us, the ability to edit genes in primary tissues and primary cell types is a big breakthrough,” says Judd Hultquist, a postdoc in the lab of Nevan Krogan, professor of cellular and molecular pharmacology at the University of California, San Francisco (UCSF).

Hultquist, in collaboration with Kathrin Schumann, a postdoc in the lab of Alexander Marson, assistant professor of microbiology and immunology in the UCSF School of Medicine, used Dharmacon’s Edit-R synthetic gRNAs in RNPs to edit human primary T cells, which are the main target of HIV. Now they are developing an RNP-based discovery platform focused on finding genetic changes that improve the resistance of T cells to HIV infection.

Latest CRISPR technologies also catch the worm

The use of CRISPR RNPs has also revolutionized nonhuman model systems such as that of the worm Caenorhabditis elegans. “For C. elegans, CRISPR has been a real game changer,” says Brian Kraemer, research associate professor in medicine at the University of Washington, who uses IDT’s DNA-free CRISPR reagents.

Injecting RNPs into the gonadal region of the worm allows for genome editing of germ cells—the resulting progeny include some animals with the edited phenotypes, which he can isolate for further study. Kraemer’s lab uses C. elegans as a discovery tool to identify genes required for pathogenic mechanisms in protein-aggregation diseases such as Alzheimer’s and atrophrophic lateral sclerosis (ALS).

Kraemer believes that the new CRISPR tools will fuel the next generation of transgenic models in C. elegans, including customized alleles—genes that encode a protein altered to suit an experimental purpose, such as changing a targeting sequence on an intracellular trafficking protein that could reroute it to a different membrane compartment.

Better reagents, better editing

One of the keys to improving CRISPR in primary cells, as well as other cell types, is the recent enhancement of reagents. IDT developed chemically modified gRNAs that are resistant to nuclease degradation inside cells. The company also manufactures gRNAs in the form of two shorter RNAs (as in the original bacterial system) that form a complex, instead of a single, longer gRNA. MilliporeSigma also plans to offer two-part synthetic gRNAs as “SygRNAs.”

Joey Riepsaame, head of the genome engineering facility in the Sir William Dunn School of Pathology at the University of Oxford, uses IDT’s Alt-R CRISPR/Cas9 RNP system to help run gene editing experiments. Riepsaame appreciates IDT’s two-part gRNAs, which also minimize the triggering of unnecessary immune responses. “For me, this was a very important factor, because my project involves using CRISPR/Cas9 to correct disease-causing mutations in immune cells,” he says. “So far, we haven’t encountered any major challenges with CRISPR/Cas9, and have been able to target every region of interest.”

Optimized CRISPR reagents such as RNPs are also creating new opportunities for researchers. One problem with using DNA-based CRISPR, or even Cas9 messenger RNA (mRNA), is a lag phase before editing begins, during which the cellular machinery transcribes and/or translates the active CRISPR reagents. Injecting DNA- or mRNA-based CRISPR reagents into embryos, for example, can result in so-called “mosaic” animals, which have more than one set of genetic information. “The RNP approach has reduced mosaicism because reagents are active the minute you introduce them; they quickly degrade after editing, and the rapid degradation has the added benefit of reducing off-target effects,” says IDT’s Behlke.

Other new tools include transfection chemistries for better delivery of CRISPR reagents into cells. MITO-GlobalStem’s new Edit-Pro Stem Transfection Reagent supports the delivery of CRISPR tools into stem cells, and its EditPro Transfection Reagent enables delivery into human primary cells and cell lines. “The new EditPro chemistry has a wide range of tunable dosage, in terms of the amount of mRNA that will translate into higher protein translation,” says James Kehler, director of scientific alliances at MITO-GlobalStem (now part of Thermo Fisher Scientific).

Researchers are also moving beyond optimizing CRISPR reagents to using the CRISPR/Cas9 system in new and creative ways. For example, removing the “scissors” portion of Cas9 turns it into an efficient molecular targeting tool that can bring an attached effector molecule to a specific location in the genome. Different effector possibilities, such as activators, repressors, or modifiers, are also being investigated. MilliporeSigma’s dCas9-p300 activator construct is a noncutting version of Cas9 fused to a p300 histone acetyltransferase domain. Upon binding, the construct acetylates nearby histones, opening up the chromatin to allow for increased and sustained gene expression.
CRISPR for functional screening

Despite the recent success with RNP-based CRISPR, there’s still a place for plasmid-based technology when it comes to functional screening. Several companies offer lentiviral CRISPR-based libraries for knocking out genes to identify those genes that are responsible for diseases. Simone Treiger Sredni, associate professor in pediatric neurosurgery at Northwestern University’s Feinberg School of Medicine, recently used Thermo Fisher Scientific’s LentiArray CRISPR Libraries to screen 160 different kinases for mutations that affect cell proliferation. Sredni’s research focuses on finding therapeutic options for children with atypical teratoid/rhabdoid tumors (AT/RTs), aggressive and lethal types of pediatric brain tumors.

