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Programmable Gene Expression in Gene Therapy: Engineering Regulatory Circuits for Tissue Specificity, Dose Control, and Durability

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Abstract

Programmable expression control can determine whether a gene-therapy product remains spatially selective, dose-appropriate and controllable after delivery. This critical translational review examines regulatory circuits as a distinct engineering layer, rather than treating vector targeting or initial transduction as sufficient evidence of controlled therapy. Peer-reviewed experimental studies and focused methodological literature published through 1 November 2026 were selected purposively to compare tissue-restricted transcription, microRNA-mediated detargeting, inducible RNA and protein switches, and circuit architectures that suppress basal leakage. Evidence was interpreted according to the biological model, delivered construct, measured output and observation period. Across these approaches, specificity emerges from multiple non-equivalent barriers: entry into a cell, transcriptional competence, RNA fate and protein production. Large induction ratios may coexist with residual basal expression, heterogeneous cellular responses or incomplete reversibility. Studies in mice and nonhuman primates further show that persistence of vector genomes does not guarantee persistence of expression, while transient expression loss does not always mean permanent vector clearance. The central translational requirement is therefore a linked validation package: tissue-resolved exposure and output, basal and induced dose, withdrawal and rechallenge, and longitudinal measurements of circuit state, immunity and function. These considerations guide condition-specific design choices without implying clinical validation of preclinical switches.

Keywords: Therapeutic transgene regulation, Synthetic promoters, MicroRNA detargeting, Inducible RNA switches, Adeno-associated virus, Expression durability

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Introduction

A therapeutic transgene needs more than delivery to the right anatomical compartment. Benefit may depend on expression in an appropriate cell population, at a tolerable abundance, for a suitable duration, and sometimes with the option to terminate exposure. A vector can enter an undesired cell without producing detectable protein; conversely, modest transduction of an off-target population may matter if a potent secreted payload is made there. The design variable is thus the joint distribution of vector genomes, transcriptional activity, RNA processing and protein output, not any one assay in isolation. Synthetic-promoter research illustrates the breadth of available regulatory strategies but also their context-specific compromises (Artemyev *et al*., 2024).

The clinical relevance of this distinction is particularly apparent for dose-sensitive products. In a mouse model of Rett syndrome, a microRNA-responsive element incorporated into a miniMECP2 cassette was used to mitigate problems arising from excess expression (Sinnett *et al*., 2021). That experiment does not establish a universal safe dose of MECP2; it demonstrates why control architecture must be considered alongside delivered vector dose. An inducible cassette creates another burden of proof: investigators must show not merely that an external input raises a reporter signal, but also what expression persists without the input, whether switching works across relevant tissues, and whether the intervention improves function without unacceptable exposure. This review asks how those requirements can be compared across otherwise dissimilar platforms.

Review Approach and Translational Evidence Boundaries

A question-driven, targeted search of publicly accessible PubMed, publisher and full-text-indexed records was completed to the search cutoff of 1 November 2026. Searches combined gene therapy, regulatory circuit, promoter, tissue restriction, inducible RNA, reversibility, persistence and immune-response terms; selected works were checked against journal identity and DOI records. The curated allocation consists of 29 distinct peer-reviewed publications spanning mechanistic demonstrations, disease-model experiments, translational counterexamples and focused synthesis. It is an intentionally selected critical-review corpus, not a systematic review, an exhaustive inventory or a basis for pooled effect estimates.



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A regulatory claim was considered interpretable only with its experimental unit and readout attached. Cell-line fluorescence, primary-cell function, animal secreted-protein concentration and therapeutic phenotype occupy different evidentiary levels. For instance, synthetic gene circuits have been used to program multiple functions in engineered human T cells, an important cellular proof of concept that should not be recast as systemic in vivo vector control (Li *et al*., 2022). The manuscript distinguishes measured induction from inferred therapeutic dosing, and reporter restriction from clinically meaningful selectivity. Some source assessments are abstract-bounded; therefore, numerical comparisons not independently validated against primary results are intentionally omitted. A favorable experiment is weighed alongside restricted tissue responsiveness, immunity-associated expression loss and assay limitations rather than being taken as a general platform property.

