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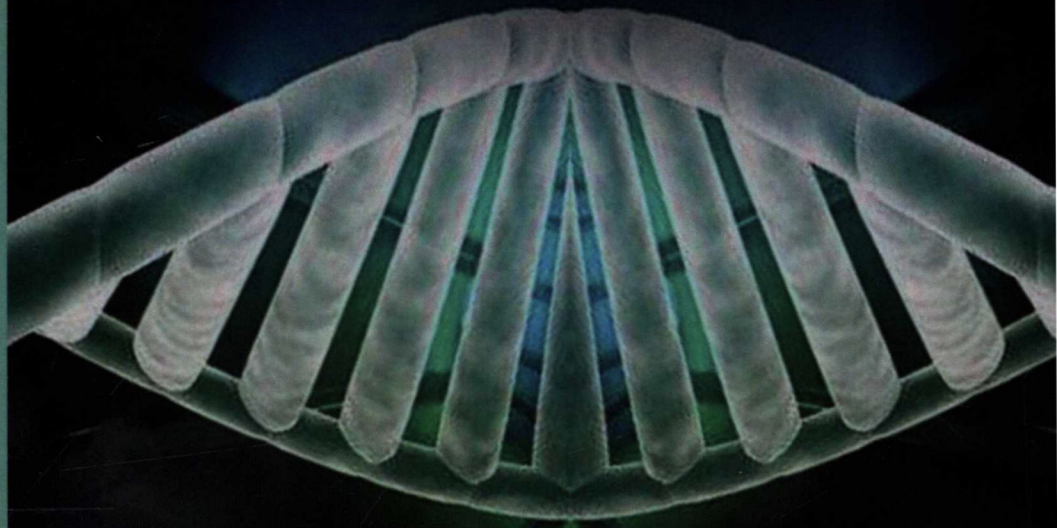

CRISPR

Advances in Research and Applications

Biochemistry Research Trends

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Genome editing using CRISPR has been identified as one breakthrough technology in recent research. Today, it is difficult to open a journal or a newspaper without reading about the application of CRISPR gene editing technology to basic research, public health, therapeutics or diagnostics. Although some problems of CRISPR remain to be solved, such as bio-safety and ethical issues, it may change people's futures. This book is to meet the needs of basic molecular biochemists, pharmacologists, medical students, clinical practitioners and scientists, as well as a broad spectrum of readers who wish to understand the advances in research and applications of CRISPR. The contributing authors are basic scientists as well as students who major in biochemistry and pharmacology. The book presents the current research in the CRISPR model, focusing on its advances and applications. Topics discussed in this compilation include: Targeting of hepatic diseases using CRISPR; applications and advances of CRISPR in animal models; gene targeting on the Cyp2c Locus in rats via CRISPR; applications of CRISPR for therapy in human genetic diseases; utilization of CRISPR in gene function and drug target validation; applications of CRISPR in plant genome editing; and genome editing on human embryos using CRISPR.

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Xin Wang, Ph.D. (Editor)

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BIOCHEMISTRY RESEARCH TRENDS

CRISPR

ADVANCES IN RESEARCH AND APPLICATIONS

XIN WANG
EDITOR

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CRISPR

ADVANCES IN RESEARCH AND APPLICATIONS

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This book is dedicated to my wife, Jinyu, and son, Ruiyang

PREFACE

CRISPR, an abbreviation of clustered regularly interspaced short palindromic repeats, is a family of DNA sequences in bacteria that contains snippets of DNA from viruses. CRISPR plays a key role in a bacterial defence system and form the rapidly developing genome editing technology. Today, CRISPR has been successfully applied in many organisms, including diseases models, animals and crop plants. Compared with previous techniques such as ZENs and TALENs, the CRISPR shows distinct advantages in editing multiple genes simultaneously in the same cell. Therefore, currently genome editing experiments have been widely performed by using CRISPR technology. Advances in research and applications of CRISPR include disease research, gene therapy, drug target identification and validation, stem cell research, research using model organisms, development of CRISPR-based therapeutics, and so on. Genome editing using CRISPR has been identified as one breakthrough technology in the biomedical research.

This book is to meet the needs from basic molecular biochemists, pharmacologists, medical students, clinical practitioners and scientists, as well as broad readers who wish to understand the advances in research and applications of CRISPR. The contributing authors are basic scientists, major in biochemistry and pharmacology. The book presents the current research in the CRISPR, focusing on its advances and applications. Topics

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Chapter 1

ADVANCES IN RESEARCH AND APPLICATIONS OF CRISPR

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ABSTRACT

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), one of rapidly developing genome editing technologies, has been successfully applied in many organisms, including diseases models, animals and crop plants. Compared with previous techniques such as ZENs and TALENs, the CRISPR shows distinct advantages in editing multiple genes simultaneously in the same cell. Therefore, currently genome editing experiments have been widely performed by using CRISPR technology. Advances in research and applications of CRISPR include disease research, gene therapy, drug target identification and

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validation, stem cell research, research using model organisms, development of CRISPR-based therapeutics, and so on.

