Molecular Therapy
Review

Gene regulation technologies for gene and cell therapy

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Gene therapy stands at the forefront of medical innovation, offering unique potential to treat the underlying causes of genetic disorders and broadly enable regenerative medicine. However, unregulated production of therapeutic genes can lead to decreased clinical utility due to various complications. Thus, many technologies for controlled gene expression are under development, including regulated transgenes, modulation of endogenous genes to leverage native biological regulation, mapping and repurposing of transcriptional regulatory networks, and engineered systems that dynamically react to cell state changes. Transformative therapies enabled by advances in tissue-specific promoters, inducible systems, and targeted delivery have already entered clinical testing and demonstrated significantly improved specificity and efficacy. This review highlights next-generation technologies under development to expand the reach of gene therapies by enabling precise modulation of gene expression. These technologies, including epigenome editing, antisense oligonucleotides, RNA editing, transcription factor-mediated reprogramming, and synthetic genetic circuits, have the potential to provide powerful control over cellular functions. Despite these remarkable achievements, challenges remain in optimizing delivery, minimizing off-target effects, and addressing regulatory hurdles. However, the ongoing integration of biological insights with engineering innovations promises to expand the potential for gene therapy, offering hope for treating not only rare genetic disorders but also complex multifactorial diseases.

INTRODUCTION

Gene therapy has created new treatment options for once-untreatable genetic diseases, with recent successes enabled by learnings from decades of progress. The field encompasses three main pillars: gene replacement, gene silencing and activation, and gene editing—each aiming to restore missing protein or RNA, provide compensatory factors, or deplete pathogenic molecules in disease-relevant cell types through the delivery of nucleic acids or gene-editing systems.[1] These treatments can be delivered directly to the patient or applied to cells *ex vivo* as a cell therapy for subsequent infusion or transplantation. The past decade has witnessed a wave of approved gene and cell therapies. Luxturna, approved in 2017, was the first U.S. Food and Drug Administration (FDA)-approved gene therapy, utilizing adeno-associated virus (AAV) to treat inherited retinal dystrophy by providing the RPE65 gene to retinal cells.[2] That same year marked the beginning of the chimeric antigen receptor (CAR)-T cell therapy era of personalized immunotherapy. In CAR-T therapy, delivery of a CAR to T cells allows the targeting of T cells' cytotoxic response to tumor cells based on presence of a target antigen.[3] Kymriah—the first-in-class CAR-T cell therapy now approved for various B cell leukemias and lymphomas was followed closely by Yescarta, also indicated for multiple lymphoma subtypes. As of December 2024, seven CAR-T cell therapies have received FDA approval.[4] Other gene and cell therapy approvals have quickly followed. For example, Zolgensma was approved in 2019 for spinal muscular atrophy (SMA), delivering a functional SMN1 transgene to motor neurons using AAV9. Skysona followed in 2022 as an *ex vivo* lentiviral gene therapy delivering the ABCD1 gene to hematopoietic stem cells for the treatment of cerebral adrenoleukodystrophy, although recent concerns have emerged regarding potential associated hematological cancer.[5] In another critical turning point in the field, the first gene-editing therapy using CRISPR-Cas9, Casgevy, was approved for sickle cell disease and transfusion-dependent β-thalassemia in late 2023.

The clinical successes of *in vivo* gene delivery have thus far primarily relied on recombinant AAV (rAAV) vectors, which can achieve stable expression in post-mitotic cells via AAV episomes. However, maintaining stable expression is not guaranteed and may be dependent on vector integration into chromosomal DNA.[6] In an early phase 1/2 dose-escalation clinical study of AAV2-mediated delivery of factor IX for hemophilia B, the duration of therapeutic expression was limited to 8 weeks due to destruction of transduced hepatocytes by immune cells targeting AAV capsid antigens.[7] Researchers have since developed strategies to mitigate these immune responses while maintaining clinical viability through carefully selected immunosuppression regimens that maintain functionality of regulatory T cells[8] and the use of AAV serotypes with higher efficiency and more selective tissue tropism. In 2022, uniQure and CSL's Hemgenix (factor IX) and Biomarin's Roctavian (factor VIII), were approved for hemophilia B and A respectively, both utilizing AAV5.[9–11]

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Both Hemgenix and Roctavian are single-dose therapies, demonstrating that with an appropriate serotype and cell system AAV episomes can enable stable expression for years, although the ultimate durability of expression remains unclear.[12,13] In the case of Roctavian, this uncertainty has limited patient uptake. In particular, endoplasmic reticulum (ER) stress associated with the unfolded protein response[14] has been observed in mouse hepatocytes expressing high levels of factor VIII, highlighting the importance of gene regulation in determining therapeutic efficacy.[15] This is attributed to the unstable nature of factor VIII compared with factor IV, as factor VIII has been shown to misfold into amyloid-like structures in the ER.[16] This ER stress may cause the decline in factor VIII expression seen over time in hemophilia A patients treated with Roctavian.[17] Engineered factor VIII variants with improved stability may overcome these issues,[18] although clinical evaluation of these variants remains to be performed.

The complex dynamics enabling stable AAV expression are still being explored. Some long-term expression may result from genomic AAV integration during double-strand break repair processes.[19–22] AAV integration in hepatocytes of non-human primates has been observed at frequencies of up to 1.6 copies per 100 cellular genomes, stabilizing at 0.1–0.7 integration events per 100 genomes after 77 days.[6] These integration events can be heterogeneous and include complex concatemers across the genome with a preference for highly expressed loci.[6,23] While rare, AAV integration events have been linked to hepatocellular carcinoma (HCC) in mice, including at the mouse *Rian* locus, where integration resulted in overexpression of proximal microRNAs (miRNAs) and retrotransposon-like 1 (*Rtl1*).[24] While so far only observed in mice, the incidence of HCC after AAV delivery is influenced by factors including AAV vector dose and enhancer/promoter selection, highlighting the importance of careful selection of these elements to mitigate the potential risks associated with these therapies.[25]

To address the challenges posed by semi-random AAV integration, investigators are increasingly exploring targeted genomic integration strategies. Although CRISPR-Cas9 enables targeted transgene integration, it remains inefficient for large transgenes.[26] Therefore, alternate strategies for reaching therapeutic thresholds despite efficiency limitations are being considered. For example, zinc finger nuclease-mediated integration of transgene into the albumin locus achieved stable therapeutic expression of factors VIII and IX in mouse models of hemophilia.[27] Notably, this approach utilized the endogenous albumin promoter to (1) drive protein expression at high levels thus requiring lower efficiency editing, (2) decrease cargo size, and (3) attenuate the risk of aberrant gene expression after potential off-target AAV insertion. In the CAR-T context, targeted insertion of a CD19 CAR from an AAV vector into the *TRAC* locus with CRISPR-Cas9 displayed decreased T cell exhaustion and tonic signaling, and enhanced tumor control compared with random integration by conventional retroviral vectors.[28]

These innovative strategies highlight the potential for promoters, fusion proteins, and targeted insertions to improve gene therapy efficacy, but this also holds true for other diverse strategies including the integration of regulatory elements within vectors and leveraging *cis*- and *trans*-regulatory interactions in the genome. As gene therapy continues to evolve, the focus is shifting toward more precise control of gene expression and cellular function. In this review, we discuss the technologies that enable precise control of gene and cell therapies, from inducibility and cell type specificity to dynamic sensing and perturbation of gene regulatory networks (GRNs). These technologies have the potential to usher in a new era in which gene therapies move beyond constitutive expression systems and treatment of Mendelian diseases to address more complex and dose-sensitive conditions. Only when these regulatory interactions are incorporated will gene therapy reach its full potential, maximizing its ability to cure disease and improve lives.

