Henceforth CRISPR

In less than a decade, the genome-editing technology now recognized by the Nobel Prize in Chemistry has impacted the biological and biomedical sciences widely. What's next for CRISPR in biomedicine?

Emmanuelle Charpentier, from the Max Planck Unit for the Science of Pathogens in Germany, and Jennifer Doudna, from the University of California, Berkeley, in the United States, were awarded the [Nobel Prize in Chemistry 2020](#) for the original discovery of how clustered regularly interspaced short palindromic repeats (CRISPR; a molecular mechanism providing adaptive immunity to bacteria and archaea) can be programmed, by using RNA guides, to precisely edit DNA at specific loci in vitro. CRISPR's efficiency and fidelity and, most of all, its ease of implementation compared to preceding genome-editing technologies, has engendered a rush of research activity. The versatility, controllability and biological and biomedical applications of genome editing across cell types, tissues and organisms have been refined and expanded, mostly through newly discovered and engineered bacterial CRISPR-associated (Cas) endonucleases, via the design of RNA guides that ensure on-target activity and minimize off-target effects, and by improving the targeted delivery of endonucleases and guides to the cells to be edited.

Beyond loss-of-function and gain-of-function changes upstream of a short sequence adjacent to the region targeted for editing (known as a protospacer adjacent motif), catalytically impaired Cas proteins (which bind to the desired DNA locus without cutting it) fused to functional domains can perform genetic and epigenetic modifications: base editing uses adenosine and cytidine deaminases fused to CRISPR–Cas nickases to make transition point mutations; prime editing uses a Cas9 nickase fused to a reverse transcriptase to make all types of substitutions, transitions and transversions; and deactivated Cas nucleases fused with transcriptional activators or repressors can upregulate or suppress the expression of target genes.

Barely eight years after Doudna's and Charpentier's original publication¹, it is difficult to find an area of biology left untouched by CRISPR. The applications for CRISPR technologies in biomedicine are also myriad: screens (for the discovery of drugs or gene functions, in particular), new cell lines and animal models of disease, gene therapies (for sickle-cell anaemia or muscular dystrophy, for instance), engineered cells (such as those for adoptive

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T-cell immunotherapy), the engineering of organs for xenogeneic transplantation (via the inactivation of retroviruses in the genome of donor pigs), diagnostic assays, and more.

In fact, CRISPR's exquisite specificity makes it outstanding for the detection of nucleic acids in biofluids. Notably, target genes in buccal samples can be detected electrically via inactivated Cas9 immobilized on a graphene field-effect transistor without the need for amplification², and Cas13 has been used to monitor opportunistic viral infections in blood and urine samples following organ transplantation, and to detect transplant rejection in urine samples³. And, most recently, fast and inexpensive lateral-flow fluorescence assays containing Cas12a or Cas13a can detect SARS-CoV-2 RNA accurately in nasopharyngeal samples^{4–6}.

Naturally, CRISPR diagnostics have a much easier path towards clinical deployment than applications for treating or preventing disease by editing somatic cells (or, when ethical, gametes or embryos); in diagnostic assays, the nucleases and RNA guides do not need to cross tissue and cellular barriers, and the consequences of off-target activity stay with the sample. In the ex vivo modification of cells for therapeutic purposes, viral delivery of the nucleases can be easily controlled and off-target activity measured. Somatic editing

in vivo demands much more controllability and higher levels of safety, and in this respect synthetic nanoparticles as delivery vehicles⁷ (pictured) are more apt than viruses. In fact, CRISPR faces challenges akin to those seen in protein-based therapeutics: mainly, targeted delivery, timed release, and immunogenicity. The usually low levels of gene-correction efficiency, the multicomponent nature of gene-editing therapies, and the non-monogenic and multifactorial causes of diseases with the highest burden to healthcare — cardiovascular and metabolic, and most cancers — all add to the challenges CRISPR faces if it is to have wider societal impact. Cells with genomes edited to produce a therapeutic under certain stimuli and protected within implanted materials or devices would bypass some of these problems⁸. Combining permanent edits with the transcriptional regulation of multiple genes will open up new applications in molecular and cellular bioengineering. Scientists in academia and in the burgeoning companies developing genome-editing technologies will find solutions that will largely be bespoke to certain types of application. The programmability of CRISPR is highly versatile; solutions to long-standing challenges in the delivery, safety and efficiency of therapeutics are not.

The recognition of the impact of CRISPR genome editing with a Nobel Prize was highly expected. Delightfully, the award honours two highly deserving female pioneers, and does so earlier than usual for Nobel prizes. What's next? Will the twenty-first century be marked by achievements in precision genome engineering? The answer may end up being succinctly outlined in an announcement of a Nobel Prize in Medicine. □

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