Biological and Engineering Layers of Expression Control

Expression systems operate as partly independent biological filters. Vector dose, route and capsid properties influence which cells receive a genetic cassette. Promoter and enhancer sequences determine which of those cells can transcribe it. Endogenous microRNAs, engineered ribozymes and small-molecule-responsive RNA elements then influence RNA stability, splicing, editing or translation. Protein turnover and secretion finally shape the concentration and duration of the effective payload. These filters may be combined, but their effects cannot be inferred by multiplying isolated fold changes from different studies: the same tissue, delivery amount, assay and time window are needed for a meaningful combined estimate.

The engineering consequence is that exposure, selectivity and regulation require separate measurements. A reporter confined to a tissue does not establish that every transduced cell is transcriptionally silent elsewhere; an apparently selective promoter may simply have been tested in a model with uneven vector delivery. Conversely, high promoter activity in cultured cells does not establish the spatial distribution of output in a living animal. Large in vivo screens of regulatory elements can compare tissue-associated activity while making the uneven distribution of vector exposure itself a relevant interpretive concern (Brown *et al*., 2025). The hierarchy in **Figure 1** is therefore a map of conditional control opportunities, not a validated universal molecular sequence.

Tissue Restriction and Context-Dependent Expression

Transcriptional selectivity depends on regulatory DNA and the transcription-factor environment of the target cell. Designed promoters tested in retinal pigment epithelial cells have demonstrated that strength and desired cellular preference may not rise together (Johari *et al*., 2021). Promoter comparisons within a mucopolysaccharidosis mouse model likewise motivate matching promoter choice to the disease tissue and output requirement, rather than treating a ubiquitous promoter as inherently superior or inferior (Khan *et al*., 2025). In epilepsy-oriented work, an activity-responsive promoter was designed to detect hyperactive neuronal states; its reporter behavior is evidence about an input-dependent expression pattern, not itself proof that seizure outcomes can be controlled clinically (Burke *et al*., 2023).

MicroRNA detargeting offers a complementary negative filter. Liver-associated endogenous microRNAs have been used to suppress vector-derived transcripts within particular cellular contexts (Dhungel *et al*., 2018). More recent mouse experiments combined capsid, promoter and microRNA-response elements to improve expression targeting in adipose tissue and skeletal muscle while reducing selected off-target expression (Blahetek *et al*., 2025). These results support layered restriction but remain model- and cassette-specific. Similarly, a microglia-directed AAV strategy integrating vector and expression regulatory elements has been investigated across experimental species; specificity claims still depend on which cell populations and readouts were sampled (Yu *et al*., 2026). Cross-model discrepancies in lung-directed promoter performance reinforce that a successful mouse tissue signal cannot simply be transferred to other species or tissue preparations (Zielinska *et al*., 2026).

Tunability, Reversibility, and Input–Output Behavior

The first question for an inducible system is what the control input actually changes. A CRISPRa-responsive promoter library alters the transcriptional response of tested mammalian constructs; input-dependent reporter behavior should not be equated with pharmacologically reversible therapy (Fu *et al*., 2025). Circuit topology may alter this relationship: CASwitch incorporates feedforward and mutual-inhibition logic to reduce uninduced leakage while preserving induced output in mammalian cellular experiments (De Carluccio *et al*., 2024). Its performance is informative about leak reduction and network design, but its biosensor and vector-production demonstrations are not clinical gene-therapy outcome tests.

At RNA level, different molecular controls offer different operational trade-offs. A ribozyme-based on-switch controlled AAV-delivered transgene output with an antisense inducer and was repeatedly inducible over prolonged observation in mice (Zhong *et al*., 2020). The reported dynamic range belongs to that system and its tested assays; it does not define a guaranteed response for an unrelated payload. A compact ADAR-dependent adaptamer uses ligand-responsive RNA structure and endogenous editing to permit transgene expression, with FGF21-dependent metabolic effects investigated in AAV-treated mice (Bryant *et al*., 2026). Another system, RisdiON, employs a small molecule to alter RNA splicing; reversible AAV-delivered expression was demonstrated in mice alongside cell-based validation (Mendel *et al*., 2026). These mechanisms differ in trigger, RNA intermediate and reversibility conditions, despite sharing the broad label of an inducible switch.

Post-transcriptional regulation is not confined to RNA stability or splicing. A split RNA switch can combine pre- and post-translational control to create cell-context-selective output in human cells (Abe *et al*., 2025). A responsive RNA circuit has also been investigated as a way to link intracellular microRNA information to imaging and treatment-related outputs in experimental models (Shi *et al*., 2025). At a different level, a trimethoprim-responsive endoplasmic-reticulum-associated degradation architecture regulates secreted protein output after AAV delivery in mice (Gao *et al*., 2026). Each platform demands

a different measurement of both the controlled molecular state and the final active product. Drug availability, response delay, residual basal activity and capacity for repeat activation cannot be collapsed into a single headline induction ratio.

