Main Submission

An Overview of CRISPR – From Germs to Giants

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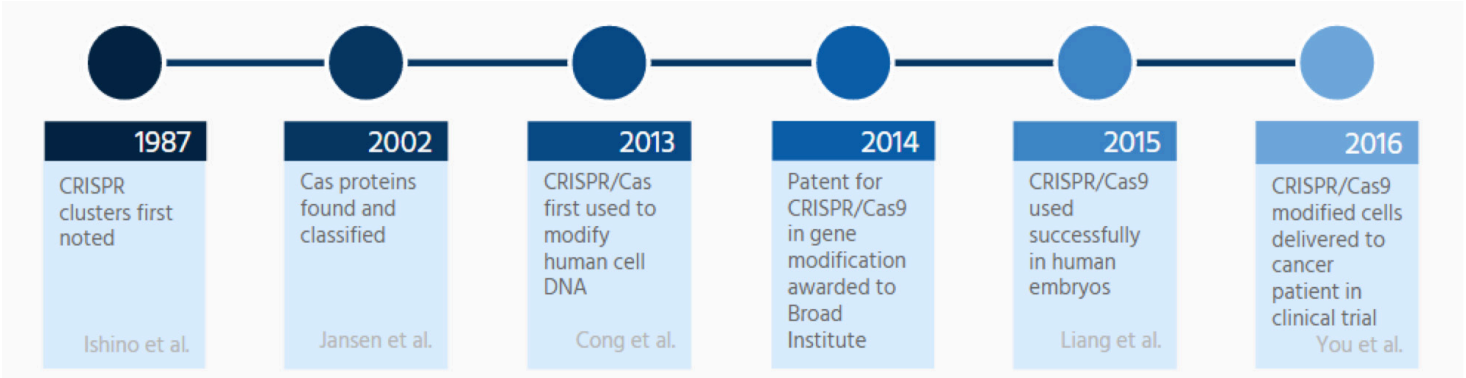
Introduction

In 1987, Yoshizumi Ishino and his colleagues noticed a set of regularly repeating genomic sequences within *E. coli* DNA (1,2). While repeats in DNA were common, these repeats were separated by different, irregular sequences. Other researchers began noticing the same oddity in all kinds of bacteria, and academic interest grew (1). The body of literature on CRISPR, or clustered regularly interspersed short palindromic repeats, grew over the course of the next 15 years (Figure 1), but its function was not fully understood (3). By 2002, proteins that regularly interacted with CRISPR DNA segments (Cas proteins) had been identified (1). In 2005, various research teams discovered that the gaps between the regular repeats matched up to extracellular sequences, suggesting that bacterial cells could record DNA from previous viral invaders (1). With the finding that archaea were protected from viruses whose genome matched with sequences between CRISPR segments, a picture of a primitive bacterial defense mechanism started to emerge (1).

CRISPR's gene editing capabilities in bacteria were soon discovered, and were applied to alter mammalian DNA (Figure 1) (4). CRISPR was able to overcome many problems with existing gene-altering methods. Meganucleases, for example, are very sequence-specific, but difficult to engineer correctly (5). Zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) are more straightforward to synthesize, but ZFNs lack accuracy, and the complex and time-consuming engineering process of TALENs discourages their use (2,5). The aim of this paper is to provide an overview of the mechanism of CRISPR and its current and potential applications, as well as explore some of the bioethical considerations of the technology.

Mechanism of CRISPR

The CRISPR/Cas system works in three stages (Figure 2A). First, segments of the invading viral DNA are integrated into the CRISPR array as spacer sequences, which act as genomic records of encountered infections (3). Next, these sequences are transcribed and processed into CRISPR RNA (crRNA), which guides Cas proteins to viral sequences complementary to the crRNA sequence (3). Lastly, crRNA forms a multiprotein complex that cleaves viral DNA, allowing for bacterial immunity against the virus (2,3). There are three types of CRISPR systems, but researchers have been particularly interested in the type II CRISPR/Cas9 system (2,3). CRISPR/Cas9 has been modified into the CRISPR technology known today. In prokaryotes, Cas9 is an



