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Toward tunable dynamic repression using CRISPRi

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CRISPR interference (CRISPRi) is widely utilized for regulation of target gene expression by repressing transcription. Simple design rules for the single guide RNA (sgRNA) and multiplexity won this method immense popularity. However, quantitative control of the expression levels at varying degrees in a dynamic manner using CRISPRi has been regarded difficult. To deal with this limitation, Fontana et al. modulated the expression levels of the components of CRISPRi, the deactivated Cas9 (dCas9), and the sgRNAs, using various constitutive or inducible promoters (Fontana et al., *Biotechnol. J.* 2018, *13*, 1800069). They found that the expression level of sgRNA is the key to controlling CRISPRi. Modulation of sgRNA expression levels enabled quantitative tuning of the CRISPRi-regulated gene expression level. This approach is expected to be easily applied to diverse applications owing to its simplicity compared to the conventional approaches that modified target sequence or changed the expression level of dCas9.

CRISPR/Cas systems are widely utilized for genome-wide studies in which site-specific engineering are required. Straightforward design rules for the targeting moiety, single guide RNA (sgRNA), enables easy application of this method. Beyond the wild-type Cas proteins with nuclease activity, nuclease-deficient Cas (dCas) proteins have been developed. These mutant proteins can simply bind to the target locus and repress transcription of nearby genes with the guidance of the sgRNA. This system called CRISPR interference (CRISPRi) can repress expression of multiple genes.[1,2] For fine-tuning the CRISPRi, methods for controlling the repression level of CRISPRi have been studied. First, the spacer of sgRNA was modified to change the binding region in the same gene[1] or different genes.[3] Second, several sgRNAs were simultaneously expressed to control the tightness of the repression.[1] Finally, dCas9 or sgRNA was placed under inducible promoters to regulate their expression levels in a dynamic manner.[4,5] However, several limitations of these approaches hindered their wide use. When several sgRNAs are to be used, sgRNAs with different repression efficiencies must be designed. Furthermore, the inducible regulation of dCas9 exhibited low fold repression and high leakage, and fine-control of the repression level has not been demonstrated.

To overcome the drawbacks of the conventional methods, Fontana et al.[6] regulated the expression level of sgRNA or dCas9 using combinations of constitutive and inducible promoters. In this study, it was shown that the regulation of the amount of sgRNA was more effective than the regulation of dCas9 to modulate CRISPRi-induced repression. When dCas9 or sgRNA was fully expressed with a strong promoter and the other machinery of CRISPRi was expressed at varying levels by different constitutive promoters, the repression showed a linear correlation with the varied promoter strengths (**Figure 1**). Especially, when the expression of sgRNA was controlled by the inducible promoter and that of dCas9 was controlled by a constitutive promoter, the repression level changed up to 16-fold. In addition, when all machinery of CRISPRi was controlled by the inducible promoter, the fold-change was further increased to 30-fold. However, when the expression of dCas9 was not enough, the repression level of CRISPRi could not be regulated by the expression level of sgRNA. Therefore, it was shown that the dCas9 is the limiting component for CRISPRi. Additionally, CRISPRi was able to maintain the repression even when the competing sgRNA that could bind with the dCas9 but not bind with any genomic locus is expressed.

In summary, the expression level of each part of CRISPRi was quantitatively controlled to investigate its effect on the repression. The amount of sgRNA significantly contributed to the overall repression level of CRISPRi. This work opens new possibilities of the CRISPRi based on tunable dynamic regulation of gene expression. Since only model systems were demonstrated in this work, practical applications of this approach are expected in future. In metabolic engineering, fine-tuning of gene expression is very important to improve overall productivity, titer, or yield. Therefore, CRISPRi system can be effectively used for quantitative control of multiple pathway genes at the same time. Moreover, inducible control of sgRNA could be adapted for dynamic control of metabolic flux. Finally, the system will also be applicable for predictable design of genetic circuits for synthetic biology.

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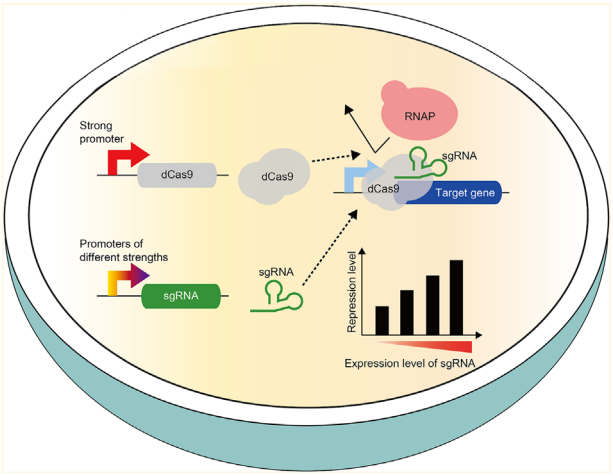

Figure 1. Modulation of CRISPRi repression by controlling the expression level of sgRNA. For CRISPRi repression, dCas9 and sgRNA form a complex and bind to a target locus to block RNA polymerase (RNAP) for gene expression. When dCas9 was fully expressed, the amount of sgRNA determines the repression level of CRISPRi.

Conflict of Interest

The authors declare no commercial or financial conflict of interests.

Keywords

CRISPRi, dCas9, dynamic regulation, sgRNA, tunable gene expression

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