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Review paper

Molecular basis, applications and challenges of CRISPR/Cas9: a continuously evolving tool for genome editing

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Abstract

The clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) system is a recently discovered tool for genome editing that has quickly revolutionized the ability to generate site-specific mutations in a wide range of animal models, including nonhuman primates. Indeed, a significant number of scientific reports describing single or multiplex guide RNA microinjection, double-nicking strategies, site-specific knock-in and conditional knock-out have been published in less than three years. However, despite the great potential of this new technology, there are some limitations because of the presence of off-target genomic sites, which must be taken into consideration. To address this issue, various research teams have tried to improve the efficiency of the system through enzymatic modifications of the Cas9 protein or by the introduction of alternative strategies. Although several review articles are available that singly describe the molecular mechanism(s), applications and challenges of each of these strategies, a concise compilation of approaches is lacking. In the current review, we describe and evaluate most CRISPR/Cas9 approaches available at present, describing both mechanism of action, in addition to advantages or disadvantages. The primary goal of this work is to serve as a guide for not skilled researchers, facilitating the selection of the best strategy to target their gene of interest and allowing optimization of particular applications to the specific aims of the study. The present article also offers a unique perspective, focusing on the fact that CRISPR technology is opening a new genomic era, providing the means to manipulate specific genes in a targeted manner in all animal models, an endeavor previously considered to be difficult.

Key words: genome editing; CRISPR/Cas9; guide RNA (gRNA); knock-in; Conditional knock-out

Introduction

Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 is a new system of genome engineering that has transformed our ability to manipulate genes in many different animal models [1]. This technology uses the properties of an ancient adaptive immune system present in archaea and eubacteria [2], used as a defense mechanism to protect the host cell against the presence of exogenous nucleic acids, such as viral DNA [3–5]. Even if different types of CRISPR are present in nature, one of the best characterized is the type II from *Streptococcus pyogenes*, which acts via the introduction of double-strand breaks (DSBs) in the target genomic DNA [5]. At present, the workings of this system can be reproduced *in vitro* through the synthesis of a guide RNA (gRNA) plus the messenger RNA (mRNA) encoding the Cas9 protein, which are all co-transfected in cells [6, 7] or co-injected in the one-cell-stage embryos [8–13].

Owing to the simplicity of the CRISPR/Cas9 system, it is becoming widely used and accessible also to nonexperts in molecular biology; however, despite the significant potential and

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