Controlling expression of exogenous transgenes

Cell-type-specific promoters

It was recognized early in the gene therapy field that restricting expression to the tissue of interest could be highly advantageous (Figure 1A). Initial approaches focused on repurposing the sequences of native promoters to drive tissue-specific expression, such as gamma-globin for erythroid cells,[29] myosin light or heavy chain for cardiac tissue,[30] pancreatic amylase for pancreatic tissue,[31] or muscle creatine kinase for muscle tissue.[32] The importance of tissue-specific promoters was emphasized by the discovery that off-target expression of a therapeutic transgene in dendritic cells could trigger an immune response against the transgene.[33,34] This motivated a search in the Duchenne muscular dystrophy field as well as others for muscle-specific promoters to circumvent this issue.[34–36] Researchers have engineered improved promoters by combining known enhancer elements with core promoter regions, creating highly active and tissue-specific promoters compatible with viral vector packaging limits. For instance, a modified MCK promoter with enhanced muscle-specific activity was generated by linking the MCK core promoter with the E-box enhancer elements and replacing a weaker native E-box with a second copy of the stronger native E-box.[35,37] It was also found that independent enhancer elements control expression in fast and slow twitch muscle, highlighting a challenge in promoter design wherein improved specificity ablates off-target expression but must be fine-tuned to preserve expression in multiple cell types within the tissue of interest.[37,38] Other work has engineered improved promoters through the systematic combination of known muscle-specific enhancer elements.[38,39] This approach aims to develop promoters that maintain high specificity for muscle tissue while ensuring expression across various muscle fiber types, thereby optimizing the therapeutic potential of gene therapies targeting muscle disorders.

More recently, the combination of high-throughput genomic assays and novel computational approaches have enabled sophisticated engineering of promoter sequences. Massively parallel reporter assays[40,41] have allowed simultaneous activity measurements for thousands of enhancers. More recently, this technique has been applied to enhancer elements encoded within AAV vectors and transduced into

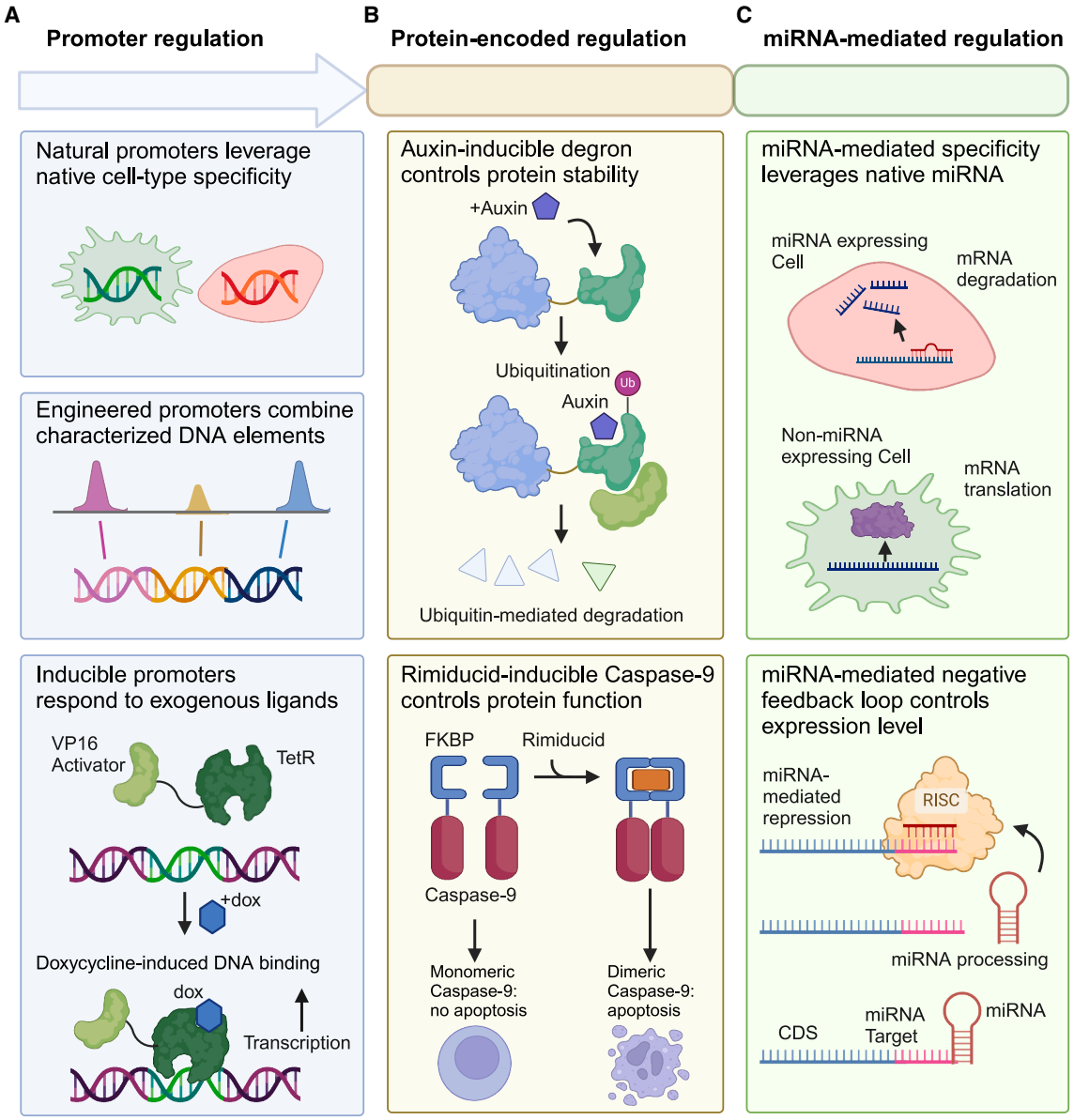

Figure 1. Methods to control expression and function of transgenes

(A) Promoter-encoded regulation may leverage the intrinsic properties of native promoters, combine useful properties of multiple characterized DNA elements, or utilize inducible DNA-binding proteins to activate or repress expression in the presence of an exogenous ligand. (B) Protein-encoded regulation can function either through inducible protein degradation, as in the case of AIDs, or inducible protein function, as in the case of rimiducid-inducible caspase-9 safety switches. (C) miRNA regulation of transgene expression may either leverage cell-type-specific miRNA expression to repress expression in off-target cells, or create a negative feedback loop by including both a miRNA and its target site in the transgene mRNA to tightly control expression levels.

multiple tissues *in vivo*, facilitating the systematic discovery of tissue-specific promoters and enhancers.[42,43] Bioinformatic methods have been used to identify gene regulatory regions that mediate high levels of muscle-specific expression[44] when combined with the established muscle-specific desmin promoter,[45] highlighting the effectiveness of both bioinformatic promoter identification and strategic combination of multiple regulatory elements. Similar studies have screened libraries of repeating tiles of transcription factor (TF) binding sites and used the results to build predictive models for cell-type-specific expression.[46]

Current and future work in tissue-specific promoter design is increasingly driven by computational innovations, including the application of machine learning. Early machine learning approaches

to promoter design have primarily focused on optimizing parameters such as overall expression strength in bacteria[47] and yeast cells.[48] More recent developments have enabled the generation of fully synthetic, cell-type-specific promoters.[49] This work combines a cell-type-specific expression scoring algorithm, trained on MPRA data from multiple cell types in culture, with an iterative design process to generate and evaluate candidate synthetic promoters. Future work will focus on applying these computational techniques to clinically relevant cell types and addressing key remaining challenges such as the need for large empirical datasets for training and the inherent complexity of tissues composed of diverse cell populations.

