Talk

On the "CRISPR" of the wave: Development of a new CRISPR-Cas9 application.

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ABSTRACT

The recent emergence of the prokaryotic CRISPR-Cas9 system provides the opportunity for efficient genome engineering in eukaryotic cells by simply specifying a 20-nt targeting sequence within its guide RNA (1). Apart from its use in genome editing, CRISPR-Cas9 system can be used as a programmable RNA-dependent DNA-binding protein by using a catalytically inactive Cas9 protein (dCas9) (2). Precisely recruiting of dCas9 to desired regions of the genome offers numerous applications such as fusion of dCas9 to transcriptional effector or repressor domains, upregulating or downregulating the transcription of endogenous target genes (3). On the other hand, dCas9 can be fused to a fluorescent protein allowing the visualization of endogenous genomic elements in living cells (4). Recently, this system has been implemented in the fission yeast Schizosaccharomyces pombe, a potent genetic model organism. Genome editing in S. pombe with CRISPR/Cas9 involves the manual identification of unique PAM and spacer sequences (sgRNA) and cloning of these sequences into an expression plasmid (5).

This project proposes the validation of a novel idea that can represent a valuable tool in the study of the location and dynamics of genomic regions of interest. To achieve that, the new-fangled system combines the Cas9 nuclease with the bi-molecular fluorescent complementation technique (BiMFC) in the yeast Schizosaccharomyces pombe. The catalytically inactive Cas9 nuclease (dCas9 from S. pyogenes harbouring D10A and A840A substitutions; Addgene #46920) is fused to the yellow fluorescent protein (YFP), both the whole protein and the N-terminal and C-terminal ends. The resulting protein is directed to the centromeric regions of S. pombe through the introduction of sgRNAs specific to those regions into plasmids derivate from the S. pombe plasmid pMZ376 (Addgene #74213). On the other hand, the protein Cnp1, a variant of the H3 histone that binds to the centromeres, is fused to the cherry protein and the two halves of the YFP. As a result, we will be able to determine if there is a colocalization between the YFP signal of dCas9 and the red signal of the cherry protein fused to Cnp1. In the case of YFP halves, the colocalization of the dCas9 and Cnp1 will allow to recover the full structure of the yellow fluorescent protein, resulting in the emission of yellow fluorescence. The development of this system allows an accurate study of the DNA-protein interactions at cell level.

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