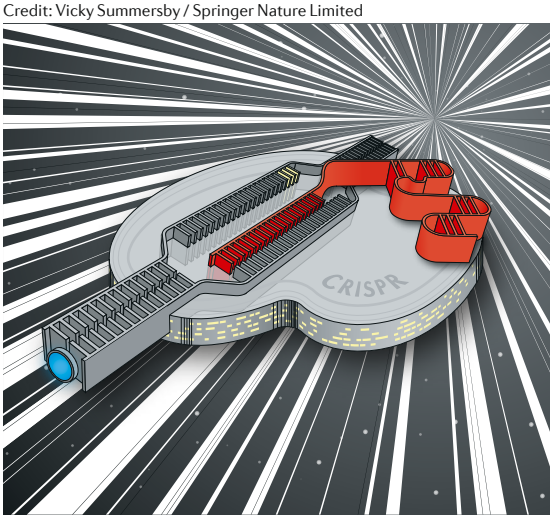
MILESTONE 12

Gene editing by CRISPR–Cas

Credit: Vicky Summersby / Springer Nature Limited



One of the most revolutionary developments in biology has its origins in the RNA-based defence system of bacteria, which encodes clustered regularly interspaced short palindromic repeats (CRISPR) along with CRISPR-associated (Cas) proteins. CRISPR–Cas has been adapted to function as a programmable genome-engineering tool that has enabled easy targeting and manipulation of precise genomic sequences in bacteria, plants, fungi and mammals, including humans.

Prior work with programmable genome-engineering tools focused on the use of various nucleases for gene targeting. Though precise, the reliance on protein-based recognition of a target DNA sequence meant that each new target required redesigning the tool, which is often a laborious task. CRISPR–Cas has the advantage of being precise and highly adaptable. This precision is derived from base-pairing between the complementary CRISPR–Cas guiding RNA and target DNA, which creates a straightforward and easy-to-adapt targeting system.

Foundational work was performed in 2012 by groups studying the function of CRISPR and the associated protein Cas9 in bacteria. It was known that these CRISPR–Cas systems function as a bacterial immune system against viruses. Jinek et al., and complementary work from Gasiunas et al., demonstrated that two small RNAs — the CRISPR RNA (crRNA) in a complex with a *trans*-activating CRISPR RNA (tracrRNA) — function together in targeting the nuclease Cas9 to specific DNA sequences, where it can generate a double-stranded DNA break (DSB). Jinek et al. had the insight that the two RNAs could be joined together to form a single guide RNA (sgRNA). The groups speculated that RNA could also be used to guide Cas9 to specific genomic sequences and thus enable gene editing and genome engineering.

The concept was demonstrated in a set of papers published in early 2013, when Jinek et al., Mali et al. and Cong et al. adapted the bacterial CRISPR–Cas system to function in mouse and human cells. Using sgRNAs and optimising the system for expression in a mammalian context, they demonstrated that Cas9 could be guided to specific target sequences in the larger and more complex mammalian genomes where, as in bacteria, it cleaved the DNA.

Mammalian cells attempt to rapidly repair these DSBs and this process can be co-opted for tailored genome engineering. The non-homologous end-joining DSB repair pathway attempts to stitch the DNA ends back together, often resulting in the introduction of deleterious mutations in genes. Furthermore, if a

MILESTONE 13

Chemical optimization improves oligonucleotide delivery

Forty years after their initial discovery, oligonucleotide therapeutics had begun to show potential to reach clinical maturity. By 2014, several drugs had been approved for disease treatment, and many others were undergoing clinical trials at various stages. However, being able to deliver effective doses of oligonucleotides to a specific set of diseased cells or organs was still challenging.

Small interfering RNAs (siRNAs) had been successfully used to inhibit the expression of disease-causing genes, and the implementation of antibody-mediated and lipid-nanoparticle-mediated delivery had significantly improved their efficiency. However, these treatments often required high doses and repeated intravenous injections to be therapeutically effective, which limited their clinical applicability in cases where intravenous drug administration was not feasible. Therefore, researchers worked to develop chemically modified oligonucleotides to enable efficient delivery to target cells by subcutaneous administration.

How such chemical optimisation could improve siRNA delivery was demonstrated in 2014 by Manoharan and colleagues, who covalently conjugated siRNA to *N*-acetylgalactosamine (GalNAc), a ligand of the asialoglycoprotein receptor (ASGPR). This receptor is expressed on the surface of liver cells and is responsible for the uptake of circulating glycoproteins with exposed GalNAc glycans. This design enabled targeted siRNA–GalNAc delivery to the liver owing to the high-affinity binding between receptor and ligand. The first tests were performed in cultured mouse liver cells and showed that receptor binding-affinity correlated with siRNA uptake efficiency. Encouraged by these results, the researchers tested the ability of siRNA–GalNAc conjugates to silence gene expression in vivo. They observed a robust and durable silencing of the targeted gene, transthyretin, in the liver of mice after a single or multiple low-volume subcutaneous administrations. The extent of silencing was higher following subcutaneous administration compared with intravenous administration.

