CRISPR (Clustered Regularly Interspaced Palindromic Repeat)/Cas9 System

A Revolutionary Disease-Modifying Technology

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As clinicians, we are equipped with an ever-expanding armamentarium of drug and device therapies that have extended the lifespan of countless patients with cardiovascular disease. Nevertheless, it is likely that future therapies aimed at the root cause of disease, rather than secondary effects, will improve clinical outcomes in our patients yet further. Imagine if we could restore expression of a critical protein that is reduced as a consequence of remodeling associated with heart failure? Alternatively, what if a mutated gene in one of your patients with familial hypertrophic cardiomyopathy could actually be corrected such that they were essentially cured of their disease? Although these scenarios would have been inconceivable just a few years ago, molecular genetic tools now exist in principle to modify genomes and essentially fix a variety of diseases that affect the heart and blood vessels. To achieve this seemingly impossible objective, several research groups have swiftly repurposed a bacterial system for adaptive immunity, called the CRISPR (Clustered Regularly Interspaced Palindromic Repeat)/Cas9 System, as a revolutionary genome editing tool in mammalian tissues.[1]

For a genome modification technology to be translatable to human disease, it must be able to navigate the complex milieu of the human genome and precisely cut the DNA only at the intended site. In other words, genome editing must be targeted and highly specific. Additionally, a donor template that harbors the corrected version of a particular gene is required to fix a mutated allele. In model organisms, such as mice, targeted recombination is performed routinely in embryonic stem cells to generate genetically modified animals, but this process is exceedingly inefficient. Therefore, alternative technologies for genome modification, such as zinc-finger nucleases and TAL effector nucleases have been developed to improve the efficiency of targeted cleavage. However, cumbersome design and optimization features have precluded their widespread adoption. Thus, the stage was set for the discovery of the CRISPR/Cas9 system.

After identification of the CRISPR/Cas9 system in bacteria, a series of elegant biochemical studies distilled the essential site-specific deoxyribonucleic acid cleavage activity to only 2 components (Figure): 1) an RNA guide sequence and 2) a DNA endonuclease. Based on these observations, yet another group of investigators re-engineered these components to function in mammalian cells, and it was discovered subsequently that the CRISPR/Cas9 system could be manipulated to generate either random mutations or targeted repair. Aside from cultured cells, the CRISPR/Cas9 system functions efficiently to modify the genome of fertilized mouse zygotes, and this approach has been adopted rapidly as an efficient method for creating genetically altered mice. Although these investigations established CRISPR/Cas9 as a cutting-edge genome-editing technology, it remained unclear whether this approach would be equally efficient for disease modification.

Duchenne Muscular Dystrophy is a fatal human disease caused by mutations in the dystrophin gene that result in severe muscle wasting and chronic heart failure. Several

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Key Words: adeno-associated virus ■ CRISPR ■ gene therapy ■ genome ■ muscular dystrophy, duchenne