Induction is also spatially uneven. Adult-tissue experiments comparing Tet-On and Tet-Off systems showed differing tissue and cell-type responsiveness, with inducer choice modifying the achievable behavior (Taguchi *et al*., 2025). An RNA-switch strategy used with AAV gene editing has likewise been evaluated for temporary output and reduced unwanted readouts in experimental systems, not proven as a broadly safe clinical controller (Zhang, Yang, *et al*., 2026). Accordingly, **Table 1** compares demonstrated dimensions and the interpretive limits that must accompany them, rather than ranking platforms by incomparable fold changes.

Table 1. Experimentally supported control dimensions and the evidence boundaries for representative regulatory mechanisms

Mechanism / reported setting	Directly shown control dimension	Unresolved transfer boundary
Synthetic promoter; retinal or disease-cell models (Johari *et al*., 2021)	Context-dependent transcriptional output; specificity–strength trade-off	Promoter-only comparisons do not establish biodistribution or clinical targeting
Endogenous miRNA target sites; mouse tissue targeting (Blahetek *et al*., 2025)	Post-transcriptional suppression of selected off-target expression	Species- and tissue-dependent miRNA context; incomplete silence possible
CASwitch; cultured mammalian cells (De Carluccio *et al*., 2024)	Basal leak suppression with induced output	Therapeutic durability after in vivo delivery not demonstrated by these assays
RNA ribozyme on-switch; AAV-treated mice (Zhong *et al*., 2020)	Repeated inducible output with long observation	Payload, oligonucleotide delivery and target-organ transfer not generalized
Drug-responsive splicing; cells and mice (Mendel *et al*., 2026)	Dose-responsive and reversible regulation in assessed systems	Input exposure and efficacy in human therapies remain untested
Protein-level ERAD switch; AAV-treated mice (Gao *et al*., 2026)	Small-molecule-responsive protein secretion	Secretory payload and animal context constrain generalization

Note. Entries summarize reported qualitative capabilities, not cross-study rankings; NR indicates not reported in the cited abstract or inspected material. Original review synthesis.

Figure 1 maps these layers against the validation axes that would be required to distinguish delivered genomes from actual, controllable therapeutic output.

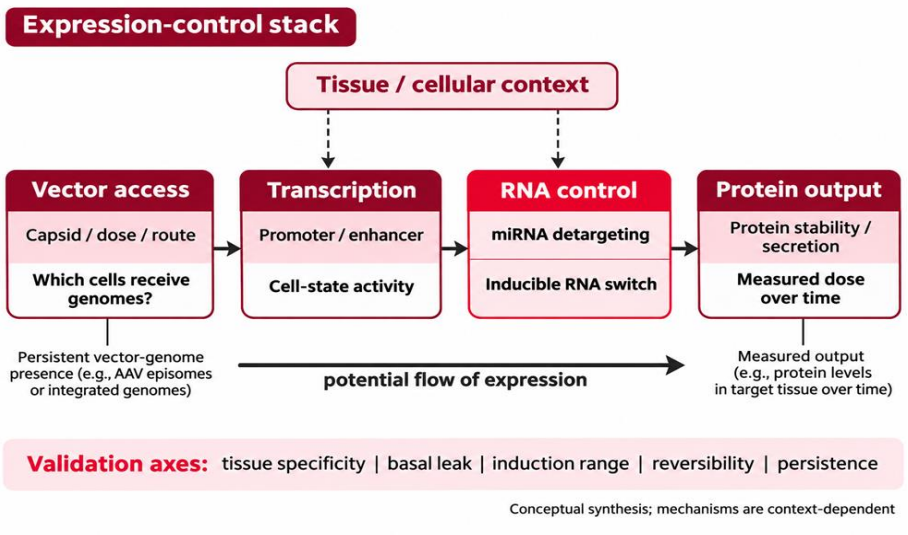


Figure 1. Spatial and temporal checkpoints separating vector access from controlled protein expression

Note. Conceptual synthesis of conditional expression-control layers. Solid horizontal arrows indicate possible progression toward measured protein output, not a proof of causal sufficiency; dashed arrows represent context dependence. Vector access, transcription, RNA processing and protein output should each be evaluated in relevant cells and at appropriate times. Mechanisms reflect the cited gene-regulation literature; the layered comparison itself is an original synthesis (De Carluccio *et al*., 2024).

