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Manipulation of Gene Transcription Using dCas9

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EDITORIAL

Essentiality may prohibit gene knockdown with any genome-editing approach, and Plasmodium, regrettably, lacks the RNAi route. Conditional knockout is now possible thanks to the Dimerizable Cre Recombinase (DiCre) technique, which involves inserting "silent" loxP sequences into the locus of interest then chemically activating a split Cre recombinase to achieve recombination. Gene knockdown has also been accomplished using FKBP, EcDHFR, TetR aptamer, or glmS ribozyme fusions. All of these techniques, however, necessitate genome editing and leave a genetic scar at the endogenous site.

CRISPR interference/activation (CRISPRi/a) is an alternative technique for transcriptional modulation that does not require genome editing. CRISPRi/a uses an enzymatically inactive Cas9, also known as "dead" Cas9 (dCas9), to attach to but not chop a gene's promoter region. The binding of dCas9 alone can stop the transcription machinery from assembling or progressing, resulting in gene knockdown. CRISPRi has been shown to be effective in P. falciparum and P. yoelii in recent research by Baumgarten, Walker and Lindner, respectively. These findings show that dCas9 gene knockdown necessitates binding at a certain 'sweet spot,' which can only be discovered through trial and error but is most likely placed on the non-template DNA strand as close to the transcriptional start point as feasible, where the chromatin is most open. Using numerous sgRNAs to tile dCas9 across the promoter may improve the CRISPRi effect. Some researchers also provide a good procedure for Chromatin Immunoprecipitation (ChIP) and dCas9 sequencing to check sgRNA specificity, which is critical in any CRISPRi/a work, dCas9 ChIP can also be used to evaluate the efficacy of possible sgRNAs for CRISPR/Cas9 research.

To change the local chromatin structure/composition, dCas9 can be joined to transcriptionally repressive or activating domains. dCas9 was coupled with histone acetyltransferase or deacetylase domains from PfGCN5 and PfSir2a, respectively, in a recent study by Xiao et al. Binding of dCas9-GCN5 and dCas9-Sir2a to gene transcriptional start sites changed local levels of histone acetylation, resulting in up- and down-regulation of the targeted genes. The dCas9-Sir2a system may give a greater knockdown than dCas9 alone, despite the fact that just two genes were targeted for CRISPRi in this investigation. To change the local chromatin structure/composition, dCas9 can be joined to transcriptionally repressive or activating domains. dCas9 was coupled with histone acetyltransferase or deacetylase domains from PfGCN5 and PfSir2a, respectively, in a recent study by Xiao. Binding of dCas9-GCN5 and dCas9-Sir2a to gene transcriptional start sites changed local levels of histone acetylation, resulting in up- and down-regulation of the targeted genes. The dCas9-Sir2a system may give a greater knockdown than dCas9 alone, despite the fact that just two genes were targeted for CRISPRi in this investigation.