Commentary: Thilo Pohle

Why synthetic gene promoters matter

In vivo gene therapies succeed or fail based on their ability to achieve the right level of gene expression in the right cells. Delivery vehicles such as adeno associated viruses (AAVs) determine where the genetic cargo arrives, but it is the promoter that dictates what happens next, whether a therapeutic gene is active and to what extent. As gene therapy enters a new phase of clinical maturity, promoter engineering remains undervalued, even though it is central to addressing the two defining challenges of targeting and dosing.

This article explores the pivotal role of promoters in determining therapeutic protein expression and why their design is critical for the success of gene therapies.

Gene therapy has emerged as one of the most promising modalities in modern medicine, offering the possibility to correct the underlying causes of genetic disease rather than treating symptoms. Over the past three decades, the field has matured significantly, leading to more than 2,200 therapies in development (preclinical and clinical) and, to date, 36 approved therapies for conditions such as spinal muscular atrophy, haemophilia B and certain haematological malignancies [1]. Ongoing successes such as uniQure's recent Huntington's trial results demonstrate the clinical viability of the approach[2], particularly for rare monogenic disorders, and inspire confidence that the technology can be extended into broader indications. At the same time, these milestones have highlighted how much more must be achieved if gene therapy is to fulfil its potential. Durability, manufacturability and the multimillion-dollar cost per dose remain considerable hurdles. Perhaps the most critical challenges for therapeutic efficacy and safety are targeting and dosing. These two have always been central to the design of *in vivo* gene therapies, but recent adverse events and evolving regulatory expectations have heightened the demand for more precise control.

Targeting and dosing

Targeting and dosing are governed by two main elements of a gene therapeutic: the delivery vehicle and the genetic payload. Traditionally, the spotlight has fallen on the delivery vehicle, particularly capsid engineering of AAVs, which have become the workhorse for *in vivo* gene therapies due to their favourable safety profile, low immunogenicity and potential for long term expression. Building on the natural diversity of AAV serotypes, researchers have developed capsid engineering techniques such as chimeric and mosaic constructs, barcoded library screening, directed evolution and machine learning-guided rational design. These approaches have yielded synthetic capsids with refined specificity, improved transduction efficiency and reduced immunogenicity[3]. The progress has been substantial, leading to major investments for companies such as Dyno Tx for individual capsids or libraries[4], underscoring the value of capsid engineering and reflecting the recognition that dosing and targeting are fundamental.

Yet delivery is only half of the equation. A capsid can decide where the genetic material goes, but it does not dictate what happens once inside the cell. That function is governed by the promoter, the short stretch of DNA that regulates transcription and determines when, where, and how much of a therapeutic protein is produced. Gene promoters determine whether transcription is constitutive or regulated, tissue-specific or ubiquitous, and how durable or stable the output will be. In other words, promoters provide a second layer of targeting that is just as decisive as the first. Without the right promoter, even the most advanced capsid cannot deliver optimal therapeutic precision.

Despite this central role, promoter choice has often been treated as a secondary consideration. More than 50% of clinical stage AAV-based programmes still rely on strong viral or ubiquitous promoters such as CMV or CAG[5]. These can drive high levels of expression but also provoke immune responses, lead to expression in tissues where the protein is neither needed nor wanted, and are prone to silencing. In some cases, such as systemic metabolic disorders, broad expression may be desirable. However, even with engineered capsids, systemic delivery often results in high vector uptake by the liver, which can cause hepatotoxicity[6]. Hence, even systemic diseases require a more nuanced expression profile than CMV or CAG can provide. For most monogenic diseases, expression must be targeted to specific tissues or cell types and minimised elsewhere.

The traditional alternatives to ubiquitous promoters are endogenous promoters. Derived from genes that are naturally enriched in particular tissues, they can show high specificity but are often large, relatively weak or dependent on distal elements. Their fixed characteristics usually make them difficult to use within the restricted packaging capacity of AAV vectors. The regulatory behaviour of endogenous promoters can also be unpredictable in therapeutic contexts and they cannot easily be tuned to the needs of a specific programme. As a consequence, scientists often modify natural promoters by deleting sections, as in the clinically used hSyn1 and MeP426, or by combining segments of different promoters, as in tMCK and ApoE/hAAT[7].