Durability and Failure Modes

Durability is ambiguous unless the measured entity is named. AAV vector genomes may persist without detectable protein expression; transgene product may persist after transcription ceases; and a clinical phenotype may improve or decline on a different timescale again. A circuit can remain inducible after months yet still generate

heterogeneous basal output or gradually alter its apparent drug response. Consequently, expression duration, regulatory stability and lasting therapeutic benefit are distinct endpoints requiring separate evidence.

Delivery Constraints, Immune Responses, and Cellular Turnover

Immune processes complicate the interpretation of regulation. In a mouse hepatic gene-transfer model, pre-existing reactivity toward a contaminant-associated antigen contributed to liver inflammation and reduced transgene output, illustrating that reduced protein need not indicate a successfully switched-off promoter (Brimble *et al*., 2024). In a nonhuman-primate liver study, vector genomes remained detectable despite immune response and loss of hepatic transgene expression (Pichard *et al*., 2025). Genome persistence therefore cannot, without an output assay, be used as a proxy for an intact control circuit. Nor can a single immune-cell assay determine the spatial history of all transduced populations.

The relationship can also run in the opposite direction. Following intramuscular AAV administration in nonhuman primates, loss of transgene expression in an immune context was subsequently followed by re-expression while vector genomes persisted (Journou *et al*., 2025). Such observations challenge the assumption that every period of low output reflects irreversible elimination of all expressing cells. Ocular vector administration provides a distinct immune setting: experimental subretinal delivery showed that peripheral anti-transgene T-cell measurements depend on local exposure and immune context (Vendomèle *et al*., 2024). For proliferating tissues, cellular division can further alter vector-genome representation, so stability must be tested in the actual target cell turnover environment rather than inferred from a quiescent model. The available results justify measuring vector DNA, RNA, protein, tissue injury and immune markers together, not attributing all loss of expression to a failed regulator.

Feedback Behavior, Heterogeneity, and Loss of Control

Even when a circuit has been designed with feedback-like motifs, its performance depends on the cellular state in which those motifs operate. The cellular CASwitch results indicate that engineered topology can suppress basal leakage under specified test conditions (De Carluccio *et al*., 2024). They do not show that every transduced cell has the same response function after long-term delivery. Copy-number variation, cell-state transitions, altered input exposure and selective loss of high-expressing cells could all change an average population signal. These are plausible failure mechanisms requiring experimental tests, not outcomes automatically demonstrated by an induction curve.

A separate mouse experiment using temporally controlled OSK expression in glaucoma underscores the need to follow transcription and function on distinct schedules: transgene expression after inducer withdrawal and visual benefit did not share an identical trajectory (Karg *et al*., 2023). Repeated activation, withdrawal and rechallenge are therefore more informative than a single on/off observation. Basal leakage should be measured before induction and after withdrawal; cell-resolved readouts should accompany population averages; and a protein-level time series should be paired with a physiological endpoint when a payload has a therapeutic rationale. **Table 2** organizes these failure modes by the observations needed to distinguish circuit instability from confounding biology. The table is a proposed validation synthesis, not a claim that every cited study performed every test.

Table 2. Interpreting apparent loss of expression control across biological conditions

Observed pattern / context	Competing interpretation	Proposed discriminating measurement
Reduced output with hepatic immune injury (Brimble *et al*., 2024)	Transduced-cell injury or immune loss rather than switch-off	Pair vector DNA, RNA, protein and tissue-injury measures
Genome persists, expression falls in primate liver (Pichard *et al*., 2025)	Genome presence without active expression	Cell-localized transcription, protein and inflammatory readouts
Expression returns after muscle immune-associated loss (Journou *et al*., 2025)	Temporary silencing rather than universal cellular clearance	Longitudinal output with tissue genome persistence
Tet systems differ across adult tissues (Taguchi *et al*., 2025)	Drug sensitivity or cellular competence differs by site	Matched tissue-wise basal, induction and withdrawal curves
Cell-average output after engineered regulation (De Carluccio *et al*., 2024)	Population mean masks heterogeneous cells	Single-cell distribution and repeat activation tests
Function outlasts measured transcription (Karg *et al*., 2023)	Therapeutic effect and expression run on different clocks	Joint temporal protein and functional readouts

