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Research Highlights

Exploiting synthetic regulatory elements for non-dominant microorganisms

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Engineered genetic devices and gene circuits can reprogram cellular behaviors towards different applications in synthetic biology domains, such as detection of small molecular metabolites, biosynthesis of chemicals or proteins valuable for agriculture, food, chemical and pharmaceutical fields. One of the main barriers for these approaches is the lack of genetic regulatory elements with ideal characteristics in different organisms. Developments of these synthetic elements has been rapidly boosted in the last few years, fueled mostly by generating of artificial promoters, libraries of ribosome binding site (RBS) or 5'untranslated regions (UTRs), genetic switches, protein degrons, etc. Because of the flexibility of usage and great number of regulatory components accessible, many synthetic biology studies and applications have been conducted in microorganisms utilizing the model *Escherichia coli* or *Saccharomyces cerevisiae*. On the opposite, despite several modular regulatory elements were developed in other microorganisms [1,2], the use of chassis cells such as *Bacillus* and *Streptomyces*, has so far been limited in synthetic applications (see Fig. 1).

Recently, Gang et al. reported an operator-based inducible regulatory system (MATE) in *Bacillus subtilis* which capable of expressing heterologous proteins and fine-tuning of pathway genes when exposed to maltose [7]. This study provides a comprehensive genetic regulatory system in engineering of *B. subtills* as workhorse for production of valuable proteins in industrial, agriculture and pharmaceutical fields, as well as shows the great potential metabolic engineering in fine-tuning of biosynthetic pathways for chemicals production. *B. subtilis* has been well-studied as gram-positive model bacterium for a long time, and being routinely used as a biotechnology workhorse to produce enzymes and chemicals. In comparison with popular host cells such as *E. coli* or *S. cerevisiae*, one reason for the modest usage of *B. subtilis* in synthetic biology is the lack of well-characterized, collections of regulatory elements to control gene expression levels stringently and flexibly in different pathways. Although recently a collection of genetic elements including promoters, RBSs and protein degron tags with wide-range strength was developed [3], the robustness and stringency of these elements are still incomparable with tools currently available in *E. coli* [4,5]. Besides, in contrast to constitutive genetic elements, an ideal regulatory system should allow flexible controlling of target genes between "ON" or "OFF" status, thus achieving balance between cell growth and production yields [6]. The development of MATE system in *B. subtilis* provides us an ideal toolbox for robust gene expression and flexible gene regulation in synthetic biology applications.

In their work, through an approach to modulate the sequence, numbers, and position of *malO*, a *malA* promoter-driven operator box, maltose inducible activated and repressive systems (MATE-ON/OFF) were established with both ideal strength, stringency, homogeneity and reproducibility. Without compromising the robustness, the carbon catabolite repression (CCR) effect was fully alleviated in this system even in the presence of 20 g/L glucose, and the induction rate was remarkable improved to 790-fold by intercalating of ON-type riboswitch elements. To prove the stringency of their system, gene *divIVA* which encodes a cell division-initiation protein in *B. subtilis* and violacein biosynthetic pathway which is toxic for the host cell was tightly controlled by MATE-ON and MATE-OFF system as an example. In addition, expression levels of nine proteins originate from mammals, insects or bacterium were tested by MATE-ON system. And the average expression level of intracellular or secreted proteins could achieve 60% or 80% of host cellular proteins respectively, which is comparable with the widely used pET/T7 expression system in *E. coli*. The MATE system is a feasible option for selecting robust expression systems in protein production due to its stringency and strength, and the system has potential application value for solving intellectual property risk of some industrial protein producing strains in China.

Another innovation design of this work is the strategy to exploit *malO* operator as attenuator or enhancer to modify the strength of activities of bacterial native promoters. Activities of synthetic promoters were gradually decreased by positioning duplicated operators upstream of them, which can be employed as a strategy to rapidly generate a series of synthetic promoters with different activities. On the other hand, the operator could serve as an inducible enhancer when positioned at an optimized distance upstreaming of the target promoters. In comparison to CRISPRa systems developed in bacteria [8], similar fold change (2.68-fold) was achieved by this low-complexity strategy with negligible influence on promoter architecture and minimal burden on host cell [7].

