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Preview

Designing quantitative gene therapy on ComMAND

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Gene replacement therapies can generate unnaturally high levels of transgene expression, potentially compromising their safety or efficacy. Variable gene delivery compounds this problem, leading to heterogeneous expression. To address this limitation, ComMAND, a microRNA-based biomolecular circuit assisted by computational models, reduces cell-to-cell variation in gene expression.

Advances in DNA delivery, especially virus-based vehicles, have greatly accelerated the field of gene therapy and now enable us to treat diseases with an underlying genetic cause. Many of these genetic disorders are haploinsufficient, caused by a mutation or deletion that renders only one copy of a gene nonfunctional while the remaining copy is unable to compensate. Previously, patients with haploinsufficient disorders such as beta thalassemia would have limited treatment options. But now, there is an FDA-approved gene replacement therapy (Zynteglo), which uses viral vectors to deliver a functioning gene to replace the patient's nonfunctional copy in autologous hematopoietic stem cells.[1] However, many potential gene therapies still fail due to having variable safety and efficacy profiles that limit their translation to the clinic.

Though improving viral vector efficiency has been extensively researched and our ability to target specific cells and deliver DNA has improved immensely, superphysiological expression of potentially therapeutic transgenes can induce off-target effects. For example, in a mouse model of fragile X syndrome, a form of mental disability caused by *Fmr1* deficiency, delivering *Fmr1* led to behavioral abnormalities.[2] In another mouse model for Friedrich ataxia, a neuromuscular disease caused by *Fxn* deficiency, *Fxn* overexpression led to cardiac toxicity.[3,4] In both cases, the safety and efficacy of the treatment can benefit from considering the effect of dosage or gene copy number delivered to any one cell.

However, the solution to unnaturally high transgene expression is not as simple as lowering the total number of viruses administered to the patient. Tuning the total viral dose would allow us to control the average expression level across a cell population and has been done extensively before. But at the single-cell level, gene delivery is a stochastic process, and the number of viruses that interact with any one cell is uncontrollable. When decreasing the number of viruses administered, delivery efficiency can be compromised, leaving many target cells without a copy of the healthy gene. Furthermore, some gene therapies are administered via injection, which will naturally have a dosage gradient as cells closer to the injection site will get more copies of the gene. Lastly, the body is made up of complex tissues, which naturally leads to uneven biodistribution of the viruses. Therefore, a synthetic biomolecular circuit offers a solution that is built into the delivered cargo itself.

One circuit used to buffer gene expression against dosage variability is the microRNA-based incoherent feedforward loop (IFFL). (Due to the reference limit, refer to Love et al. for references regarding microRNA-based IFFLs.) An IFFL is a network motif that can maintain an output level against variation in input. It senses changes in the input strength and compensates by proportionately regulating an activating arm and a repression arm (Figure 1A). In the context of gene delivery, dosage (e.g., gene copy number) acts as the input and transgene expression level as the output. MicroRNAs, small single-stranded non-coding RNAs involved in RNA silencing, can be used to implement the repression arm. These components are non-immunogenic and small, having a minimal resource burden on the target cell. But despite the potential for microRNA-based IFFLs to improve the success of gene replacement therapies, there remain major gaps toward their therapeutic applications.

To better understand and optimize miRNA-based IFFLs across therapeutically relevant delivery methods and cell types, Love et al. introduce compact microRNA-mediated attenuator of noise and dosage (ComMAND).[5] ComMAND uses a single-transcript design, placing an intronic microRNA within the transgene and the microRNA target site in the 3′ untranslated region (Figure 1B). It uses orthogonal miRNAs such that they do not target endogenous mRNA transcripts. Furthermore, the intronic design both controls the stoichiometry of the mRNA and microRNA species and acts as a safety feature, since failure of the microRNA to properly splice leads to a nonfunctional mRNA transcript. This compact design adds only ∼450 base pairs to the transcript length—a desirable feature since many therapeutically relevant viruses like adeno-associated viruses and lentiviruses have packaging limits. Love et al. demonstrate how ComMAND can reduce the mean and variability of the transgene and can be tuned through modulating different components of the system. These features make ComMAND extremely adaptable for gene therapies that are currently under development.

