# The Biology and Application Areas of CRISPR Technologies

Most prokaryotes use Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) and CRISPR-associated (Cas) proteins as an adaptive immune systems to cleavage the invading foreign genetic elements. Understanding how CRISPR/Cas system works resulted in one of the most powerful biotechnological advances. Due to their efficacy, efficiency, and ease of implementation, CRISPR-based tools have changed the rules of molecular efforts to target and manipulate genetic information in a wide range of organisms. The ever-expanding CRISPR toolbox now enables researchers to precisely target DNA, edit genetic information, control gene expression, visualize chromatin, and perform high-throughput loss and gain of function screenings. In this CRISPR special issue, we present six review articles and two primary research articles. They cover multiple important topics ranging from the molecular organization of native CRISPR systems, their identifications, and repurposing as technological tools for DNA and RNA targeting.

There is a remarkable complexity and diversity of the CRISPR–Cas systems in nature. Faure *et al*. [1] discusses the functional links between CRISPR–Cas systems, their effect on horizontal gene transfer, DNA repair, and programmed cell death in unicellular organisms. Furthermore, the article discusses the signal transduction mechanisms and potential role of CRISPR–Cas systems in transposon integration and plasmid maintenance. The review article demonstrates the interplay between the primary function of CRISPR–Cas as an adaptive immunity mechanism and these other roles, which defines the richness of the biological effects of CRISPR systems and how they spread among bacteria and archaea during evolutionary process [1].

In a complementary review article, Hidalgo-Cantabrana *et al*. [2] summarize computational and experimental efforts to identify and repurpose a CRISPR/Cas system as a biotechnology tool. They describe the process of how an endogenous CRISPR–Cas system can be repurposed for any application. Hidalgo-Cantabrana *et al*. propose several key steps in this process. The article describes how to (i) identify CRISPR–Cas systems with currently available bioinformatics tools, (ii) characterize their functionality based on transcriptomic data and interference assays, and (iii) repurpose endogenous and develop heterologous systems for a wide range of applications in bacteria [2].

CRISPR/Cas system is providing unprecedented versatility to target DNA. Beyond the gene editing applications of wild type CRISPR–Cas nucleases, the catalytically inactive Cas molecules (dead Cas9 and dead Cas12a) offer a novel platform to target and manipulate genome function. Xu and Qi [3] discuss the invention and a wide range of application areas of dead Cas9-based tools for precise control of genome function without gene editing. Over the last several years, a diverse set of dCas tools have been developed, which constitute a comprehensive toolbox that allows for interrogation of gene function and modulation of the cellular behaviors. In their review article, Xu and Qi summarize current applications of the dCas tools for transcription regulation, epigenetic engineering, genome imaging, genetic screens, and chromatin immunoprecipitation. Furthermore, they discuss the key advantages and existing challenges of these comprehensive dCas tools and provide perspectives on future directions and applications [3].

One of the powerful application areas of CRISPR tools is high-throughput and genome scale screenings. Utilizing a library of single-guide RNA empowers researchers to rapidly investigate the functional consequences of perturbations at thousands of genomic, transcriptomic, and epigenomic loci. Ford *et al*. [4] discuss key technical and analytical steps in the design and application of CRISPR-based screenings. They highlight recent advances in CRISPR–Cas genomic screening while outlining protocols and pitfalls associated with screen implementation. Finally, they describe current challenges that limit the utility of CRISPR–Cas screening as well as future research needed to resolve these limitations [4].

Naturally accruing CRISPR systems provide immunity against invading not only DNA molecules but also RNA molecules. In a comprehensive review article, O'Connell [5] focuses on the type VI CRISPR–Cas systems, which contain a single Cas13 (formerly C2c2) protein that can be guided to target RNA molecules instead of DNA. This review compares and contrasts what is known about the molecular architecture and behavior of type VI (A–D) CRISPR–Cas13 interference complexes, how this allows bacteria to carry out RNA-targeting function, how type VI accessory proteins are able to modulate Cas13 activity, and how together all of these features have led to the rapid development of a range of RNA-targeting

tools. Critically, the review also discusses some of the outstanding questions regarding Cas13's molecular behavior, and its role in bacterial adaptive immunity and RNA-targeting applications.

With the emergence of a versatile CRISPR technology, targeting genome in a wide variety of organisms is a trivial process. CRISPR is no longer just a research tool. As the application areas of CRISPR technologies exceed beyond research and biomedical therapies, new and existing ethical concerns are arising. To this end, Brokowski and Adli [6] review fundamental ethical issues revolving around CRIPSR tools and technologies. They discuss the ethical and moral limits of CRISPR applications, access to CRISPR technologies and whether a regulatory framework(s) should be enforced to the research involving human subjects and other species at large.

In addition to these comprehensive review articles, we also include two research articles that focused on genome and epigenome editing activities of CRISPR/Cas9 system. Jayavaradhan *et al*. [7] present an interesting method to rapidly and precisely assess three different types of DNA double-strand breaks and subsequent repair outcomes in hematopoietic cells. This platform, which can be potentially adapted for other site-specific nucleases, allows for rapid enhancement of gene-editing efficiencies and manipulation of the DNA repair pathways [7]. On the other hand, Kuscu *et al*. [8] use a novel dCas9-based epigenome editing approach to temporally control the deposition of epigenetic marks and study the persistence of induced gene expression. To this end, they integrated the auxin-inducible degron technology with CRISPR tools, which enables rapid depletion of the dCas9-fused epigenome modifier complex from the target site. The temporal control over the dCas9 complex allows for investigating the persistence of a locally deposited epigenetic mark and its functional consequences.

This collection of review and primary research articles captures many aspects of CRISPR systems and the CRISPR-derived genome-targeting tools. In the near future, we will likely observe further improvement in CRISPR technologies and witness many novel CRISPR tools and application areas. Attaining maximum benefit and improving CRISPR technologies will continue to require deeper understanding of the native CRISPR systems and their natural evolution. This knowledge will empower us to take the maximal advantage from and develop additional CRISPR-based technologies for the betterment of human health and progress.

## References

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