nature biotechnology

Genome editing for all

CRISPR-Cas is about to transform how we interrogate genetic variants and model disease.

CRISPR-Cas genome editing technology is attracting a growing cadre of devotees. In the past 18 months, over 125 papers on the technology have been published. At least three commercial ventures have been founded around the platform. And last month, the University of California (UC) Berkeley, UC San Francisco and the Li Ka Shing Foundation launched the $12-million Innovative Genomics Initiative (IGI), which seeks to accelerate adoption of the technology. According to its website, IGI will be dedicated to "a revolutionary method of genome engineering based on the transformative discovery of Cas9, a programmable DNA binding and cleaving enzyme." So just how transformative is CRISPR-Cas likely to be?

CRISPR, short for clustered, regularly interspaced, short palindromic repeats, is the name of a genomic locus in some bacteria and archaea that functions as an adaptive immune system against invading phage or plasmids. The locus encodes an endonuclease and stores snippets of foreign sequence, which are transcribed into RNAs that guide the endonuclease by base complementarity to cleave foreign nucleic acids at specific sequences. Type II CRISPR systems use the endonuclease Cas9 and two RNAs—tracrRNA and crRNA. The realization that these can be fused into a single, synthetic 'guide RNA' to direct Cas9 to virtually any desired genomic sequence is what has the biology world abuzz.

CRISPR-Cas is just the latest of several customizable genome-editing approaches. Other DNA-binding proteins, such as zinc finger nucleases and transcriptional activator–like (TAL) effector nucleases, can also be targeted to specific genomic sites. Like CRISPR-Cas, they can be used to knock out genes or to introduce designed sequences into the genome with far greater efficiency than traditional genetic engineering strategies. Where CRISPR-Cas stands out is the simplicity with which it can be targeted. Zinc finger and TAL effector proteins require protein engineering to bind a desired DNA sequence, but with CRISPR-Cas all one needs to do is design and synthesize a guide RNA. The system also readily targets multiple genomic sites at once.

In the under two years since Cas9 was first combined with an exogenous guide RNA, CRISPR-Cas has been used for targeting single loci in at least 19 fungal, bacterial, plant and animal species, including nonhuman primates, and multiplexed targeting has been shown in various systems, including mammalian cells. It has been adapted to diverse applications, including gene activation and silencing, visualization of genome dynamics and genome-wide functional screens.

Nevertheless, the system remains a work in progress. Cas9 targeting specificity in various cell types from different organisms remains to be assessed on a genome-wide scale. Strategies for enhancing specificity beyond that of native Cas9 are likely to be important in some contexts, for example, to avoid deleterious mutations in clinical settings or when using cell proliferation–based assays in which a clone with an off-target mutation might rapidly outgrow the rest of the population. As yet, CRISPR-Cas systems from only a small number of bacterial species have been studied—it is possible that other, uncharacterized nucleases have more useful properties. For applications that aim to introduce specific DNA sequences, methods for controlling the balance between homology-directed repair and nonhomologous end-joining, which results in indels, could also be beneficial. Once these issues have been ironed out, Cas9 promises to be a fundamental tool for interpreting and exploiting the massive amounts of sequence variant data and for modeling genetically complex diseases.

Genome-wide association studies have already identified at least 2,000 mutations associated with ~300 conditions (*Nat. Rev. Genet.* **14**, 549–558, 2013). The challenge of interpreting the function of each mutation is complexity as well as scale, given that most genetic variants don't act alone. With traditional methods of genetic manipulation in mammalian animal or cell models, introducing single mutations is difficult enough, let alone testing myriad combinations on diverse genetic backgrounds. CRISPR-Cas should facilitate multiplex assays in various experimental formats.

In developing models of complex disease, patient-to-patient heterogeneity in genotype and phenotype remains a challenge. For *in vitro* disease modeling, induced pluripotent stem cells derived from patients and differentiated to the appropriate lineage have been used with some success to recapitulate the pathology of neurodegenerative and other diseases. Combining such cellular models with CRISPR-Cas would allow researchers to start to pinpoint specific genetic contributors to phenotypic features.

Cas9 will also galvanize generation of *in vivo* disease models. Modifying the germline of a mouse can take six months using conventional methods, requiring gene targeting in embryonic stem cells, screening to identify correctly targeted cells, implantation of targeted cells into blastocysts, and birth and subsequent breeding of chimeric mice to identify animals harboring the desired germline modification. With efficient genome-editing methods, things become much simpler and faster as targeting constructs can be injected directly into the mouse zygote, enabling birth of germline-modified mice just three weeks later. Considering the ease of generating Cas9 guide RNAs, this sounds like a transformational technology.

For cancer research, one could study different combinations of candidate tumor driver mutations by introducing them into nonmalignant human cells and implanting the cells in immunodeficient mice. Alternatively, one could implant patient-derived tumor cells in which various mutations have been corrected one by one.

One day, CRISPR-Cas may be used for corrective gene therapy—in a similar manner to CCR5 zinc finger nuclease *ex vivo* gene therapy for HIV-positive patients. The use of catalytically inactive Cas9 fusions to DNA- or histone-modifying proteins may even make epigenetic therapy attainable. But in the meantime, CRISPR-Cas can have an immediate impact onvariant analysis and disease modeling. Further optimization of the CRISPR-Cas system will be needed, which is why IGI, and other efforts like it, are important. In the era of high-throughput sequencing, the maturation of a tool for facile manipulation of the genome cannot come soon enough.