Development of promoter-based tools for precise regulation of transgene expression in mammalian cells

By Sneha R. Kabaria

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Rewiring mammalian cells through transgene delivery has revolutionized biomedicine, with powerful demonstrations including cell fate reprogramming, cell-based therapies, and gene-replacement therapies. In many systems, strikingly, even subtle changes in transgene levels can direct cells to distinct cellular states. Therefore, recognizing and controlling dose-dependent transgenes can be essential in directing cells towards complex behaviors.

Fine-tuned control is required for identifying dosage-sensitive transgenes, but remains a challenge. To support dosage titration, we developed a highly modular, extensible framework called DIAL for building editable promoters that allow for fine-scale, heritable changes in transgene expression. Using DIAL, we increase expression by recombinase-mediated excision of spacers between the binding sites of a synthetic zinc finger transcription factor and the core promoter. By nesting varying numbers and lengths of spacers, DIAL generates a tunable range of OFF/Low/High unimodal setpoints from a single promoter. Through small-molecule control of transcription factors and recombinases, DIAL supports temporally defined, user-guided control of transgene expression. To enable more direct inducible transactivator control, DIAL architecture is also extensible to binding sites of the Tet-ON system. For the broadest impact across research and therapeutics, genetic control systems need to perform in multiple contexts. Lentiviral delivery of DIAL generates multiple setpoints in primary cells and iPSCs and additionally can control diverse transgene identities. As promoter editing generates stable DNA states, DIAL setpoints are heritable, facilitating mapping of transgene levels to phenotype and fate. As a compelling demonstration, we titrate the cell fate regulator HRasG12V in direct conversion of primary fibroblasts to induced motor neurons and track changes in proliferation and yield that emerge over weeks. Collectively, the DIAL framework opens opportunities to tailor transgene expression and improve the predictability and performance of gene circuits across diverse applications.

Constitutive promoters, particularly those that leverage native transcription factors, remain preferable for setting known optimal transgene levels and driving sustained expression. However, the mammalian synthetic biology toolkit contains a limited library of high-performing promoters to encode distinct setpoints. To address this gap, promoters

encoded in the antisense direction of the protein-encoding sequence can be leveraged to tune protein expression through sense-antisense transcript hybridization. With varying combinations of sense and antisense promoters of different strengths, more setpoints were achieved than with the sense promoter panel alone across transfection and site-specific integration delivery methods. Output levels trended in accordance with promoter strength, and spacer length and identity between promoters provide additional tunable parameters. However, there is also some non-modularity: only some promoter identities enable antisense-mediated suppression, and upstream sense promoter identity affects the mechanism of RNA-level regulation. Finally, recombinases support switching between expression levels from the sense-antisense cassettes. In sum, the approach presented here not only expands the number of setpoints from existing promoter libraries but also provides essential characterization to further leverage antisense transcription in mammalian synthetic biology.

Overall, this thesis introduces two novel promoter-based methods for titrating and setting precise transgene levels. In future engineering of mammalian cells, these tools can enable more effective identification, encoding, and leveraging of dosage effects across a range of applications.

Thesis supervisor:
Kate E. Galloway

Title:
W. M. Keck Career Development Professor in Biomedical Engineering
Assistant Professor of Chemical Engineering