Dead Cas Systems

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The gene editing tool CRISPR-Cas has become the foundation for developing numerous molecular systems used in research and, increasingly, in medical practice. In particular, Cas proteins devoid of nucleolytic activity (dead Cas proteins; dCas) can be used to deliver functional cargo to programmed sites in the genome. In this review, we describe current CRISPR systems used for developing different dCas-based molecular approaches and summarize their most significant applications

Cas9 dCas transcription epigenetics chromatin cancer hereditary diseases inflammatory diseases infectious diseases editing

1. Introduction

In recent years, precise genomic and epigenomic editing has transformed into a fast-growing area of research, with lucrative applications in medicine and biotechnology. Targeted modifications of genomes in various organisms, from bacteria to plants to mammals, can be applied to treating human diseases and to developing bacterial strains and genetically engineered organisms with desired properties. Discovery of site-specific CRISPR-Cas nucleases and adapting these bacterial tools for gene editing applications have revolutionized genetic engineering and molecular biology. Robust activity, easy design, and capacity to target virtually any DNA or RNA site has put CRISPR-Cas at the forefront of gene editing techniques, with yet-undiscovered potential applications of optimized CRISPR-Cas components and novel CRISPR-Cas systems. In simplest terms, CRISPR-Cas systems are based on nucleolytic activity of Cas9 protein guided by a chimeric RNA molecule (guide RNA; gRNA) to the desired site in the genome. An important property of CRISPR-Cas is the high specificity defined by gRNA sequences that recognize the nucleic acid target and the protospacer adjacent motif (PAM) sequence, adjacent to the target, and required for CRISPR-Cas activity.

2. Dead Cas Systems: Types, Principles, and Applications

Type II CRISPR-Cas systems are most commonly used, while CRISPR-Cas systems of other types (e.g., types V and VI) have also been leveraged for genomic and epigenomic editing. The Cas9 protein of the type II CRISPR-Cas system harbors two nucleolytic domains (RuvC and HNH) that cleave target DNA strands and generate double-stranded breaks (DSB) [1]. Introducing point mutations into each domain (D10A and H840A, correspondingly) blocks nucleolytic activity of Cas9 but does not impact its binding to its target [2]. This mutant protein, called dead Cas9 (dCas9), has significantly broadened the application of CRISPR-Cas9 technology.

Chimeric dCas-X molecules, in which X is, in principle, any functionally active domain, can be used to deliver virtually any cargo (functionally active domains) to specific loci in the genome. Functionally active domains may include (a) epigenome remodeling factors for activating or suppressing gene expression; (b) domains for investigating chromatin structure; (c) domains for directly remodeling three-dimensional (3D) chromatin structure; and (d) base editing enzymes, among others (Figure 1).