Small molecule-inducible control of gene expression

Transcriptional response to chemical signals is both a key feature of native metabolic processes and a highly useful tool for biomedical research. The first widely used chemically inducible promoter system was the tetracycline-regulated Tet-off system wherein tetracycline prevents binding of an activating TF, enabling the repression of an engineered transgene expression cassette by the addition of tetracycline.[50] Further development allowed doxycycline-dependent DNA binding, resulting in a system in which expression is activated by doxycycline addition (Figure 1A).[51]

Chemically inducible dimerization is a powerful approach for controlling gene expression by inducing proximity of transcriptional activators or repressors. The most widely used system is S-(+)-abscisic acid-induced dimerization of the PYL1 and ABI domains.[52] Fusion of ABI to a DNA-binding domain and PYL1 to a transcriptional activator domain allows chemically controllable regulation of gene expression of both exogenous and endogenous genes.[52]

Inducible protein degradation systems offer a rapid and controllable means of regulating protein levels. The classic example of chemically inducible protein degradation is the auxin-inducible degron (AID).[53] This system relies on fusion of the auxin-binding protein IAA17 to the protein of interest. This is in turn bound by auxin, which permits binding to the endogenous TIR1 protein followed by ubiquitination and proteasomal degradation. An improved AID2 system has been generated to reduce leakiness and increase sensitivity.[54] Similar systems such as dTAG and IKZF3, which rely on chemically induced binding to native ubiquitin ligases, have been developed and used to control the expression of a CAR.[55] However, each of these systems has variable activity depending on the protein to which they are fused (Figure 1B).[56]

Due to the significant potential of Cas9-based therapeutics, significant effort has focused on creating molecules to control Cas9 activity. Naturally occurring anti-CRISPR proteins derived from phage genomes have been explored as competitive Cas9 inhibitors.[57] Nucleic acid-based inhibitors that bind to the single guide RNA (sgRNA) sequence have been used to control Cas9 activity.[58] Small molecules have also been developed that inhibit the interaction between Cas9 and the genomic PAM sequence and allow efficient temporal control of Cas9 or dCas9 activity.[59]

The clinical potential of these systems is exemplified by the rimiducid-inducible caspase 9 dimerization system, which allows rimiducid-inducible apoptosis as a safety switch for engineered T cell therapies (Figure 1B).[60] This has been used successfully in the clinic to control graft-versus-host disease after T cell transplantation (NCT01494103)[61] and reduce side effects from CAR-T therapy (NCT06096038).[62] Similarly, rimiducid-inducible MyD88/CD40 (iMC) dimerization has been used to activate CD40 signaling pathways[62,63] to drive T cell activation, although clinical trials were halted due to toxicity concerns (NCT02744287 and NCT02743611).

miRNA-regulated cassettes

In addition to tissue-specific promoters, miRNA-mediated control of mRNA levels has been leveraged to mediate tissue specificity and tightly control expression levels (Figure 1C). This approach has proven particularly valuable in cases where transgene expression must be tightly regulated to avoid toxicity in non-target tissues. For example, the calpain3 transgene is therapeutic for limb-girdle muscular dystrophy type 2A in skeletal muscle, but toxic if expressed in the heart. To avoid cardiac expression but preserve skeletal muscle expression, binding sites for the cardiac-specific miR-208a were included in a calpain3 transgene and successfully restricted expression to the skeletal muscle.[64] Other examples include use of a neuron-specific miRNA binding site in the transgene of a CNS-targeted lentivirus to ablate neuron expression and bias toward astrocyte expression.[65] Alternatively, miRNA regulation has been leveraged to avoid transgene expression in antigen-presenting cells, reducing transgene immune responses and promoting tolerance.[66]

Regulation by miRNA has also been used in Rett syndrome therapy to prevent the toxicity associated with overexpression of the therapeutic MeCP2 transgene.[67,68] One approach being pursued creates a negative feedback loop using endogenous miRNAs upregulated by MeCP2. In this system, MeCP2 expression leads to increased miRNA levels, which in turn repress MeCP2 RNA, resulting in precisely calibrated protein levels.[69] A similar approach has been developed wherein a synthetic miRNA is included in the vector to directly regulate the MeCP2 transgene (NCT05898620).[70]

Methods of delivery: Optimizing efficiency, specificity, and expression duration

The method of delivery can greatly alter the efficiency, cell-type specificity, and duration of expression of a therapeutic gene of interest. Lentiviral and retroviral vectors have seen considerable clinical use, for instance to modify hematopoietic stem cells and introduce CARs into T cells for immunotherapy.[71] While these vectors enable persistent expression after cell division due to semi-random genomic integration, they carry a potential risk of oncogenic transformation.[72] rAAV vectors offer a potentially safer alternative by primarily remaining as non-chromosomal double-stranded DNA episomes, decreasing the risk of insertional mutagenesis.[22,73] The episomal nature of rAAV allows stable expression in non-dividing cells but leads to dilution through cell division. This characteristic makes rAAV vectors ideal for targeting post-mitotic cells, such as muscle tissue,[74]

but less suitable for modifying dividing cells such as hematopoietic stem cells in the absence of chromosomal integration.

Liposomal delivery of mRNA or protein is often preferred for applications requiring transient delivery, such as genome editing. This approach minimizes potential off-target effects or immune responses associated with the long-term expression of bacteria-derived genome-editing reagents. A notable example is the clinical use of liposomal delivery for Cas9 mRNA and sgRNA to reduce *TTR* gene expression in amyloid transthyretin amyloidosis.[75]

Delivery methods also offer opportunities for enhancing cell-type specificity. Significant efforts have been made to alter the tropism of rAAV vectors, expanding their tissue-targeting capabilities while reducing off-target expression. For example, inserting short peptides into the exposed loops of the AAV capsid can redirect viral tropism.[76–79] More recently, large domains such as nanobodies have been incorporated into capsids for enhanced targeting, using either careful interrogation of insertion sites[80] or non-canonical amino acid-mediated conjugation.[81] Directed evolution approaches have also been applied for engineering AAV capsids with enhanced tissue-specific tropism. This approach has yielded capsids with improved transduction efficiency in many tissues of interest.[82–85] However, early efforts often resulted in capsids with species-specific tropism, limiting their broader applicability. To address this challenge, researchers have developed sophisticated strategies to incorporate multi-species selection into directed evolution campaigns. These approaches successfully produced AAV capsid variants with high muscle tropism across multiple species,[86,87] enhancing their potential for clinical translation. Another challenge in AAV-directed evolution for altering tissue tropism is distinguishing viral genomes resulting from successful transduction events from DNA within bystander viral particles that may be incidentally present in the target tissue. This has been addressed by either measuring RNA expression levels[87] or by utilizing Cre-based reporter systems that measure the activity of the AAV cargo inside of transduced cells.[88] The latter approach enables the recovery of only those sequences that have successfully transduced Cre-expressing cells. It also allows cell-type-specific selection based on restricted Cre expression. The Cre-based strategy has been particularly effective in generating AAV capsids with enhanced CNS tropism.[88] While these approaches have generated AAV capsids with significantly improved tropism for many tissues of interest, the field remains highly dynamic. Modern machine learning techniques have also been applied to increase the structural viability of modifications to the AAV capsid.[89,90] This allows the generation of libraries with much greater numbers of viable variants as compared with random library generation. This improved viability of individual substitutions also enables the generation of highly combinatorial libraries, which are more likely to alter tropism but would be infeasible in random screening due to the high probability of incorporating variants which disrupt AAV function. Ongoing research continues to refine and expand upon these methods, aiming to develop even more specific and efficient AAV vectors for gene therapy applications across a wide range of tissues and diseases.

Regulating endogenous gene expression

Antisense oligonucleotide therapeutics

Since the initial discovery of RNA interference,[91] the therapeutic potential of RNA oligonucleotides has been apparent. This breakthrough led to the development and approval of siRNA therapeutics such as Patisiran for transthyretin-mediated amyloidosis and Givosiran for acute hepatic porphyrias.

