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Editors Letter

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Special Issue on the Chemical Biology of CRISPR

In the battle for survival and dominance, organisms often resort to heated molecular warfare, deploying molecules big and small, some of which have changed the world. For example, the molecular warfare between the fungus *Penicillium notatum* and bacteria yielded penicillin. The warfare between bacteria and bacteriophages continues to furnish transformative molecules, including restriction endonucleases and the subject of this special issue.

Phages attack and infect bacteria by hijacking bacterial proteins to express their genetic material, producing new virions for further infection. For their part, bacteria wield an innate and adaptive immune defense system to resist viral invasion. Bacteria evolve proteins that impede phage adhesion and/or uptake, in addition to expressing restriction endonucleases that can recognize and block replication of intruding DNA. But for every bacterial defense mechanism that emerges, the phage will evolve in turn to overcome and evade these barriers. The bacteria and the phage are locked in a molecular arms race, continuously coevolving to outsmart one another.

It is within this primordial conflict where the origins of CRISPR lie. CRISPR, an acronym for Clustered Regularly Interspaced Short Palindromic Repeats, is the adaptive immune response to phages in bacteria and archaea. Following viral infection, short segments of the foreign genome are incorporated into the CRISPR array as spacers and then transcribed by CRISPR-associated proteins (Cas) into CRISPR RNAs. The resulting ribonucleoprotein complex can then recognize and inhibit future invasion by phages bearing sequences complementary to the spacers. Remarkably, the CRISPR-Cas system boasts complex weaponry incorporating all three elements of the central dogma—DNA, RNA, and protein—in a sophisticated and fascinating defense mechanism.

Chemical biologists use chemical concepts, tools, and molecular approaches to understand or manipulate biological systems. Though a relatively recent discovery, the modularity and programmability of the CRISPR-Cas system makes it a powerful platform for chemical biology research. In this special issue of *ACS Chemical Biology*, our authors delineate the many ways in which chemical biology and genome engineering intersect. Neena K. Pyzocha (MIT) and Sidi Chen (Yale) give a comprehensive overview of the CRISPR-Cas system, focusing specifically on the type II systems most commonly employed for genome engineering, including CRISPR-Cas9 (DOI: 10.1021/acschembio.7b00800). Erik J. Sontheimer and colleagues at the University of Massachusetts Medical School, led by first author Aamir Mir, provide further in-depth review of the Cas9 effector proteins (DOI: 10.1021/acschembio.7b00855). Taken together, these reviews provide essential background for those who may be less familiar with the mechanisms of how CRISPR works. Each Article, Letter, Review, and Perspective in this collection focuses on the significant discoveries, the development of new tools, and the questions that remain surrounding this extraordinary molecular machine.

In this issue, chemical biologists present creative new ways to coopt the CRISPR-Cas platform for chemical biology approaches to cellular imaging, mechanism, and small molecule target discovery. Work by Marie K. Schwinn, Keith V. Wood, and colleagues at Promega and the University of Utah demonstrate how CRISPR can be used to integrate luminescent reporters directly into the genome of endogenous proteins, thereby enabling protein labeling while preserving native biological activities (DOI: 10.1021/acschembio.7b00549). Alexey N. Butkevich and co-workers, based at the Max Planck Institute and the University of Göttingen, also employed a CRISPR-Cas9 approach in the endogenous expression of SNAP- or Halo-tagged fusion proteins for live-cell, near-IR STED imaging (DOI: 10.1021/acschembio.7b00616). Jordan Meier's team at the National Institutes of Health, including first author Jonathan H. Shrimp, employ a dCas9 fusion protein as a reporter of lysine acetyltransferase activity. They use this system to successfully identify small molecule inhibitors of acetylation-dependent transcription (DOI: 10.1021/acschembio.7b00883). Marco Jost and Jonathan Weissman at UCSF review the role that CRISPR has played in chemical genetics, wherein gene editing is used to identify novel targets of small molecules and gain a better understanding of their mechanism of action (DOI: 10.1021/acschembio.7b00965).

Several of our authors demonstrate the role that chemistry can play in controlling CRISPR itself. Many elements of CRISPR-Cas gene engineering must be carefully regulated for successful therapeutic applications; fine-tuning of activity, delivery, timing, and localization of CRISPR are all critical for carrying out genome editing with surgical precision. Jinyue Pu, Kaitlin Kentala, and Bryan C. Dickinson at the University of Chicago employ RNA polymerase-based biosensors to modulate the production of guide RNAs, regulating Cas9 activity as a result (DOI: 10.1021/acschembio.7b00532). A team led by Douglas M. Fowler and Dustin J. Maly at the University of Washington report a chemically inducible Cas9 variant that can be activated and tightly regulated by small molecules, providing further insight into the kinetics of Cas9 genome editing (DOI: 10.1021/acschembio.7b00652). Scientists at Stanford University, led by Michael Z. Lin and Stanley Lei Qi, engineer a photoswitchable Cas9 architecture for optical control of transcription and gene editing (DOI: 10.1021/acschembio.7b00603). Ross C. Wilson (UC Berkeley) and Luke A. Gilbert (UCSF) review emerging methods in the delivery of gene editing components to cells and tissue, which is essential for translating CRISPR technology into therapeutic applications (DOI: 10.1021/acschembio.7b00680). Kazuo Takayama and Hiroyuki Mizuguchi (Osaka University) tackle the issues of both spatiotemporal control and delivery in their report of the Opt/Cas-Ad system, in which CRISPR-Cas9 gene editing is carried to its targets *via* an optogenetically regulated adenovirus vector (DOI: 10.1021/acschembio.7b01058). Validation of guide RNAs, which direct Cas9 to its target gene, is another crucial aspect of precision genome editing that can be brought under chemical control, as demonstrated by Tara R. deBoer, Niren Murthy, and their

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colleagues at UC Berkeley (DOI: 10.1021/acschembio.7b00899). A review from Digvijay Singh and Taekjip Ha (Johns Hopkins University) demonstrates how single molecule techniques such as fluorescence particle tracking, FRET, and other chemical biology approaches can be harnessed to interrogate CRISPR-Cas systems for optimized gene editing (DOI: 10.1021/acschembio.7b00905).