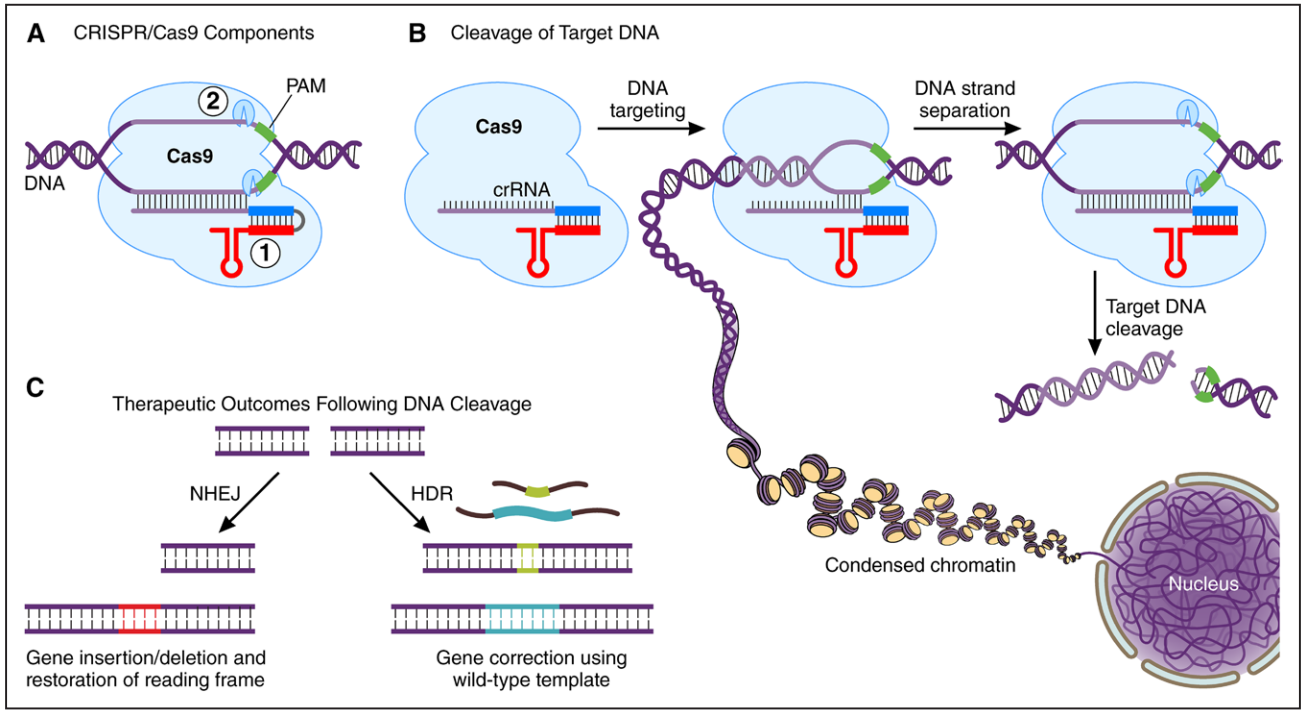

Figure. The nuts and bolts of the CRISPR (Clustered Regularly Interspaced Palindromic Repeat)/Cas9.
A, The CRISPR/Cas9 system can be distilled to 2 essential components: 1) an ribonucleic acid guide sequence to target host deoxyribonucleic acid (DNA) containing a protospacer adjacent motif (PAM) and 2) the Cas9 endonuclease enzyme. **B**, DNA cleavage involves target recognition amid the complex genomic milieu followed by DNA strand separation and precise enzymatic scission of the DNA backbone. C, After DNA cleavage, nonhomologous end joining (NHEJ) can potentially lead to restoration of an out-of-frame transcript without needing a DNA template. Alternatively, the homology-directed repair (HDR) pathway can use an error-free template to correct a gene mutation.

therapeutic approaches have been explored, including gene therapy to express wild-type dystrophin and exon-skipping to avoid premature transcript termination. However, neither strategy results in sustained correction of the mutant phenotype. To overcome this hurdle, the CRISPR/Cas9 system recently has been adapted for use in the mouse Mdx model of Duchenne Muscular Dystrophy.[2] In a seminal study, investigators at once introduced into Mdx zygotes a guide RNA targeting a dystrophin exon that contains a premature stop codon, the Cas9 endonuclease, and a repair template. Remarkably, they found that CRISPR/Cas9 could repair the Mdx gene in a significant proportion of skeletal myocytes and that genome-corrected, dystrophin-expressing cells had a clear survival advantage. Although this study represents an important demonstration of proof-of-concept, translation to patients ultimately will require genome correction in somatic cells rather than the germ line.

In the next phase of the CRISPR/Cas9 revolution, several groups recently attempted somatic genome correction in neonatal and adult Mdx mice.[3–5] Rather than utilize a homology repair template, however, these researchers took advantage of the fact that deletion of the mutant exon 23 of dystrophin leads to exon-skipping and restoration of the reading frame. As a result, a partial dystrophin transcript is produced, similar to what is observed in the clinically milder Becker's Muscular Dystrophy, and targeting efficiency is increased substantially as homologous recombination is not required. The challenge of somatic delivery, however, is the requirement for an efficient and specific delivery vehicle, such as a virus. Taking advantage of the skeletal and cardiac muscle tropism of adeno-associated virus, the investigators introduced a guide RNA and Cas9 into postnatal mice using multiple delivery routes. Collectively, these studies demonstrated that somatic genome correction of Mdx mice leads to partial restoration of dystrophin expression and meaningful improvement in muscle strength.

The rapid progress in genome modification technology that has been achieved in the past 4 years is truly remarkable. The CRISPR/Cas9 system has evolved quickly from the initial discovery of an adaptive immune system in bacteria to a 2-component genome editing tool to a global disease-modification strategy. Although beyond the scope of the current review, CRISPR/Cas9 technology also has revolutionized how gene perturbation experiments are conducted at a genome-wide scale and has enabled the unprecedented creation of genetically altered nonhuman primates for preclinical studies. Eventual therapeutic translation, however, will require identification of appropriate disease targets and development of robust methods for introducing CRISPR/Cas9 components into specific cell-types without off-cell-type

Circulation. 2016;134:777–779. DOI: 10.1161/CIRCULATIONAHA.116.024007

and off-target effects. Despite these immediate hurdles, CRISPR/Cas9 technology is poised to revolutionize cardiovascular disease management in ways that were once difficult to imagine.

DISCLOSURES

None.

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FOOTNOTES

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Circulation. 2016;134:777-779
doi: 10.1161/CIRCULATIONAHA.116.024007
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 0009-7322. Online ISSN: 1524-4539