Notably, Love et al. explore the design space and characteristics of ComMAND using the analytical model they developed. ComMAND can be tuned by adjusting promoter strength, but additional knockdown through increasing miRNA binding strength or the number of miRNA binding sites has diminishing returns. ComMAND is robust to changes in splicing, which improves the safety profile of the system across cell types with different microRNA processing regimes. Lastly, they note that ComMAND is limited by the RNA-induced silencing complex (RISC) endogenous to

the cell. When RISC becomes saturated with microRNA, dosage control becomes compromised. However, most therapeutic applications use lower copy numbers typical of viral transduction. Thus, this limitation is less crucial. Such understandings offer useful guidelines for future adopters of ComMAND.

To demonstrate how ComMAND might address the safety and efficacy issues of potential gene replacement therapies, Love et al. show how the system can be used to control a transgene in therapeutically relevant cells and how it can maintain a transgene expression similar to physiological levels. ComMAND leads to decreased average transgene expression and more uniform expression across cells in a remarkable variety of primary cells: rat cortical neurons, mouse embryonic fibroblasts, human T cells, and induced pluripotent stem cells. Most excitingly, they demonstrate how ComMAND can control genes involved in haploinsufficient disorders, specifically *Fmr1* and *FXN*, in which *in vivo* studies for gene replacement therapy have led to mixed outcomes and toxicities. Love et al. then take it a step further using lentivirus, a more therapeutically relevant delivery modality, to deliver *FXN* and show expression remains within an order of magnitude of the endogenous expression, which was previously identified to a safe range.[4]

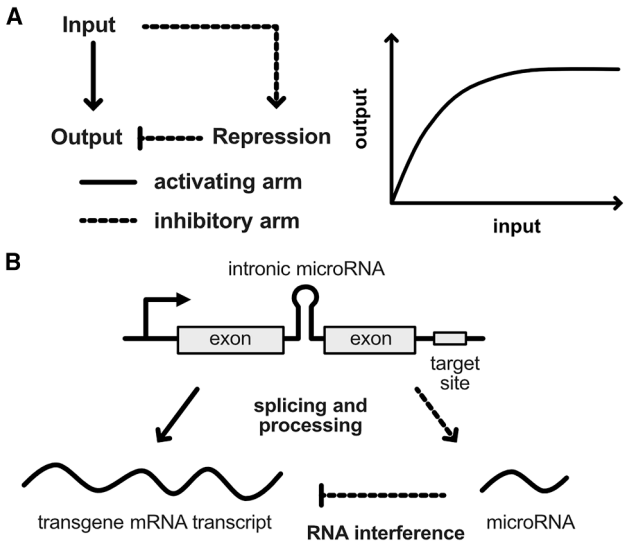

Figure 1. Incoherent feedforward loop and ComMAND microRNA implementation

(A) Schematic of incoherent feedforward loop (IFFL). An IFFL buffers against input variability.

(B) Schematic of ComMAND, a miRNA-based IFFL, as designed by Love et al.

The development of promising gene replacement therapies for haploinsufficiency can fail due to unexpected phenotypes and toxicities resulting from overcorrection of the deficiency. With ComMAND, Love et al. have brought us one step closer to a rigorously characterized and easily adoptable system to solve this problem. By simply including an intron and a microRNA binding site, any gene therapy currently in development can benefit from the IFFL's ability to buffer against dosage variability. Our ability to treat genetic disorders that used to be considered incurable is expanding, and tools such as ComMAND enable us to precisely and robustly work toward that goal.

DECLARATION OF INTERESTS

X.J.G. is a co-founder of and on the advisory board of Radar Therapeutics.

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