As new tools and approaches for harnessing the CRISPR-Cas system emerge, programmable genome engineering technologies continue to advance at a rapid pace. Alexis C. Komor (UCSD), Ahmed H. Badran (Broad Institute), and David R. Liu (Broad Institute and Harvard) coauthor a review of recent advances in base editing, a new gene editing strategy in which a Cas9 protein is engineered to convert one single base pair into another, circumventing the introduction of double-strand breaks (DOI: 10.1021/acschembio.7b00710). Katherine S. Pawelczak and colleagues at NERx Biosciences and Indiana University also address the challenges associated with double-strand breaks in gene editing, reviewing advances in modulating the DNA repair pathways that have been critical for mediating genome engineering: homology directed repair and nonhomologous end joining (DOI: 10.1021/acschembio.7b00777). Danielle N. Gallagher and James E. Haber (Brandeis University) review the fundamentals of how DNA repair pathways have traditionally been used in gene editing, signifying that we still have much to learn about these earlier models (DOI: 10.1021/acschembio.7b00760).

Additional applications of programmable gene editing lie in advancing multiplexed engineering and high-throughput screening. Martin Kampmann (UCSF) reviews the roles of CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) in next-generation genetic screens for precision medicine applications (DOI: 10.1021/acschembio.7b00657). Jason C. Klein, Jay Shendure, and their colleagues at the University of Washington offer a Perspective on the potential for CRISPR screens to identify enhancers, or noncoding regions of DNA that distally control gene expression, thereby advancing our understanding of how gene expression is regulated (DOI: 10.1021/acschembio.7b00778). George Church's team at Harvard Medical School, led by first author David Thompson, gives an overview of multiplexed genome engineering, or the introduction of multiple edits simultaneously, as well as its challenges, applications, and future directions in eukaryotes (DOI: 10.1021/acschembio.7b00842).

In addition to new tools and applications associated with CRISPR-Cas, there is still much to learn about the system's mechanism and biological pathway. Eugene V. Koonin and Kira Makarova at the National Center for Biotechnology Information characterize a key pathway in the type III CRISPR-Cas system that raises new questions about the mechanism (DOI: 10.1021/acschembio.7b00713). Brian Bothner, Blake Wiedenheft, and their colleagues at Montana State University and Utah State University elucidate the conformational dynamics of the CRISPR-associated complex for antiviral defense (Cascade) to gain a better understanding of how this complex associates with DNA and recruits the Cas3 protein (DOI: 10.1021/acschembio.7b00649). In Shengdar Q. Tsai's (St. Jude Children's Research Hospital) Perspective, he addresses the global nature of genome editing, in which billions of cells can potentially be modified by a single treatment, and its implications for precision targeting of genome engineering therapeutics (DOI: 10.1021/acschembio.7b00847). And a review from Joseph Bondy-Denomy (UCSF) reminds us of CRISPR's archaeal origins as an immune response to phage attacks, detailing the protein inhibitors of CRISPR that bacteriophage invaders use to fight back (DOI: 10.1021/acschembio.7b00831).

This issue encompasses many of the exciting and promising directions that CRISPR technology can take us, but there are risks involved as well. Renee D. Wegrzyn (Defense Advanced Research Projects Agency) and co-workers provide an in-depth analysis of best practices for genome editing to maximize biosafety, with a focus on the role that small molecules can play in modulating gene editing activity and reducing off-target effects (DOI: 10.1021/acschembio.7b00689). Austin Burt and Andrea Crisanti (Imperial College) introduce us to the concept of gene drive, wherein genes, aided by genome editing technology, are rapidly inherited through generations of a species regardless of whether they enhance overall fitness or survival (DOI: 10.1021/acschembio.7b01031). John M. Marshall (UC Berkeley) and Omar S. Akbari (UCSD) offer additional perspectives on gene drive: its potential to eradicate disease-carrying species, unintended consequences for ecosystems, and whether the technology can be truly controlled (DOI: 10.1021/acschembio.7b00923).

In this issue and beyond, there are a number of outstanding researchers working tirelessly to advance, harness, and understand CRISPR gene editing technology. We asked many of them for their insights into some of the most pressing questions regarding CRISPR and its future, covered in our In Focus piece (DOI: 10.1021/acschembio.8b00135). Laura Kiessling, Editor-in-Chief, interviewed Jennifer Doudna (former Associate Editor) about her own experience in pioneering the CRISPR revolution (DOI: 10.1021/acschembio.8b00108). Additionally, we highlight our early career scientists at the heart of these discoveries, generally the students and postdoctoral fellows at the bench, in our Introducing Our Authors article (DOI: 10.1021/acschembio.8b00136).

The genetic engineering capability of CRISPR-Cas represents one of the most extraordinary discoveries. This special issue spans a diverse array of the molecular curiosities that comprise this system: from the biological mechanisms of the enzymes and nucleic acids that drive this bacterial defense method, to the role of CRISPR in shaping how we modulate the activity of small molecules in biological systems, to the chemical challenges of delivery, control, and therapeutic applications of CRISPR, it is clear that chemists and chemical biologists have an exciting role to play in the future of the CRISPR revolution.

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