More advanced oligonucleotide therapeutics have been developed to address specific genetic defects, as exemplified by exon-skipping strategies for Duchenne muscular dystrophy.[92,93] This approach has resulted in FDA-approved treatments such as Amondys 45, Exondys 51, and Vyondys 53, which lead to the production of partially functional dystrophin protein by skipping mutated exons. Similarly, splicing modulation has been successfully applied in treating SMA, wherein an antisense oligonucleotide biases the splicing of the SMA2 gene to create a functional gene product to compensate for the disease-causing loss of SMA1.[94] This has led to the FDA-approved oligonucleotide therapeutic Spinraza. In Friedreich's ataxia, multiple oligonucleotide approaches aim to upregulate frataxin expression. One strategy uses an antisense oligonucleotide targeting the expanded frataxin repeats to prevent R-loop formation by the RNA transcribed from the expanded repeat.[95] This approach seemed to significantly restore frataxin protein in cell culture.[95] An alternative approach has explored DNA-targeting polyamides that bind the expanded repeat and recruit BRD4 using the BRD4-binding molecule JQ1, leading to the restoration of frataxin expression,[96] an approach that was also subsequently tested clinically (NCT05285540).

A novel approach to upregulating protein expression involves modulating splicing to bias against the baseline level of incorrect splicing which occurs in cells, leading to increased protein levels due to increased levels of correctly spliced mRNA.[97] The subtle increase in gene expression that results from this approach is particularly suitable for treating haploinsufficiency disorders, where only a modest increase in target protein expression is desirable. This strategy is currently being evaluated in clinical trials for Dravet syndrome, a condition characterized by SCN1A haploinsufficiency (NCT04442295).

Epigenome editing

Gene expression can also be altered at endogenous gene loci by modulating native epigenetic control. First described by Conrad Waddington as "changes in phenotype without changes in genotype," the greater understanding of epigenetics has bloomed into a complex network of biochemical marks and interactions that regulate gene expression. These epigenetic mechanisms are central to development, response to environmental stimuli, and disease progression.[98–100] Epigenetic mechanisms encompass a complex interplay of histone modification, DNA methylation, three-dimensional chromatin structure, and interactions with noncoding RNA and DNA/RNA-binding proteins such as TFs, all of which collectively and dynamically regulate gene expression.[101,102]

Histone modifications

The tails of histone proteins interact electrostatically with adjacent DNA, and various chemical modifications of specific amino acids within these tails—including acetylation, methylation, ubiquitylation, phosphorylation, and SUMOylation—are associated with distinct genomic elements and transcriptional states.[103] For example, H3K4me3 (trimethylation of lysine 4 on histone H3) is a hallmark of transcription start sites of active genes, while H3K79me2/3 is characteristic of gene bodies.[104–106] Notably, different modifications on the same amino acid can lead to contrasting effects on gene expression, such as the association of acetylation and trimethylation of lysine 27 on histone H3 with enhancer activation and repression, respectively.[107]

DNA methylation. Cytosines at CpG sites in the genome can be methylated or hemi-methylated. Locally dense regions of CpG sites, known as CpG islands, occur in the majority of gene promoters and, when heavily methylated, cause stable silencing of genes that is copied through cell division and DNA replication by DNA methyltransferases.[108]

Three-dimensional chromatin structure. Chromatin loops, mediated by proteins such as CTCF and cohesin, result in proximity-based enhancer-promoter interactions which regulate gene expression. On a larger scale, topologically associated domains (TADs) define extensive genomic regions with dynamic interaction potential. These TAD compartments can be sequestered in densely packed heterochromatin, creating a repressive state that inhibits TF accessibility.[109]

Interactions with RNA and DNA/RNA-binding proteins. Noncoding RNAs can act as scaffolds for activating or repressive DNA/RNA-binding proteins, either directly at gene promoters through sequence homology or distally from promoters as decoys, wherein long noncoding RNAs (lncRNAs) compete for binding of TFs.[110,111]

Epigenetic regulation has emerged as a promising target for therapeutic interventions, with several small molecule drugs that inhibit writers or erasers of these epigenetic modifications approved for clinical use. These include DNA methyltransferase inhibitors such as azacitidine and decitabine, as well as histone deacetylase inhibitors such as valproic acid, vorinostat, romidepsin, belinostat, and panobinostat. While effective, these drugs act globally, lacking specificity for individual genes implicated in disease processes. To address this limitation, researchers have developed epigenetic editing tools that offer precise, targeted modulation of gene expression.[112] These systems utilize DNA-targeting proteins, such as zinc finger proteins, transcription activator-like effectors (TALEs), or most commonly nuclease-inactive Cas proteins (dCas), fused to epigenetic effectors, allowing for site-specific alterations of the epigenome.[113–117] CRISPR-based transcriptional silencing (CRISPR interference [CRISPRi]) is achieved through the fusion of repressive effectors, primarily KRAB domains, to dCas9. Conversely, transcriptional activation (CRISPR activation [CRISPRa]) uses fusions of activating viral peptides (e.g., VP16 or its multimers, Rta) or activating domains (e.g., p65), either alone or in combination (Figure 2A).[114,117–119] The versatility of these CRISPR-based tools is further enhanced by the ability to fuse effectors directly to Cas proteins or recruit them to associated scaffolds. This latter approach allows for higher copy numbers and modularity, albeit at the cost of increased system complexity.[120,121] Systems that precisely modulate more complex epigenetic states have also been developed, such as the fusion of dCas to enzymatic writers and erasers of various epigenetic marks (e.g., TET1 or p300) or CTCF.[122–125] These various epigenetic editing tools can be targeted to gene promoters or distal regulatory elements, enabling both direct and indirect gene regulation. This level of precision and flexibility represents a significant advancement over global-acting small molecule inhibitors, offering new possibilities for targeted epigenetic therapies in diverse settings, including rare genetic diseases, common conditions such as cardiovascular disease, regenerative medicine, cancer, and infectious diseases.

Epigenetic modifications induced by CRISPR-based tools are typically transient, dissipating with the loss of Cas expression or through cell division. However, programmable epigenetic memory has been attained through the writing of methylation to CpG islands using fusion of KRAB, DNMT3A, and DNMT3L domains with engineered TALEs or Cas9.[126–128] Durable methylation has also been achieved by fusion of a zinc finger protein to a DNMT3L domain that recruits DNMT3A and an unmethylated histone H3 tail to circumvent autoinhibition.[129] The compact nature of this system enabled efficient *in vivo* silencing of the prion protein via AAV delivery. Conversely, removal of DNA methylation via the activity of the DNA methylcytosine dioxygenase TET1 can lead to stable gene activation.[128] The therapeutic potential of this approach has been demonstrated by the activation of *Mecp2*[130] or *CDKL5*[131] on the inactive X chromosome for Rett syndrome and infantile epilepsy, respectively, activation of the methylated *FMR1* locus for fragile X syndrome,[131] and activation of the imprinted maternal Prader-Willi syndrome locus as a strategy to compensate for the lost paternal allele.[132] Thus, the ever-expanding toolkit of epigenome-editing tools enables the precise regulation of endogenous genes via the programming of many distinct epigenetic states, including more nuanced and lasting modifications to gene expression patterns. Looking forward, the focus is shifting toward the development of even more compact editors.[133,134] This emphasis on size reduction aims to enhance *in vivo* deliverability, a crucial factor in broadening the therapeutic applications of these technologies.

