Molecular Therapy
Nucleic Acids
Commentary

Genome editing before CRISPR: The gnostic bible

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The emergence of programmable nucleases has provided significant and perhaps realistic hope for the development and transformation of gene editing technologies into clinical application. The activation of a cascade of cellular pathways that respond to double-stranded breaks and DNA damage, and the cell's capacity to repair that damage, are at the heart of genetic modification. Accolades surrounding the evolution and development of programmable nucleases as gene editing tools are well deserved. But where the experimental objective is to repair a mutated gene, many of the foundational mechanics were previously established by gene editing studies executed by a lone agent, a single-stranded oligonucleotide. These studies took place in the before CRISPR (BC) era of gene editing. Herein, I provide context of early gene editing fundamentals, knowledge that was memorialized in the gnostic bible(s) of the BC era.

A brief literature search conjures up a series of reviews centered on recent advancements in gene editing of mammalian cells.[1,2] Cleverly, certain authors define that early-stage period, preceding the democratization of CRISPR-Cas, as…"BC", or "before CRISPR".[3] Many gene editing originalists have watched with great satisfaction as these advances have propelled the field into the scientific mainstream and offered hope for the future. Widely cited summations and reviews focus on how DNA breakage, executed by primitive (Mega) nucleases and early-stage programmable nucleases, paved the way toward therapeutic application.[4] While there is little doubt that the initiation of and response to double-strand breaks and subsequent repair are critical players in the process of human gene editing, a section of the history of gene editing appears to have been minimized or simply left out.

Elucidation of mechanistic steps of DNA repair following a double-strand break is central to our foundational understanding of the evolution of precise and imprecise genome modification. CRISPR-Cas complexes, in fact all programmable nucleases, do one thing: they cleave the DNA efficiently, often in a "precise" fashion, although we are aware of error-prone activities. We also know that gene knockout, gene knockin, or gene correction or repair involves enzymatic activities that function in the DNA repair, recombination, and/or replication pathways. Sometimes working in unison, these pathways orchestrate critical cellular activities that are, no doubt, at the heart of genetic modification. But if the objective is to correct a mutation, so-called genetic spellchecking, then a donor DNA template must be added to the reaction.

Like other molecular mechanisms in mammalian cells, at least some of the foundational information about gene repair arose from studies in the baker's yeast, *Saccharomyces cerevisiae*. Sherman and colleagues carried out pioneering experiments in the 1980s introducing single-stranded DNA to disable or restore functionality in an auxotrophic mutant strain.[5] These experiments demonstrated the feasibility of gene repair using an exogenously added DNA template. Experimentation in human cells utilizing a variety of different forms and chemistries, including hybrid structures of single-stranded oligonucleotide, containing both DNA and RNA or tethered molecules with mixed results[6] followed shortly thereafter. One of those prominent modifications was the chimeric oligonucleotide, which consisted of an RNA strand specifically designed to bind to the genomic target site and a DNA strand, which would direct single-base repair. But the gene repair results were not robust, were marginal at best, and were largely inconsistent.

Taking a decidedly reductionist approach, workers focused on the single-stranded DNA component of the hybrid and demonstrated more consistent levels of directed gene repair. Hence, the field was re-directed back to the observations of Sherman and colleagues[5] described above. Reproducible and novel experimental systems began to appear as workers re-engineered the single-stranded oligonucleotide with base and linkage modifications. So while improvement in reproducibility and sustainability were achieved, the frequency of gene repair itself remained at levels that enabled mechanistic studies but clinical application remained elusive.

Ironicall, within the same time frame, details surrounding the mechanism, enzymology, and regulatory circuitry of how mammalian cells repair double-strand breaks emerged. It had become clear that DNA ends, single stranded or double stranded, ignite a DNA damage response pathway(s) that utilizes enzymatic activities supporting DNA repair, replication, and recombination, often acting in concert. In parallel to the activation of enzymatic pathways whose goal is to repair the break, the mammalian cell also senses the presence of broken DNA and slows down cell-cycle progression.

Taking that information into consideration, workers began to design experiments to purposely activate the DNA damage repair pathways in mammalian cells using chemo-toxic reagents that cause chromosomal breakage. Surprisingly, the activation of the damage response pathway led to significant increases in the levels of gene editing directed by single-stranded DNA oligonucleotides. Double-stranded DNA breaks were introduced simply by adding anticancer drugs such as camptothecin, VM26, or VP16 to cells in culture.[7] When double-stranded DNA breaks occur and the DNA damage pathways are activated, the cell cycle slows down, and

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replication forks stall. The stalled replication forks provide open access to single-stranded DNA molecules, and, if the target site of the oligonucleotides is exposed, the efficiency of gene repair is naturally enhanced.

As mentioned above, the introduction of double-strand DNA breaks often slows the progression of cells through S phase, early- through mid- to late-S phase, which can ultimately lead to stalled or collapsed replication forks. Chromatin disruption and an increase in target accessibility follows, creating a hospitable and fruitful environment for single-agent gene editing. Thus, workers recognized that by simply synchronizing the targeted cell population at the G1/S border and then releasing them for a slow burn through the S phase, gene editing/repair frequencies were elevated.[8]

If these revelations sound familiar, they ought to; similar molecular strategies form the mechanical and regulatory basis surrounding CRISPR-directed gene editing. In certain cases, the same enzymatic activities that were stimulated and/or regulated by crude and primitive chemicals and/or reagents appear to regulate gene repair activity directed by programmable nucleases.[9,10]

Despite the primitive nature of these early studies, a few bricks of the gene editing foundation were laid during these early years. And, as the field evolves, it's safe to say that accumulated wisdom from old BC and new after Doudna (AD) testaments will light the way toward successful therapeutic applications, perhaps of biblical proportions.

DECLARATION OF INTERESTS

This commentary was prepared by the author in his personal capacity. The views and opinions expressed in this commentary are solely those of the author and do not reflect the views, opinions, or position of his employer or any subsidiaries.

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