In a study published a year later, the same research group optimised the design of the conjugates to improve therapeutic effectiveness. The researchers tested various attachment sites for the GalNAc ligand on the RNA molecule, and evaluated silencing activity both in vitro and in vivo. They found that placing three monovalent GalNAc units in close proximity to each other

Credit: Sebastian Kaulitzki / Alamy Stock Photo

> …CRISPR–Cas has opened up new avenues in our understanding of how cells repair DNA damage, in our ability to engineer cells and in the possibility of developing new, RNA-dependent therapies for previously intractable genetic diseases

DNA template is available the cell will attempt to copy genetic information from it during repair, thereby inserting new genetic information into the genome, as demonstrated by Mali et al. and Cong et al.

From its origins as a bacterial immune system, CRISPR–Cas has been developed into an all-purpose tool for tailored engineering of genomes in a range of species. In the span of less than a decade, CRISPR–Cas has opened up new avenues in our understanding of how cells repair DNA damage, in our ability to engineer cells and in the possibility of developing new, RNA-dependent therapies for previously intractable genetic diseases.

Ross Cloney,
Senior Editor, *Nature Communications*

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> This seminal work established siRNA–GalNAc as a promising therapeutic delivery approach to treat diseases involving liver-expressed genes

on the RNA sense strand resulted in a higher-affinity binding to ASGPR on liver cells, and a more robust silencing in vivo.

This seminal work established siRNA–GalNAc as a promising therapeutic delivery approach to treat diseases involving liver-expressed genes. Despite halting of the development of the first siRNA–GalNAc-based drug (Revusiran) during clinical trials in 2016, the impressive silencing efficiency, good safety profile and encouraging results from more recent clinical trials of drugs for acute hepatic porphyria (Givosiran) and cardiovascular disease with elevated LDL cholesterol (Inclisiran), established GalNAc conjugation as a promising solution for therapeutic siRNA delivery to the liver.

Alfredo Sansone,
Senior Editor, *Nature Communications*

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MILESTONE 14

An antisense oligonucleotide splicing modulator to treat spinal muscular atrophy

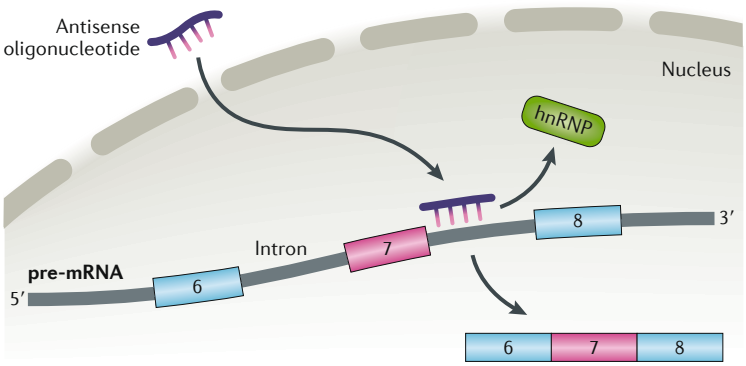

Figure adapted with permission from Rigo, F. et al. *J. Cell Biol.* **199**, 21–25 (2012)

On 23 December 2016, the United States Food and Drug Administration (FDA) approved the antisense oligonucleotide (ASO) drug nusinersen (Spinraza) to treat spinal muscular atrophy (SMA), a fatal genetic disease that can affect children and adults. The approval was the culmination of a successful collaboration between researchers in academia and industry, with support and assistance from patient advocacy groups and regulatory agencies.

SMA is a devastating neuromuscular disease that affects 1 in 10,000 people and is caused by mutations in the gene survival of motor neuron 1 (*SMN1*). Without functional SMN protein, the motor neurons in the spinal cord and brain stem degenerate, resulting in muscle weakness and atrophy. Of the infants born with SMA, 60% show symptoms before six months of age, with median life expectancy of less than two years. A paralog of *SMN1* in the human genome, *SMN2*, encodes an identical SMN protein. However, its pre-mRNA undergoes aberrant splicing, with 90% of mature *SMN2* transcripts lacking exon 7 and producing a truncated, unstable polypeptide. Some individuals with SMA carry multiple copies of *SMN2* and can thus produce higher levels of full-length SMN protein, which reduces the severity and delays the onset of the disease.

The molecular basis of *SMN2* exon 7 skipping was elucidated by several groups, including those of Ravendra Singh at University of Massachusetts Medical School and Adrian Krainer at the Cold Spring Harbor Laboratory, in the late 1990s to early 2000s. *SMN2* contains a synonymous C-to-T substitution in exon 7 that weakens the binding of splicing activators, thereby reducing the efficiency of the 3′ splice site. In 2003, Cartegni and Krainer engineered bifunctional ASOs that operate as synthetic splicing activators: a peptide mimicking a splicing activator was covalently linked to an ASO that hybridized to exon 7. This chimeric effector was able to promote exon 7 inclusion in cell extracts. Those findings prompted C. Frank Bennett, from Isis (later Ionis) Pharmaceuticals, to contact Krainer and initiate a collaboration, as recounted by Rigo et al. in 2012.

Over the next years, the strategy to control exon 7 inclusion was optimized for use in cells and