Promoter editing generates stable setpoints of gene expression

Precise control of transgene expression remains a challenge in engineering primary cells for diverse applications. We developed DIAL, a promoter editing framework that transmits transient inputs into stable setpoints of expression in primary cells and human induced pluripotent stem cells, paving the way for predictable programming of gene circuits in therapeutically relevant cells.

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The problem

Synthetic biology aims to harness the power of native biology by interfacing endogenous and synthetic gene regulatory networks through transgenic circuits. For over two decades, the field has constructed synthetic gene circuits such as toggle switches, pulse generators, bandpass filters and oscillators that dynamically control transgenes to direct cellular processes, states and identities. Although tools for control in mammalian systems are expanding, rational de novo design of synthetic circuits remains challenging[1]. Limiting translation in research and medicine, tools and gene circuits developed in cell lines often suffer poor performance in primary cells due to silencing and unpredictable patterns of transgene expression. Even simple inducible promoters can exhibit emergent, undesirable behaviors such as bimodality that impede predictable, uniform expression across a population. Gene circuits need to buffer variation across populations, over time, and in primary cells to ensure robust, uniform performance. Building stable, heritable setpoints would support titration of gene expression and its mapping to phenotypes.

The solution

To achieve this, we developed DIAL, a modular, extensible framework for building editable promoters that allows fine-scale, stable titration of transgene expression. Through a defined combination of programmable inputs, we designed DIAL to generate multiple unimodal setpoints of expression via recombinase-based promoter editing (Fig. 1a). As predicted by previous work[2], editing the promoter increased transcriptional activity by excising a spacer between transactivator binding sites and the core promoter. As the transactivator induces transcription from the core promoter, editing increased the proximity of the binding sites to the core promoter and shifted transactivator-induced expression of the transgene to a higher setpoint. Increasing the length of the DIAL spacer increased the setpoint range by reducing the level of basal transgene expression from the pre-edited promoter. Nesting orthogonal recombinase sites generated four stable setpoints from a single promoter. As promoter editing is genetically encoded, DIAL translates user-defined inputs into stable, heritable setpoints.

We demonstrated that setpoints are robust over a large range of transactivator levels. To further explore the generality of the spacer-excision architecture and expand it to new transactivators, we integrated the TET-On system — a doxycycline-inducible promoter and transactivator pair — into the DIAL framework, generating TET-DIAL. We found that TET-DIAL generated doxycycline-inducible setpoints, allowing compact, reversible control of expression. For broad translation to diverse cell types, we demonstrated that DIAL can be delivered via lentivirus and generates setpoints of transgene expression in primary cells. We demonstrate how DIAL can set expression levels that are stable during and after a cell-fate transition. By controlling a cell-fate regulator, we showed that DIAL setpoints influence the rate of direct conversion from primary fibroblasts to induced motor neurons (Fig. 1b).

Future directions

Control via a single promoter offers scalability for generating multiple setpoints from libraries of transgenes. Importantly, a single promoter inherently controls for bias in clonal founder lines that may obscure or confound expression-based differences in phenotypes. Thus, as we demonstrated in direct conversion, DIAL can identify and control transgenes with subtle dose-dependent effects on cellular states and fates[3]. Understanding the dose dependence of transgenes will guide development of improved genetic control systems for programming desired functions and phenotypes. Integrating new synthetic[4] and native transcription factor binding sites into the DIAL framework may support cell state- and pathway-responsive promoters with tunable setpoints.

The current DIAL framework offers stability, but promoter editing induces irreversible changes that increase the setpoint. Addition of new recombinases and optimization of spacer sequences may support reversibility as well as promoter editing to achieve lower setpoints.

DIAL setpoints are robust at high transactivator levels. However, variation in the dosage of the promoter construct itself affects expression, requiring alternative controllers such as incoherent feedforward loops[5]. Layering DIAL with circuits that control for varying promoter dosage offers the potential for improved stability of transgene expression. By combining these controllers, stable titration of cell-fate transcription factors and regulators will enable rapid cell-fate programming.