In summary, compared to other gene regulatory elements established

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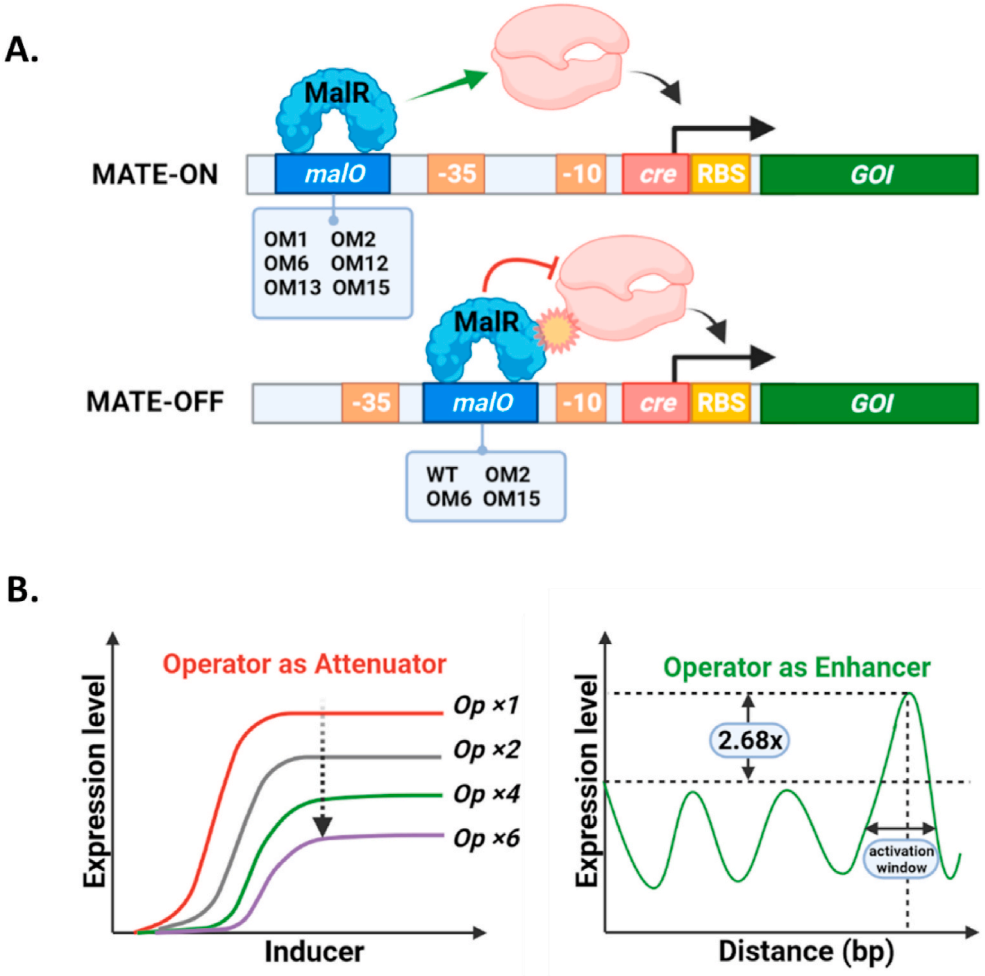

Fig. 1. Schematic illustration of the MATE inducible regulatory system in *Bacillus subtilis*. A, Construction of the activated- or repressive regulatory system by modification of sequence or position of operator *malO* in *B. subtilis*. B, operator *malO* was exploited as a promoter attenuator or enhancer to inducible upregulate or downregulate the expression of target genes without affect the basal activity of promoter.

in *B. subtilis*, this MATE-ON/OFF system shows remarkable advantages on robustness, stringency, and richness of genetic elements simultaneously. Besides, the inducer of MATE system is safe and inexpensive, and the CCR effect which is the main drawback of sugar-inducible system has been alleviated in MATE system. These features are critical for commercial scale-up synthesis of valuable proteins and chemicals. This system provides a one-stop gene ON/OFF regulatory toolkit and strategy, which is useful for expanding the use of *B. subtilis* in synthetic biology. Hence, this study therefore represents an important landmark in gene expression and regulatory system in non-dominant microorganisms and provides a new path ahead in the development of more flexible and orthogonal operator-based genetics devices and components, as well as address various biological challenges and boost the applications of *B. subtilis*.

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References

[1] Bai C, Zhang Y, Zhao X, Hu Y, Xiang S, Miao J, et al. Exploiting a precise design of universal synthetic modular regulatory elements to unlock the microbial natural products in Streptomyces. Proc Natl Acad Sci U S A 2015;112(39):12181–6.
[2] Kushwaha M, Salis HM. A portable expression resource for engineering cross-species genetic circuits and pathways. Nat Commun 2015;6:7832.
[3] Guiziou S, Sauveplane V, Chang HJ, Clerte C, Declerck N, Jules M, et al. A part toolbox to tune genetic expression in Bacillus subtilis. Nucleic Acids Res 2016;44(15):7495–508.
[4] Morra R, Shankar J, Robinson CJ, Halliwell S, Butler L, Upton M, et al. Dual transcriptional-translational cascade permits cellular level tuneable expression control. Nucleic Acids Res 2016;44(3):e21.
[5] Zheng Y, Meng F, Zhu Z, Wei W, Sun Z, Chen J, et al. A tight cold-inducible switch built by coupling thermosensitive transcriptional and proteolytic regulatory parts. Nucleic Acids Res 2019;47(21):e137.
[6] Wang W, Li S, Li Z, Zhang J, Fan K, Tan G, et al. Harnessing the intracellular triacylglycerols for titer improvement of polyketides in Streptomyces. Nat Biotechnol 2020;38(1):76–83.
[7] Fu G, Yue J, Li D, Li Y, Lee SY, Zhang D. An operator-based expression toolkit for Bacillus subtilis enables fine-tuning of gene expression and biosynthetic pathway regulation. Proc Natl Acad Sci U S A 2022;119(11):e2119980119.
[8] Fontana J, Voje WE, Zalatan JG, Carothers JM. Prospects for engineering dynamic CRISPR-Cas transcriptional circuits to improve bioproduction. J Ind Microbiol Biotechnol 2018;45(7):481–90.