Targeted RNA regulation

Significant advances have been made in regulating gene expression by targeting endogenous RNA. In addition to well-established siRNA technologies, CRISPR-Cas-based approaches have seen extensive development. While other CRISPR-Cas families cleave DNA, CRISPR-Cas13, a class 2 type VI RNA endonuclease, recognizes and cleaves RNA, enabling gene knockdown with varying durability and offering a flexible approach to gene regulation.[135] Since its initial description as a programmable RNA-editing tool, Cas13-based systems have been significantly utilized for a wide range of applications,

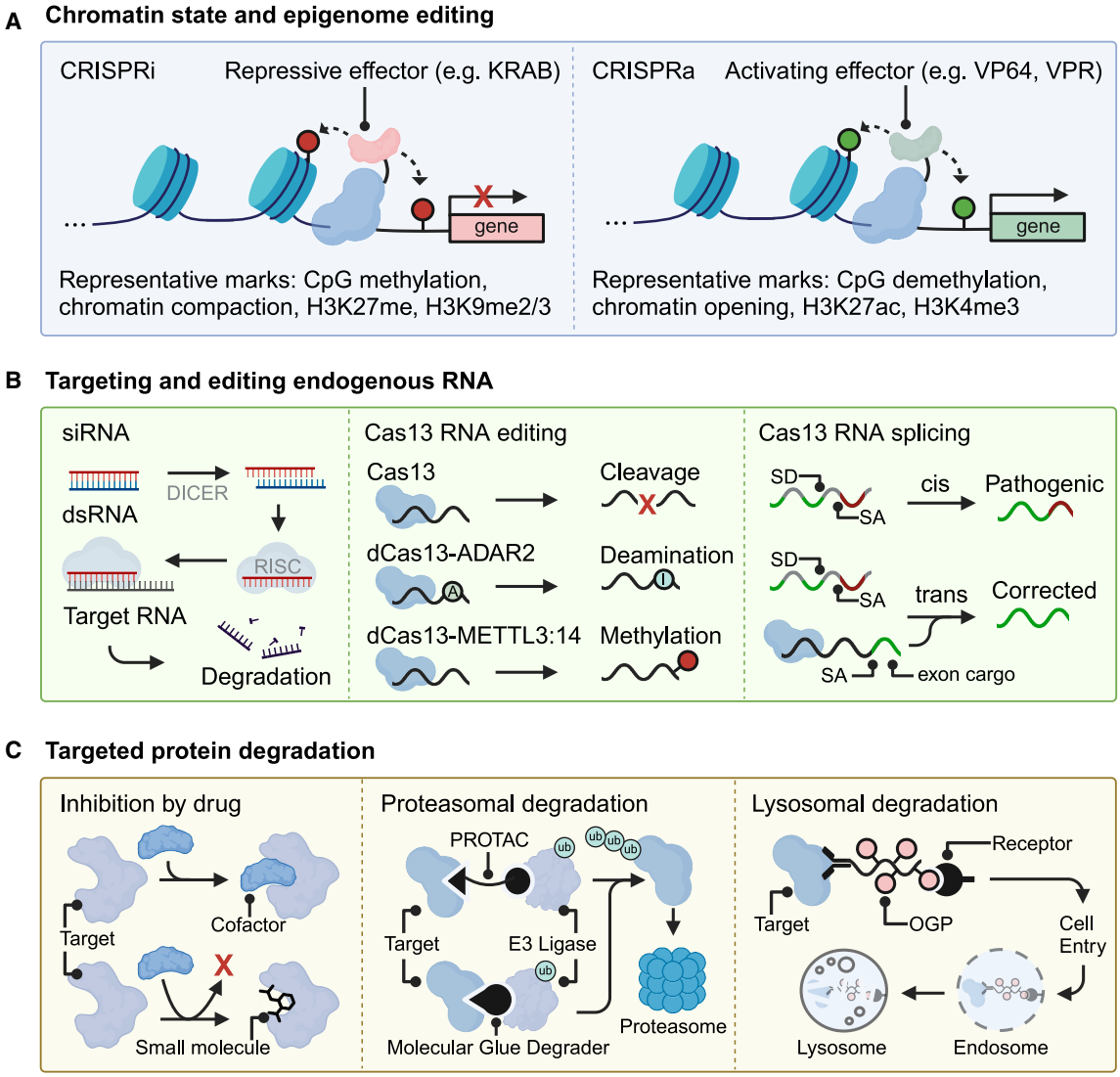

Figure 2. Targeted regulation of endogenous gene expression

(A) Regulating gene expression with CRISPR-based epigenome editing. CRISPR interference (CRISPRi) utilizes dCas fusion(s) to repressive effectors that deposit repressive epigenetic marks and/or promote heterochromatin formation. CRISPRa utilizes fusion(s) to effectors that deposit marks associated with active transcription and/or open chromatin. (B) Methods for targeting and editing endogenous RNA including siRNAs, wherein a delivered dsRNA is processed into siRNAs, which are then recruited to the RISC complex and subsequently bind complementary single-stranded mRNA, resulting in degradation. CRISPR-Cas13 can regulate endogenous RNAs by cleavage, deamination, or methylation, depending on catalytic activity and fused effector domains. Cas13-based systems have also been leveraged for editing RNA splicing (*trans*-splicing) by delivering an exon cargo to the 5′, 3′, or internal splice sites to correct or skip mutation-containing exons. (C) Targeted degradation of endogenous proteins by small molecules, proteolysis-targeting chimera (PROTAC) and similar systems which result in proteasomal degradation, or lysosome-targeting chimaera and similar systems which result in lysosomal degradation. OGP, oligoglycopeptide.

including single base edits, RNA cleavage, methylation, deamination, nucleic acid sensing and diagnostics, and high-throughput screening (Figure 2B).[136–142]

RNA base editing has been a particularly exciting application of Cas13 technology. Efficient RNA base editing has been achieved by fusion of dCas13 with an adenosine deaminase acting on RNA (ADAR).[136] ADARs mediate adenine-to-inosine editing of cellular double-stranded RNA,[143,144] with inosine being read as guanine by cellular machinery. Further work has enabled cytosine-to-uracil conversion by directed evolution of a cytosine deaminase from an adenine deaminase.[145] Endogenous ADARs can also be used to engineer RNAs to direct RNA editing activity to sites in transcripts of interest without necessitating use of a Cas13 molecule,[146–149] and this approach has now entered clinical testing for correction of genetic mutations (NCT06677307 and NCT06405633).

Recent advancements have expanded the capability of CRISPR-Cas13 to control RNA splicing.[150–152] Here, Cas13-mediated RNA targeting is combined with cellular RNA-splicing machinery to achieve exon skipping, replacement of large transcript sections, or *trans*-splicing between RNA species. The efficiency of *trans*-splicing, however, depends on factors such as transcript abundance and the nature of the intended edit. Cas13-based RNA *trans*-splicing has gained significant attention in both academic and biotechnology settings due to its reversibility and ability to avoid the genomic instability associated with DNA cleavage. This technique may mature into a powerful tool for RNA-targeted regulation of gene expression, offering new possibilities for manipulating gene function without permanent genetic alterations.

Targeted protein degradation

Protein degradation represents another important strategy for the targeted regulation of gene expression. Conventional drug discovery has largely focused on directly targeting and inhibiting protein activity. However, emerging technologies that harness native protein degradation pathways have been developed to offer a solution for proteins resistant to traditional small molecule approaches. This was first achieved by synthesizing a proteolysis-targeting chimera, which creates interaction between a protein of interest and ubiquitin E3 ligases, triggering ubiquitylation and subsequent targeted protein degradation (TPD).[153] Other TPD strategies include molecular glue degraders, lysosome-targeting chimaeras, and biological TPDs (consisting of peptide, fusion protein, or antibody-based degraders).[154–160] While these approaches use diverse mechanisms for target affinity and ubiquitin E3 ligase recruitment, they converge on a common outcome: proteasomal or lysosomal degradation of the target protein. Compared with small molecule inhibitors, targeted degraders offer several advantages. They provide increased scope, as they do not rely on targetable active sites. They also enhance activity, with a single degrader capable of removing multiple molecules of the target protein rather than merely blocking its active site. Additionally, targeted degraders exhibit improved selectivity due to required interactions between the drug, target, and E3 ligase.[161] As a result, thousands of heterobifunctional degraders targeting hundreds of proteins have been developed, leading to a growing suite of clinical trials testing these compositions.[162–164] These technologies can be genetically encoded and delivered as a gene therapy to continuously degrade targeted proteins or be utilized as a post-translational off switch for the products of delivered transgenes.

The ability to regulate gene expression by targeting DNA, RNA, and proteins through an expanding array of technologies represents a significantly advanced capacity to modulate cellular processes and treat diseases previously considered undruggable (Figure 2C).