Sredni’s screen identified mutations in a few specific kinases that resulted in a reduction of cell proliferation in AT/RT cell lines. “An inhibitor to one of these kinases had the same effect as the absence of the gene, not allowing the tumor to grow,” she said. Although she’d previously looked at high-throughput gene expression platforms for screening, “this gene never popped up, because its level of expression is always very low.” Next, Sredni will investigate the effect of the inhibitor in mice xenografts.

MilliporeSigma also offers lentiviral-based CRISPR tools for whole-genome screening. In collaboration with the Wellcome Trust Sanger Institute, MilliporeSigma also recently constructed arrayed whole-genome CRISPR libraries for human and mouse genomes that offer flexibility in format, delivery, and scope (i.e., single genes, gene families, or whole genomes).

Agilent Technologies recently released pooled CRISPR guide libraries for screening, including the genome-scale CRISPR knockout (GeCKO) SureGuide Catalog human and mouse libraries delivered via lentiviral vector. Agilent also offers preamplified and nonamplified custom libraries for full flexibility. “Our CRISPR pooled libraries are most often used in functional screening, using CRISPR/Cas9 to generate knockouts across the genome,” says Caroline Tsou, Agilent’s global marketing director for molecular and synthetic biology in the Diagnostics and Genomics Group. “Usually these knockouts serve to identify genes involved in cellular responses, such as in signaling pathways, or to discover the function of novel genes.” Agilent also prints custom oligonucleotides of up to 230 base pairs, giving researchers “the freedom to explore other uses for the libraries,” she says.

But sometimes cells don’t thrive when forced to express a bacterial nuclelease. Dharmacon’s Edit-R inducible lentiviral Cas9 system is “a nice compromise for researchers who are uncomfortable with having the nuclelease constantly present in stable cell lines,” says Baskin. “The inducible system gives them the best of both worlds, because they can turn on nuclelease expression when they are ready to treat cells with their guide RNA, get good Cas9 expression, and then turn it off after cleavage.”

Aiming CRISPR at human diseases

Meanwhile, all manner of CRISPR reagents are on deck to fight a variety of diseases—especially using the DNA-free approach. The fast-on, fast-off nature of the RNP method, for example, is well suited to therapeutic applications where the CRISPR reagents cut where directed and then degrade quickly.

But correcting genetic defects isn’t as simple as knocking out a gene, because often the correct functional gene must also be introduced at the right location. The Porteus lab at Stanford recently published proof-of-concept work using CRISPR RNPs to target the beta-globin gene, mutations of which cause sickle cell disease. They showed that they could correct the defective beta-globin gene in human hematopoietic stem cells from patients with this disease (4). Independently, a lab at the University of California, Berkeley, accomplished a similar CRISPR editing result with the beta-globin gene, using a slightly different method to deliver the corrected gene (5).

Taken together, the work of these and other labs is promising for upcoming human trials. In June, the U.S. National Institutes of Health approved the first trial in the United States, slated for 2017, which will use CRISPR-edited human T cells to help augment cancer therapies.

Meanwhile, the Porteus lab is gearing up to manufacture CRISPR-edited cells for use in patients, in clinical trials that they hope to start in 2018. They will likely target sickle cell disease first, followed by severe combined immune deficiency (SCID). Porteus hopes to use CRISPR not just to correct mutations, but also to “give cells new properties that might treat a disease, such as an immune system that’s resistant to HIV, or to create cells that could deliver a protein to the brain,” he says. “In the ecology of science and medicine, we feel like our role is to try to bring this technology to patients.”

With the development of CRISPR research tools in hyperdrive and U.S. clinical trials set to begin next year, these goals are probably closer to being realized than we imagine.

References

Caitlin Smith is a freelance science writer based in Portland, Oregon.
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CRISPR RNA Arrayed Library

The Dharmacoon Edit-R Human Druggable Genome crRNA Library enables screening of nearly 8,000 individual targets with CRISPR/Cas9 gene knockouts. The first arrayed synthetic CRISPR RNA (crRNA) library of its kind, it provides insight into numerous biological questions and offers a powerful screening resource to identify potential therapeutic targets. The Edit-R crRNA Library delivers one-gene-per-well information by enabling high content and multiparametric assays to characterize complex phenotypes. Ready-to-use, transfecatable Edit-R synthetic crRNAs are designed using an algorithm that results in highly functional gene knockout, while also accurately identifying and eliminating sequences with the potential for off-target editing. The library offers 7,995 gene targets with four crRNAs per gene; 96- and 384-well-plate formats with quantities of 0.1 nanomole (nmol), 0.25 nmol, or 0.5 nmol per well; and subsets arranged by gene family including kinases, proteases, phosphatases, ion channels, transcription factors, G-protein-coupled receptors, ubiquitin enzymes, and other potential drug targets.

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