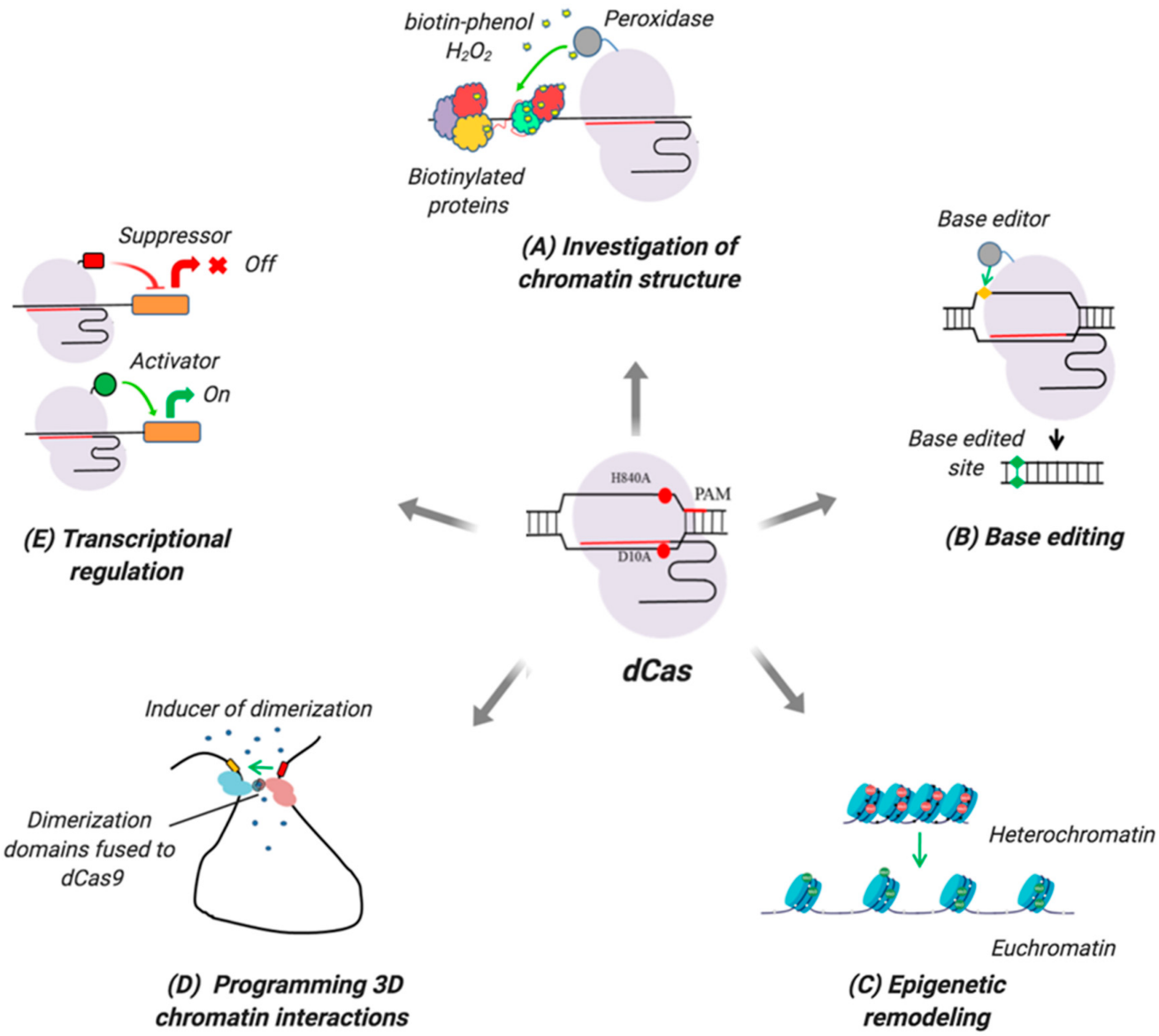

Figure 1. Types and applications of dCas-based molecular tools. (**A**) Investigation of chromatin structure. dCas proteins tethered with specific enzymes (e.g., peroxidase) enable inducible marking (biotinylation) of chromatin factors in the vicinity of the target site. These factors can be subsequently analyzed by proteomics to study chromatin organization. (**B**) Base editing. dCas proteins coupled with base editing enzymes (cytidine or adenine deaminases) can be used to modify RNA or DNA, correct genetic mutations, or knock-out genes. (**C**) Epigenetic remodeling. dCas-based epigenome modifiers can directly alter epigenetic state at a given locus, which is frequently used to annotate gene regulatory elements. Red and green spheres indicate heterochromatin and euchromatin marks, correspondingly. (**D**) Programming 3D chromatin interactions. Using two dCas proteins

targeting defined genomic loci can program 3D chromatin interactions. A chemical inducer stimulates dimerization of dCas proteins fused with dimerization domains building long-range connections between genomic elements. (**E**) Transcriptional regulation. Control of gene expression by dCas proteins tethered to transcriptional suppressors (red) or activators (green). PAM—protospacer adjacent motif; H840A and D10A are point mutations inactivating catalytic residues RuvC and HNH, correspondingly. This picture was created in BioRender software.

The publication can be found here: https://www.mdpi.com/1422-0067/20/23/6041/htm

References

1. Martin Jinek; Krzysztof Chylinski; Ines Fonfara; Michael Hauer; Jennifer A. Doudna; Emmanuelle Charpentier; A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity.. *Science* **2012**, *337*, 816-821, 10.1126/science.1225829.
2. Lei S. Qi; Matthew H. Larson; Luke A. Gilbert; Jennifer A. Doudna; Jonathan S. Weissman; Adam P. Arkin; Wendell A. Lim; Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression.. *Cell* **2013**, *152*, 1173-1183, 10.1016/j.cell.2013.02.022.