Regulating endogenous networks

In some cases, regulating genes indirectly by targeting other genomic elements can confer capabilities not possible by targeting the gene itself. A salient demonstration of this strategy's success is evident in the recently approved gene-editing therapy for sickle cell disease and β-thalassemia. This therapy does not directly target the causal mutation in the *HBB* gene but instead focuses on an enhancer of the *BCL11A* gene.[165] *BCL11A* encodes a key repressor of the *HBG* gene, which encodes fetal hemoglobin, in adult erythroid cells.[166] By targeting the erythroid-specific enhancer of *BCL11A*, these therapies achieve two crucial outcomes: they increase fetal hemoglobin to physiological levels while simultaneously repressing sickle hemoglobin, and they confer cell type specificity by mediating this switch exclusively in the disease-associated erythroid lineage.[166,167]

The concept of indirect regulation extends beyond single-gene targets to encompass broader gene expression control. TFs, which regulate gene transcription by binding to specific DNA sequence motifs, are attractive targets for manipulating gene expression on a larger scale.[168] TFs, along with TF-binding proteins, form intricate GRNs capable of dynamically controlling hundreds or thousands of genes.[169] Rewiring GRNs through the targeted manipulation of specific TFs has opened new avenues for inducing broad phenotypic changes, including reprogramming to pluripotency,[170] stem cell differentiation, and direct reprogramming of cells between differentiated cell types,[171–173] offering the potential to orchestrate complex cellular transformations. However, these approaches face a significant challenge: identifying the optimal genomic element or TF to target for achieving the desired gene expression changes.

One approach is to map the GRNs that control the gene(s) in question in a given cell state. This is commonly achieved with RNA sequencing and/or assay for transposase-accessible chromatin with sequencing (ATAC-seq) during a dynamic process (e.g., differentiation) or after perturbing a TF by knockout, repression, activation, or exogenous overexpression.[174–176] The resulting data on differentially expressed genes, changes to chromatin accessibility, and associated TF motifs are then analyzed using statistical frameworks such as regression, Bayesian inference, and logical modeling to identify key regulators.[168] Increasingly, supervised and unsupervised machine learning techniques, including decision trees, Bayesian networks, and artificial neural networks, are being applied to parse these complex networks (Figure 3A).[168]

Forward genetic screening is another valuable tool for mapping targetable elements. While many screening methods (e.g., siRNA, short hairpin RNA, cDNA, and small molecule) exist, high-throughput CRISPR screening has emerged as a popular approach. In this approach, libraries of gRNAs are delivered to cells via titered lentivirus such that each cell receives one or multiple distinct gRNAs. These cells are then subjected to selection schemes to enrich for desired phenotypes. Commonly this includes proliferation, survival of drug treatment or viral infection, or sorting based on protein expression.[177] Advanced techniques like CRISPR droplet sequencing (CROP-seq) and Perturb-seq combine CRISPR screening with single-cell RNA sequencing to link specific perturbations with their transcriptomic effects.[178–180] Perturb-seq-style experiments have been successfully used to map enhancers of specific genes, predict

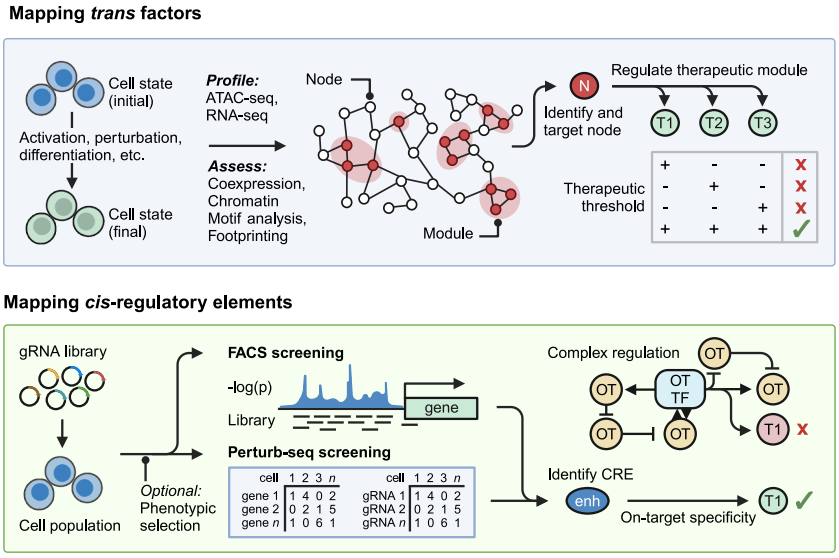

Figure 3. Mapping and perturbing GRNs to regulate target genes

(A) Mapping trans factors to regulate multiple downstream target genes. High-throughput sequencing approaches are used to profile cells during a dynamic process. Gene regulatory networks are then constructed and analyzed to determine critical node(s) (N) able to regulate target genes (T1, T2, and T3). In this example, modulation of all three genes is needed to reach a therapeutic threshold. (B) Mapping a *cis*-regulatory element such as an enhancer (enh) to specifically regulate a target gene (T1) within an otherwise prohibitively complex GRN involving off-target genes (OT) and TFs (OT TF). This can be accomplished by fluorescence-activated cell sorting (FACS) or Perturb-seq high-throughput CRISPR screening with or without a phenotypic selection.

gene functions, and identify drivers of stem cell differentiation (Figure 3B).[181–185] While Perturb-seq experiments offer valuable insights, they can be expensive and challenging to scale, especially for rare cell populations or *in vivo* studies, as they require the profiling of many cells to robustly decipher perturbations, especially when multiple perturbations are assessed combinatorially. Therefore, there has been a dedicated effort to develop computational and inferential approaches to facilitate the *de novo* identification of targetable regulators while maintaining experimental feasibility.[186–192] Taken together, these technologies aim to further our understanding of GRNs and map genetic and epigenetic interactions more efficiently. This knowledge will be crucial for developing future therapies that target regulatory elements, TFs, and other key components of cellular regulatory systems to establish new avenues for gene and cell therapy and broadly enable regenerative medicine.

Synthetic systems for interacting with endogenous gene regulation

CAR-T cell therapy is one of the most successful classes of cell-based treatments to date. In the most common form, CAR-T cells constitutively express a CAR that, when engaged with target antigens, initiates an intracellular signaling cascade. This cascade activates TFs such as nuclear factor-κB[193] and NFAT,[194] ultimately leading to perforin/granzyme-mediated lysis of targeted cancer cells.[195] Although approved CAR-T therapies utilize constitutively expressed CARs, the complex changes in T cell gene expression and function resulting in target cell lysis provide an opportunity to enhance therapeutic efficacy with more nuanced synthetic control over endogenous gene regulation.

Control of CAR-T signaling and targeting

Pharmacological control of CAR-T cell activation has allowed titratable response to a pharmacological agent using chemically induced dimerization between the CAR extracellular targeting domain and intracellular signaling domain.[196] It is also now possible to design CAR constructs which mediate logic-gated recognition of multiple antigens using a caged domain that can be exposed by a second key domain, allowing highly synergistic AND, OR, and NOT logic in response to the binding of multiple antigens.[197,198]

Synthetic receptors to control transgene expression

Recent advances have led to the development of synthetic receptors that link extracellular binding events to user-selected transcriptional outputs. The Modular Extracellular Sensor Architecture receptor system pioneered this approach by using a pair of receptors with identical extracellular domains but different intracellular components: one with a protease and another with a transcriptional regulatory domain attached via a protease-cleavable linker.[199,200] Colocalization of these receptors upon binding to a single extracellular factor triggers the release of the transcriptional regulatory domain, which can then activate or repress a synthetic promoter driving a gene of interest. Similarly, the Tango receptor system couples extracellular signals to transcriptional output via induced protease cleavage upon ligand binding, leveraging natural interactions between G-protein-coupled receptors (GPCRs) and their ligands.[201,202] The Tango system has been further refined to incorporate dCas9-mediated gene regulation in response to GPCR ligand interaction,[203] expanding its potential applications in synthetic biology and therapeutic interventions.

