Chapter 7
CRISPR-Cas9 for Studying Genetic Effects of Radiation and Heavy Metals

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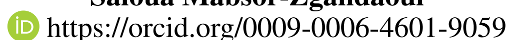
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ABSTRACT

CRISPR/Cas9 technology is a cutting-edge tool for studying the genetic effects induced by exposure to environmental stressors such as ionizing radiation and heavy metals. These agents are known to cause major molecular perturbations, including DNA damage, increased oxidative stress, enzymatic alterations and inflammatory responses in the nervous system. In this context, CRISPR/Cas9 makes it possible to identify and precisely target the genes involved in the mechanisms of response or vulnerability to these exposures. Thanks to this technology, it is possible to create cellular or animal models that faithfully reproduce the alterations observed in ex-

DOI: 10.4018/979-8-3373-4857-5.ch007

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posed organisms, and to explore strategies for correcting or inactivating genes to mitigate toxic effects. The use of CRISPR/Cas9 in this field opens up new prospects for gaining a better understanding of the interactions between the environment and the genome, and ultimately for developing targeted therapeutic approaches to pathologies linked to pollution or radiological exposure.

1. INTRODUCTION

The invention and development of the CRISPR/Cas9 system represents one of the most revolutionary advances in molecular biology, redefining scientists' ability to interrogate, manipulate and rewrite the genome with unprecedented precision. Originally discovered as enigmatic repetitive sequences in prokaryotic genomes by Ishino et al. in 1987, these loci initially had an unknown function and attracted little attention outside the field of microbial genomics. In the following years, however, comparative analyses demonstrated that these clusters consist of short, regularly spaced repeats interspersed with unique spacer sequences derived from exogenous nucleic acids, ultimately leading to the recognition of their role as an adaptive immune system in bacteria and archaea. The conceptual leap occurred in 2002, when Jansen et al. coined the term CRISPR and recognized a conserved cluster of adjacent genes, which they called CAS (CRISPR-associated genes), thus providing a basis for CRISPR-Cas systems as modular defense mechanisms (Ishino et al. 1987).

From an evolutionary and mechanistic standpoint, CRISPR/Cas9, and more specifically the subtype II-A system mediated by Streptococcus pyogenes Cas9, represents a family of programmable nucleases directed by RNAs that target foreign genetic elements for cleavage. This system is defined by three operational phases: adaptation, expression and maturation, and interference. Biochemical and structural studies of the Cas9 endonuclease have revealed its guidance by a dual RNA, a crRNA–tracrRNA, that recognises complementary DNA adjacent to a PAM motif (the protospacer adjacent motif), which triggers double-strand cleavage via its HNH and RuvC dual nuclease domains (Jiang & Doudna, 2017).

Now, its edited form, consisting of a single guide RNA fused to Cas9, has become a universal tool for inducing knockout, knock-in, transcription modulation, and epigenetic editing of target genes. Recent updates to the classification of CRISPR/Cas systems have highlighted their astonishing evolutionary diversification. Makarova et al. (2025) have expanded the classification to two classes, seven types, and forty-six subtypes that distinguish systems with a multi-protein effector complex (class I) from those that mobilize a single-protein effector, such as Cas9, Cas12, or Cas13 (class II). Although class II systems represent a minority of CRISPR loci known in

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