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EXPERT OPINION

"Overall, this is an innovative solution that addresses a broadly important problem for synthetic biology, cell engineering, biotechnology and beyond: precise, reliable control of transgene expression. The work builds upon existing tools and knowledge — specifically, recombinase-mediated promoter 'logic' and recently developed synthetic zinc finger transcription factor systems — combining them in a novel and clever way. The work offers nuances and insights that will no doubt be useful to the synthetic biology field." **Ahmad Khalil, Boston University, Boston, MA, USA.**

FIGURE

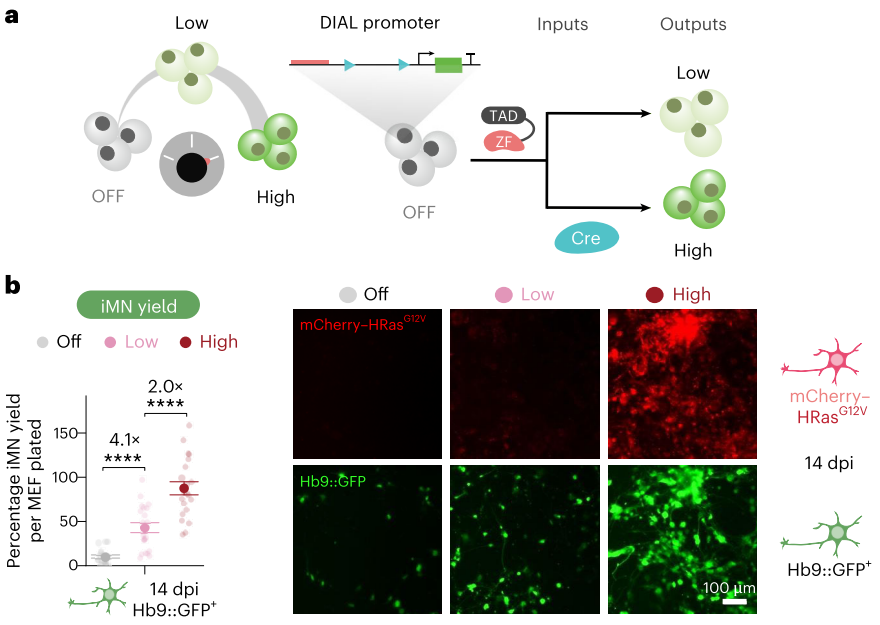

Fig. 1 | DIAL generates stable setpoints that persist through cell-fate programming. a, In the presence of the synthetic transactivator, which is composed of a transcriptional activator domain (TAD) and zinc-finger domain (ZF), the DIAL promoter expresses a transgene (green) at the low setpoint. Addition of the Cre recombinase induces promoter editing, by excising the spacer, increasing expression to the high setpoint. **b**, By controlling the setpoint of a positive cell-fate regulator (mCherry–HRas^{G12V}), DIAL induced different rates of conversion of primary mouse embryonic fibroblasts (MEFs) to induced motor neurons (iMNs), which express Hb9::GFP when the motor neuron gene regulatory network is activated. Induction to the high setpoint increased the yield of iMNs 2-fold compared to the low setpoint.

BEHIND THE PAPER

Our group has been working to build transgenic systems for predictable, stable expression in primary cells. Our original vision for DIAL was a system with heritable states that would be sensitive to the amount of transactivator. But, when we performed titrations of the transactivator, we found that the expression was bimodal, limiting the potential for titrating a dose–response to the transgene. At high levels of the transactivator, we noticed that expression from DIAL was very robust. We realized that this dosage invariance to the transactivator made the system highly stable. Thus, editing can generate distinct, stable levels of expression, which we call setpoints. These setpoints are tunable by the spacer length and transactivator. Setpoints persist through dynamic cell-fate transitions and can be used to identify subtle, dose-dependent effects of transgenes that are otherwise obscured by extrinsic noise. **K.E.G.**

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FROM THE EDITOR

"This method, DIAL, will be widely useful to the synthetic biology community. DIAL enables researchers to generate editable promoters, enabling fine-tuning of genetic expression. The authors show that DIAL can work in a variety of cell types." **Editorial Team, *Nature Biotechnology*.**