The synNotch system represents a significant advancement in synthetic receptor technology, offering a more robust alternative to previous designs that required multiple synthetic receptor molecules to bind the same target (Figures 4A and 4B).[204,205] SynNotch leverages the native Notch/Delta signaling mechanism in which Notch/Delta binding exposes a protease cleavage site, leading to the release of a transcriptional regulatory domain. Early reporter assay experiments showed that the Notch transcriptional regulatory domain could be

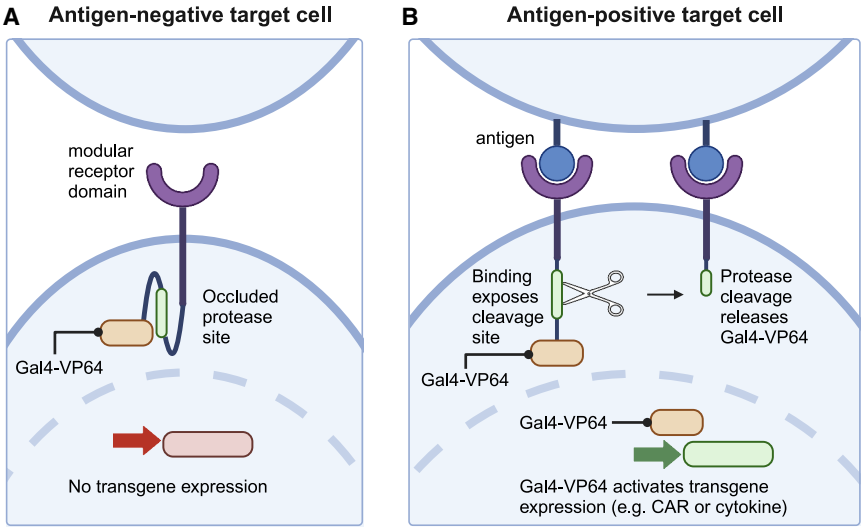

Figure 4. SynNotch mediates gene expression upon antigen recognition

(A) When in contact with an antigen-negative cell, the synNotch receptor remains in the off state, wherein the protease-cleavable linker is occluded and the Gal4-VP64 TF is not released. (B) When in contact with an antigen-expressing cell, binding of the extracellular domain leads to a conformational change, which exposes the intracellular protease domain and allows release of Gal4-VP64 to activate the transgene.

replaced with an exogenous Gal4 transcriptional activator,[206] suggesting that Notch was an excellent candidate for a synthetic receptor. Further research found that replacement of the native Notch/Delta binding interaction with alternative receptors preserved functional signaling.[204,207] Thus, the replacement of both extracellular binding and intracellular transcriptional modulator domains allowed for a fully programmable synthetic receptor in which the binding of a desired extracellular target leads to programmable transcriptional output. This versatility allows for the creation of T cells with precisely controlled responses, such as interleukin (IL)-2 production, directly regulated by binding to a desired antigen.[205] SynNotch technology has been further refined to generate genetic circuits where recognition of one antigen by synNotch induces expression of a CAR targeting a second antigen.[208] The resulting synNotch CAR-T cells exhibit enhanced specificity, selectively targeting cancer cells expressing both antigen targets of the synNotch receptor and CAR.[208–210] This approach also shows promise in addressing immune-excluded tumors.[211]

SynNotch technology has recently been extended to enable recognition of brain-enriched cell surface proteins,[212] followed by expression of either a CAR to treat CNS tumors or production of the anti-inflammatory cytokine IL-10 for the treatment of neuroinflammation. It has also been adapted for tissue-specific immune suppression, such as preventing cytotoxic T lymphocyte-mediated killing of transplanted beta cell organoids.[213] The original synNotch design was then expanded based on the observation that a variety of human receptors contained regions that could act as intramembrane proteolysis receptors, similar to the original synNotch design, resulting in a class of synNotch-like receptors known as synthetic intramembrane proteolysis receptors.[214] These new designs are compatible with humanized TF and Notch domains, overcoming limitations of the original synNotch that was restricted to the Gal4-VP64 TF, with components derived from yeast and herpes virus, and a mouse Notch IPR domain.

Sensing of intracellular RNA expression

The regulation of transgene expression via sensing of endogenous RNA levels represents a major emphasis of synthetic biology. Initial work began with ribosome-binding sites rendered inaccessible by inclusion in RNA stem-loop structures but that could be rendered active by binding of a complementary cellular RNA in *E. coli*.[215] Further work extended this concept to control a eukaryotic transgene using an internal ribosome entry site (IRES) (Figure 5A).[216] In this system, the IRES is concealed within a structured RNA, and the exposure of the IRES—necessary for translation—is triggered by the binding of an endogenous RNA to the occluding sequence. This mechanism allows for the precise control of transgene expression based on the presence of specific cellular RNAs.

An orthogonal system was recently generated based on endogenous ADAR enzymes. It was discovered that exogenous mRNA designed to base pair with endogenous transcripts could induce editing by endogenous ADARs.[147] By incorporating a stop codon within a coding sequence that is complementary to an endogenous transcript and appended to the transgene, translation of the protein product encoded by the transgene beyond the stop codon becomes dependent on the expression of the complementary native mRNA and subsequent ADAR-mediated A-to-I editing (Figure 5B).[217–219] Thus, the translation of a transgene depends on the cellular expression of a selected endogenous RNA, resulting in highly programmable, cell-type-specific expression of transgenes without the need for additional protein components.

Current challenges and future opportunities in gene and cell therapy

Specificity of therapeutics

The development of cell-type-specific promoters for targeted transgene expression remains a critical focus in gene therapy. However, an ongoing challenge is achieving the optimal level of specificity for each indication. For muscle-related conditions, the ideal targeting strategy may vary from broad (cardiac and skeletal muscle) to specific (skeletal muscle) to highly specific (myocytes only), depending on the disease context. Advancing our understanding of disease-relevant cell types is crucial for informing these targeting decisions. For

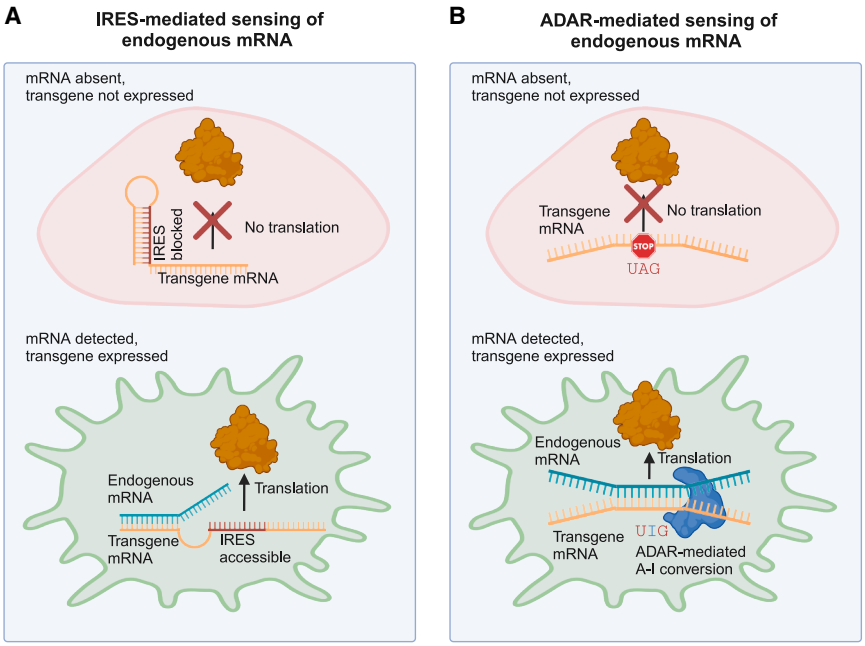

Figure 5. Sensing of endogenous mRNA expression
(A) Occlusion of an internal ribosome entry site (IRES) by an RNA hairpin structure can be alleviated by interaction with an endogenous mRNA, allowing translation only in cells which express that mRNA. (B) The intrinsic A-to-I editing performed by ADAR enzymes exclusively on dsRNA allows UAG to UIG editing only in the presence of the cognate endogenous mRNA.

instance, recent discoveries in neurodegenerative diseases have challenged the historical view that the disease etiology is driven solely by neurons by highlighting the significant roles of microglia and astrocytes.[220,221] Thus, integrating biological findings with engineering advances will be a major focus of future development for cell-type-specific promoters, regulatory elements, and vectors.