Keywords: CRISPR, genome editing, disease research, gene therapy, stem cell research, model orgainsms

INTRODUCTION

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a clustering regular interval short palindromic repetition sequence, which is part of the bacterial innate immune system, including multiple short repetitive sequences [1-3]. And Cas is called CRISPR-associated system. The CRISPR/Cas system participates in the innate immune activity of most bacteria and all archaeological bacteria, cleaving and degrading foreign nucleic acid molecules by small fragments of RNA to eliminate foreign plasmids or phage [3]. Due to its interference characteristics to deoxynucleotides, CRISPR/Cas is widely used as a gene editing tool [4-7].

CRISPR as a revolutionary gene editing technology becomes a new favorite in science. Up to now scientists have found three CRISPR/Cas systems exist, in which Type II system is mainly distributed in bacteria. The system components are simple, and the core protein is Cas9 protein. Cas9 protein and mature crRNA (CRISPR derived RNA) combined to form a complex, which has the ability to identify and cleave exogenous nucleic acid molecules, and is also the most commonly used method in cell and animal gene knockout studies [8]. After the cleavage of Cas9 protein, the target gene will form double-strand breaks (DBS) of DNA molecules. Once the DBS is formed, the eukaryotic cells will start the repair procedure. The repair program mainly includes the error-prone non-homologous end joining (NHEJ) or high-fidelity homologous recombination. When DBS is repaired by NHEJ, the cleavage site may be deletions, substitutions, or insertions [8, 9].

In recent years, CRISPR-based gene editing technology has created a lot of miracles and great achievements. Moreover, gene editing becomes

simpler and more efficient. Thus CRISPR is advancing the research and may rewrite the future of mankind. For example, researchers from around the world have published many scientific papers detailing their findings, using CRISPR to cut and replace unwanted DNA to develop therapies for the treatment of diseases such as cancer, HIV, blindness, chronic pain, muscular dystrophy and Huntington's diseases. Thus, with the support of the CRISPR, the pace of basic research has shown a spurt of development. Although CRISPR-based treatment programs will take years to test effects on the human body, almost every day, numerous publications describe the use of this new tool for new discoveries in human health and human genetics. Of course, humans are not the only species that owns the genome. CRISPR is also used in animals and plants, from helping to suppress malaria, Lyme disease parasites, to improve crop yields such as potatoes [10, 11], citrus [12, 13] and tomatoes [14-16].

CRISPR, the top ten scientific breakthroughs in 2015, has twice entered the "Top 10" Score in *Science* in 2012 and 2013. There are two high-profile achievements in 2015: one is the success of long-term dream of "gene-driven" to eliminate pests or carry the disease dream; Second, the first human embryo DNA editor to occupy the global public opinion headlines. Each of these two studies led to the development of scientific policy, especially the latter that led to the convening of the Washington International Human Gene Editorial Summit in December of 2015. What makes CRISPR look different? Compared with ZFN (zinc finger nuclease) and TALEN (transcriptional activator-like effector nuclease), which can also accurately change the DNA sequence, CRISPR technology is relatively simple, and the cost is much cheaper [9, 17]. Thus a number of companies want to use CRISPR to develop new clinical therapy. Today, applications of CRISPR gene editing include gene therapy such as cancer, genetic diseases and infectious diseases; drug target screening; and animal as well as plant genetics and breeding.

GENE THERAPY

Gene therapy is the use of gene editing technology at the genome level to achieve precise molecular modification, so as to achieve the purpose of treatment of various diseases [18]. In particular, the study of gene therapy has increased rapidly in recent years, especially after the advent of CRISPR in 2013 [19-21]. These diseases include cancer (such as leukemia, lung cancer, etc.), rare genetic diseases (hemophilia, Duchenne muscular dystrophy, thalassemia, etc.) and infectious diseases (AIDS, hepatitis B) and so on. Based on the number of 2016 gene editing drugs in the number of diseases, the cancer is second to none, followed by rare genetic disease, infectious diseases and blood diseases [20, 21].

Cancer Treatment

This new application demonstrates that CRISPR is able to play an efficient and accurate role in cancer therapy, which undoubtedly opens up new avenues for the precise treatment of cancer [22, 23]. At the same time, compared with the traditional cancer treatment, the CRISPR method has better adaptability. In particular, conventional chemotherapy not only has strong side effects, but also leads to new mutations in cancer cells, resulting in cancer drug resistance. In CRISPR way, the researchers can simultaneously target new mutations and continue to treat cancer until the tumor cells are completely eliminated and no recurrence [22, 24].

In June 2016, the NIH approved the first clinical trial of Juno's first CRISPR in the human body. First, the T cells from the immune system are extracted from cancer patients. Second, the T cells are genetically modified using CRISPR. Third, genetically modified T cells are returned to the patient so that they target the destruction of tumor cells [25, 26]. In fact, Chinese scientists carried out the first CRISPR application of human clinical trials in the world. In August 2016, China began the first use of CRISPR in clinical trials to treat the patients of chemotherapy, radiotherapy and other therapies ineffective metastatic non-small cell lung

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