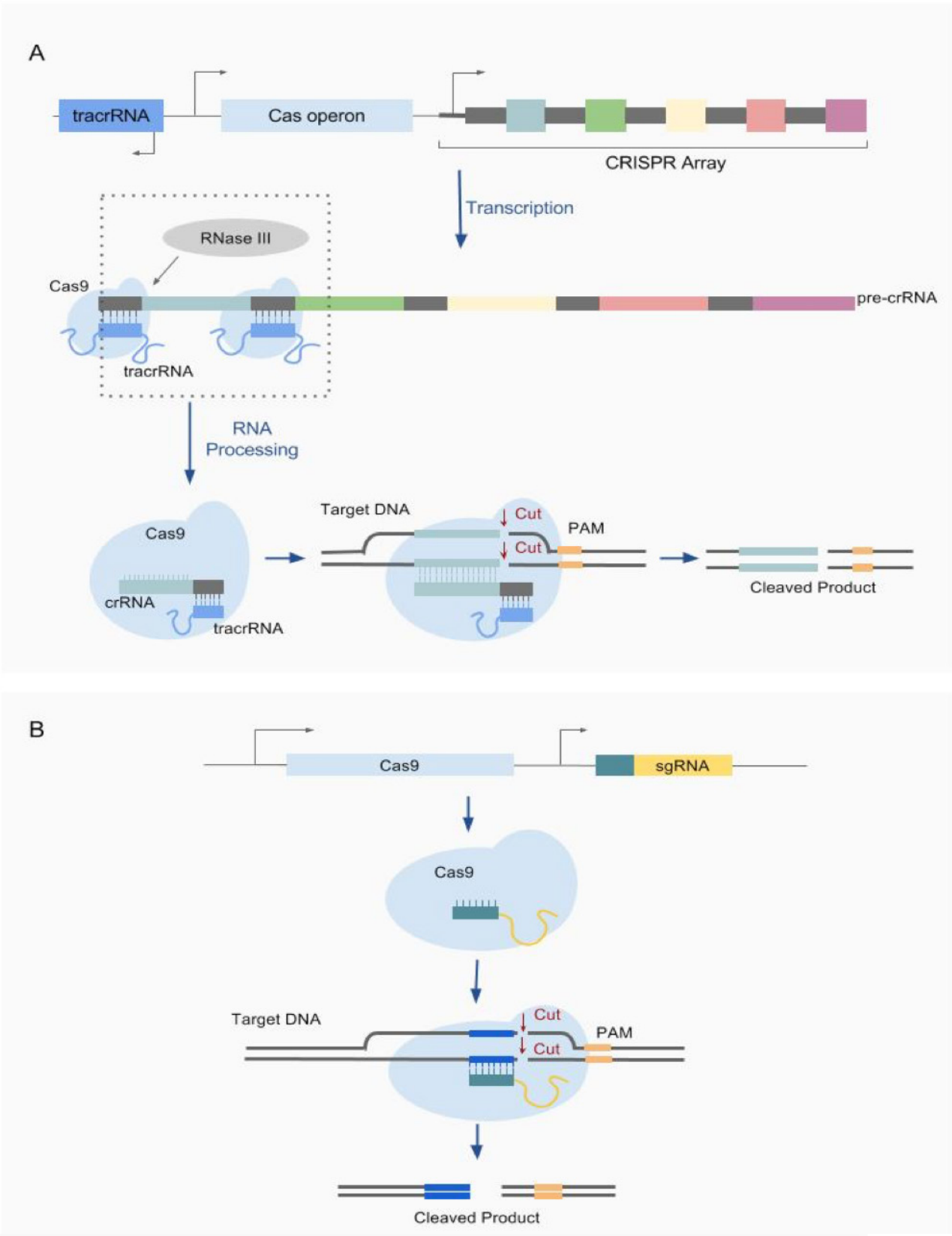


Figure 2: CRISPR/Cas mechanisms. (A) CRISPR/Cas mechanism as a bacterial "immune" response to viral DNA. Adapted from (3,2). (B) Genetically engineered CRISPR/Cas9 mechanism used as a genetic editing technique. Adapted from (2).