Identification of genomic elements that modulate disease and cell phenotypes

In addition to cell types, continued biological advances will be necessary to identify causal drivers of disease and the genomic elements that regulate them. Continuing to parse regulatory networks that govern disease processes is essential for identifying effective therapeutic targets. The success of targeting the *BCL11A* erythrocyte-specific enhancer in sickle cell disease treatment exemplifies the potential of this approach.[165] Continued efforts in functional genomics and CRISPR screening are crucial for mapping these regulatory networks and identifying key elements that control gene expression that is causal for relevant cell phenotypes. Moreover, understanding additional layers of regulatory mechanisms, including miRNAs, lncRNAs, and epigenetic marks, and the interactions between them will be critical for developing comprehensive targeting paradigms in complex disease states.

While current gene therapies primarily focus on well-characterized mutations and clear causal genes, genome-wide association studies (GWASs) have revealed that the majority of disease-associated loci are in non-coding regions.[222] Recent work in coupling functional dissection of GWAS loci with high-resolution tiling CRISPRa/i screens offers promising avenues for identifying targetable variants.[223] These approaches aim to expand the list of candidates amenable to various genome-editing technologies, including nucleases, base editors, and prime editor systems.[224]

Stability of therapies and edits: Balancing durability and safety

The quest for stable, long-lasting gene therapies presents a complex landscape of benefits and challenges. AAV-mediated delivery, while achieving semi-stability through episome formation and providing years of therapeutic transgene expression in some settings, faces limitations due to immune responses. Patients may develop immunity to AAV capsids or genome-editing reagents after initial exposure, limiting redosing strategies. Stability may also be compromised by immune-mediated clearance, promoter silencing, toxicities such as the unfolded protein response, or slow turnover of cells over time. Therefore there is tremendous need for continued development of redosable therapies, such as lipid nanoparticles (LNPs) and mRNAs, targeted to extrahepatic tissues, immunomodulation strategies that enable redosing, and studies to understand the mechanisms of AAV stability. Furthermore, the constraints of clinical trial designs, with their need for discrete, measurable endpoints, often hinder the long-term monitoring necessary to fully assess treatment durability before approval. Consequently, innovation in regulatory structures is essential for enabling these new classes of therapeutics.

Genome-editing technologies offer the promise of stable modifications after transient exposure to the therapeutic agent. However, they also carry inherent risks, including off-target edits and chromosomal translocations resulting from double-strand breaks.[225,226] Epigenome editing has emerged as a promising alternative, circumventing the risks associated with DNA damage and repair. However, achieving epigenetic memory, resulting in stable epigenetic modifications persisting through cell division or after loss of effector expression, remains challenging in some settings. Significant effort is focused on identifying novel effectors for both transient and stable epigenome modification.[227–230] Despite these efforts, few effector combinations capable of reliable epigenetic memory have been found. Although long-term epigenetic memory has been demonstrated in animal models, such as with stable repression of PCSK9 for more than 300 days,[231] extended long-term stability in human patients remains to be proven.

In cases where extended long-term expression of a transgene is readily achievable, one must also consider the ability of the cell system to maintain the desired phenotype. This nuanced relationship is exemplified in T cell therapies, where both native and CAR-T cells exhibit dysfunction following chronic antigen exposure and as a result of immunosuppressive tumor microenvironments.[232] In these cases, long-term transgene (e.g., CAR) expression does not guarantee prolonged therapeutic efficacy, although circumventing CAR-T exhaustion is an active area of research.[233] This phenomenon raises questions about similar challenges in other cellular systems. For instance, cells derived from induced pluripotent stem cells may struggle to maintain long-term functionality due to incomplete differentiation or prolonged expression of a lineage-specifying TF at non-physiological levels. These examples demonstrate the critical need for gene expression control systems that closely mimic natural regulatory networks and expression patterns. To address these challenges, researchers are expanding the toolkit of genetic regulatory elements and inducible gene expression systems. This includes the development of more sophisticated promoters, regulatory sequences, and epigenetic effectors that dynamically respond to the cell state. The goal is to create gene therapy systems that not only achieve long-term expression but also maintain cellular health and function over extended periods.

Delivery in vivo

Safe and effective delivery is arguably the greatest current challenge to gene therapy. The development of technologies to alter gene sequence and expression has far outpaced the ability to deliver these tools efficiently and specifically *in vivo*. Monumental efforts to characterize and engineer next-generation AAV vectors, non-AAV viral vectors, LNPs, and other novel delivery modalities are underway. This represents one of the largest and most active areas of preclinical development and has been reviewed comprehensively elsewhere.[234–239]

Clinical trial design and the regulatory landscape

As therapeutic modalities evolve, so must clinical trial and regulatory guidelines. Some aspects of gene therapies (i.e., potential long-term stability after single dose) pose challenges to traditional clinical trial design, as the durability of treatment and potential for redosing may be relevant for assessing relative benefit. Further, new safety and efficacy measures continue to be developed as new gene therapy and editing technologies enter clinical trials. The continued progress of these therapies into clinical testing will establish the requirements for demonstrating on-target efficacy and off-target safety for different classes of gene and epigenome editors.

These factors are particularly critical when considering permanent changes like genome editing, and highlight the importance of developing sophisticated control systems. One such example is the incorporation of a safety switch into a CAR-T cell therapy. For example, CD19.CAR T cells were engineered with a rimiducid-triggered caspase-9 safety switch that eliminates CAR-T cells in a dose-dependent manner.[240] A patient with relapsed B cell acute lymphoblastic leukemia displayed significant immune effector cell-associated neurotoxicity syndrome after infusion with these cells, but triggering of the safety switch quickly and effectively mitigated the neurotoxicity and removed the CAR-T cell population.[62] In this case, the trial design and pre-specification were designed to include criteria for use of the safety switch, representing an example of engineered control systems that may gain popularity alongside the expansion of new gene and cell therapies.

Finally, it remains to be seen how gene and cell therapies will fit into the treatment paradigms for non-life threatening conditions, and in these cases how they will be perceived by the regulatory agencies and the general public. Such issues warrant continued discussion regarding when, why, and where gene and cell therapies are warranted and justified, and gene regulation technologies to enhance safety and efficacy are likely to be important factors in that dialogue.

Conclusion

In this review, we discuss current and emerging technologies for regulating gene and cell therapies. Several gene therapies have already been developed for otherwise untreatable genetic diseases. However, rapid technological development is enabling the next wave of gene therapies to be tightly and dynamically regulated. Promoters, miRNA cassettes, and other regulatory elements incorporated in the transgene can regulate cell type specificity and expression strength. Small molecule-, oligonucleotide-, and protein-based control systems can regulate expression of endogenous and exogenous genes. Finally, native gene regulatory mechanisms and interactions can be modulated via the epigenome, epitranscriptome, and targeted degradation of proteins. Significant work is underway to turn these emerging technologies into clinical realities, which will exponentially expand the scope of gene and cell therapy and its impact on modern medicine.

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AUTHOR CONTRIBUTIONS

G.L.B., S.J.R., N.I., and C.A.G. conceptualized and drafted the manuscript.

DECLARATION OF INTERESTS

C.A.G. is a cofounder of Tune Therapeutics and Locus Biosciences and is an advisor to Tune Therapeutics and Sarepta Therapeutics. G.L.B., S.J.R., N.I., and C.A.G are inventors on patent applications related to genome engineering and gene and cell therapy.

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