These combinations, referred to as hybrid promoters, represent the first type of synthetic promoters but remain distinct from fully synthetic promoters, which are built from smaller functional units. Whereas a hybrid promoter recombines two or a few defined promoter segments, fully synthetic promoters are assembled from cis-regulatory elements (CREs), which can be either singular transcription factor binding sites (transcription factor response elements, TFREs) or dense clusters of such sites, known as cis-regulatory modules (CRMs). Both hybrid and fully synthetic promoters share an important characteristic with engineered capsids: they expand functionality beyond what nature provides, opening new possibilities for efficacy and safety,

and with the right understanding, enabling an engineering-design approach to meeting a set specification.

Modern synthetic promoter design workflows have been enabled by advances in experimental biology and computational science. Functional genomics techniques such as RNA-seq, ChIP-seq and ATAC-seq have produced genome wide transcriptional maps, chromatin accessibility profiles and transcription factor binding datasets, providing a foundation for identifying candidate elements. Machine learning, including convolutional neural networks (CNNs), generative adversarial networks (GANs), transformer models and other AI approaches, can now propose novel promoter sequences and assist in CRE assembly. DNA barcoding and next-generation sequencing support massively parallel reporter assays for high-throughput validation. Together these tools narrow down candidate sequences to a manageable test set, but the complexity of eukaryotic transcriptional regulation means that expertise in promoter biology, regulatory networks and therapeutic context remain essential.

Next generation promoters

At SynGenSys, we have designed a platform, Sypher, which uses bioinformatics analyses of genome-wide transcriptional data, combined with proprietary selection and assembly rules and expert knowledge, to engineer high-performance synthetic promoters tailored to therapeutic goals. By mapping the transcriptional landscape of on- and off-target tissues, we identify and assemble regulatory elements into compact, tuneable promoters suitable for any type of AAV, lentiviral or non-viral payload.

Next-generation synthetic promoters can go even further, offering tuneable expression profiles designed to meet the unique requirements of each indication. Such a rational and data-driven process, supported by streamlined testing, is being used to produce SynGenSys' off-the-shelf promoter lines, such as the NK.SET library for NK cell therapies, and also underpins our bespoke design offerings for therapy developers. These solutions are helping overcome expression bottlenecks, improve safety and efficacy, and accelerate the development of next-generation genetic medicines.

Promoters are universal components of DNA based therapeutics. In *ex vivo* applications such as CAR-T and CAR-NK cell therapies, promoter strength influences receptor density and hence cell potency and safety. Here, hybrid sequences such as the MND promoter have shown advantages over traditional promoters like EF1a in lentiviral systems for CAR-T therapies[8], but even here, fully synthetic designs can provide more refined control. *In vivo* applications of modified cell therapies also have cell type specificity requirements, and in multi-gene constructs, promoters must be balanced to achieve the right stoichiometry. Promoters are agnostic to the delivery vehicle, making them just as relevant for non-viral platforms such as targeted lipid nanoparticles. Regardless of delivery method, the promoter remains the element that defines expression and function.

Promoter development is gaining recognition as a key driver of precision and control among gene therapy developers, despite capsid engineering still being the dominating topic for tissue targeting of *in vivo* gene therapies in terms of visibility and investment. Looking ahead, promoter engineering will extend beyond tissue specificity. Promoters responsive to cellular states such as hypoxia are already in use but can be further refined, enabling therapeutics that adapt dynamically to their environment. Boolean logic designs can underpin gene circuits and conditional expression systems, adding a new dimension to genetic medicine. It should be noted however that promoter engineering is not applicable to all modalities classified as gene therapies. As promoters function at the DNA level, they are not relevant for mRNA-based therapeutics or genome-editing approaches that do not involve DNA delivery.

Having said this, success for most in vivo gene therapy will ultimately depend on solving the challenges of targeting and dosing. These parameters are governed by three interconnected elements: administration route, delivery vehicle and a suitable promoter. A one-size-fits-all approach does not suffice. Every genetic disease is different, and therefore every therapeutic should be optimised and individualised in every possible aspect to achieve the best outcome. The promoter is a key determinant of dosing and targeting, it should not be viewed as a secondary variable but as a strategic driver of therapeutic success.

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