RNA-mediated DNA endonuclease that forms a complex with crRNA:trans-activating RNA (tracrRNA) to cleave and form double-stranded breaks based on the presence of protospacer adjacent motifs (PAMs) on the viral DNA (Figure 2A) (2). By genetically engineering the crRNA:tracrRNA duplex into a single guide RNA (sgRNA) and including PAM in target sequences, researchers can program the CRISPR/Cas9 system to cleave any desired sequence (Figure 2B) (2,3). Cleaved sequences can then be repaired by non-homologous end joining (NHEJ) or homology directed repair (HDR), resulting in gene knockouts or gene corrections (2).

Applications in CRISPR Technology

Gene therapy is closer to reality than ever before, primarily due to the specificity and simplicity of CRISPR. For genetic conditions affecting a single protein, like cystic fibrosis, treatments are already being developed, with promising results *in vitro* (6). Some see CRISPR functioning as a primary preventative measure in disease. For example, it could sterilize all mosquitoes that carry malaria, or alter chemokine receptors expressed on CD4+ T lymphocytes to prevent HIV from spreading (6). This technology is developing quickly; countries are increasingly approving CRISPR experimentation on human embryos, a short step away from clinical applications (6). It has been incorporated into gene therapy and germline editing for both genetic diseases and cancer. In 2015, Liang *et al.* were the first to use CRISPR to edit genes in human embryos, specifically to treat β-thalassemia, a hemoglobinopathy resulting from an inherited human β-globin gene mutation (7). Moreover, an ongoing clinical trial conducted by You *et al.* (2016) has used CRISPR-modified immune cells as a treatment for patients with aggressive lung cancer (8).

Bioethical Considerations of CRISPR

CRISPR technology has been popularized due to its low complexity and cost (9). It has potential as a treatment for various diseases, but this raises ethical and safety concerns. Using CRISPR in germline editing risks causing heritable and unpredictable genetic mutations with unknown side effects (9). Before including CRISPR as a therapeutic intervention, further development of the system is required, as well as a stronger understanding of its effects on human genetics. Furthermore, the possibility of germline editing for genetic enhancement of physical and intellectual traits leads us to question where we should stop manipulating the human genome (9).

CRISPR has also created conflict over patent ownership. Jennifer Doudna (UC Berkeley) and Feng Zhang (Broad Institute of Harvard and MIT) have been engaged in legal battles with each other since 2014 over the ownership of CRISPR genome editing (9). Despite Zhang winning the patent for use in eukaryotic cells in 2017, there are still ongoing European patent battles, and CRISPR continues to advance beyond what existing patents cover (10). CRISPR's patent owner could have a stake in all therapies that emerge from one of the most remarkable discoveries of the last 100 years (9). Placing financial ownership of a scientific revolution in the hands of one individual or one institution could lead to monopolization of all resulting CRISPR treatments for the coming decades.

Conclusion

Despite CRISPR's advantages over existing gene editing technologies, concerns about its use remain. While effective in bacteria and small mammals, CRISPR's accuracy in human gene targeting is not as well studied; targeting an incorrect gene could cause unpredictable mutations and side effects. Furthermore, as a technology that could one day allow for purely aesthetic genome modification, the scientific community must ask itself where the boundary lies in terms of what we can – and should – change. As with many advances in science, we must define our own limits. ■

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List of Abbreviations

Cas – CRISPR associated proteins
crRNA – CRISPR ribonucleic acid
CRISPR – clustered regularly interspersed short palindromic repeats
HDR – homology directed repair
HIV – human immunodeficiency virus
NHEJ – non-homologous end joining
PAM – protospacer adjacent motif
sgRNA – single guide ribonucleic acid
TALEN – transcription activator-like effector nucleases
tracrRNA – trans-activating ribonucleic acid
ZFN – zinc finger nuclease

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Michelle is a 5th year Bachelor of Science (honours) candidate at McMaster University, majoring in molecular biology and genetics. In 2016, she joined McMaster's Cardio-Respiratory Research Lab to complete her honours thesis and co-op work term. Her work involves investigating the immunobiology of allergic asthma, mainly focusing on B cell biology.

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