Translational Design and Validation Priorities

Translational validation should begin with the consequence of misplaced or excessive expression, then select assays capable of detecting that particular failure. In the DB-OTO study, an engineered mMyo15 promoter was evaluated for hair-cell restriction, and comparison with a ubiquitous promoter helped connect cellular targeting to local safety and sustained auditory responses in otoferlin-deficient mice (Chung *et al*., 2025). Its functional readouts strengthen a cell-targeting argument within the studied ear model; they do not show that the promoter is universally specific or that it produces reliable expression after systemic delivery. A proposed first validation tier is therefore to measure vector exposure and transgene output separately in

intended and plausible off-target populations, using a relevant payload rather than a reporter alone.

Quantitative controllability requires distinct baseline, induced, and post-withdrawal measurements. At minimum, tests should report the input actually experienced by the tissue, absolute or appropriately normalized output, between-cell variation, the time taken to activate and decline, and behavior on rechallenge. The protein-level switch described by Gao *et al*. (2026) shows why secretion kinetics must be interpreted at the level of the therapeutic molecule, not inferred from transcript abundance. A rapid response in one secreted-payload mouse setting does not establish the same kinetics for an intracellular protein, a different organ, or repeated human dosing. No common numerical performance threshold can be defensibly transferred across these studies without matching the construct, measurement and model.

A therapeutic endpoint adds a separate evidentiary layer. In a regulated miniMECP2 program, different administration ages and routes were evaluated alongside mouse neurological and survival outcomes, rather than relying only on detectable vector genomes or protein (Sadhu *et al*., 2024). A hyperglycemia-responsive circuit similarly linked stimulus-dependent therapeutic-protein expression to improved metabolic outcomes in diabetic mice (Zhang, Deng, *et al*., 2026). Such studies motivate experiments that jointly track input, regulated output, tissue distribution and disease-relevant function. They cannot establish a universal therapeutic window: safety, input dynamics, vector persistence and therapeutic phenotype remain disease- and payload-dependent.

Table 3 translates these distinctions into a proposed validation sequence. The proposed assays are a critical synthesis, not thresholds agreed by regulators or tests performed uniformly in the cited papers. An apparently successful result at one tier permits progression to a more demanding test; it does not substitute for that test. Even a reversible reporter response must be reassessed with a therapeutic payload, relevant dosing interval, and tissue-specific exposure profile.

For practical study design, a control qualification protocol should include one deliberate stressor at a time before combining them. Candidate stressors include changes in inducer exposure, inflammatory state, target-cell proliferation, and different starting vector loads. Each should be paired with a prespecified readout that distinguishes a reversible shift in regulatory behavior from outright disappearance of transduced cells. For secreted payloads, circulating concentrations should be linked to an anatomical production source where possible; for intracellular payloads, tissue-averaged protein is an incomplete substitute for the proportion of responsive cells. These tests would make failed switches as informative as successful ones and reduce dependence on an isolated peak-response metric.

Table 3. Orthogonal evidence requirements for programmable therapeutic expression

Property to validate	Proposed measurement package	Boundary that must remain explicit
Spatial restriction	Vector DNA plus tissue- and cell-resolved RNA/protein; off-target survey	Reporter restriction differs from payload safety; delivery varies
Magnitude and leakage	Basal and stimulated output, input exposure, cell-distribution profiles	Induction ratio alone hides absolute residual dose
Reversibility	Input withdrawal, response lag, repeat induction and protein clearance	Drug delivery and payload turnover shape observed off-kinetics
Durability	Time-matched vector, RNA, protein, immunity and function	Genome persistence differs from circuit and benefit persistence
Therapeutic relevance	Matched control cassette, functional endpoint and adverse outcomes	Preclinical effects do not constitute clinical validation

Note. Prospective review synthesis; the proposed measurements are not a published consensus or clinical acceptance criteria. Evidence examples: Chung *et al*. (2025); Sadhu *et al*. (2024); Gao *et al*. (2026); Zhang, Deng, *et al*. (2026).

The evidence hierarchy in **Figure 2** keeps four control properties orthogonal. Reporting an induction ratio says little about tissue specificity; long-lived genomes are not proof of continued regulation; and a transient physiological benefit is not direct evidence of a stable on/off switch. The figure's horizontal progression denotes increasingly demanding validation contexts, with dashed boundaries acknowledging that species, dose, cellular turnover and treatment schedules can prevent transfer of an apparent effect.

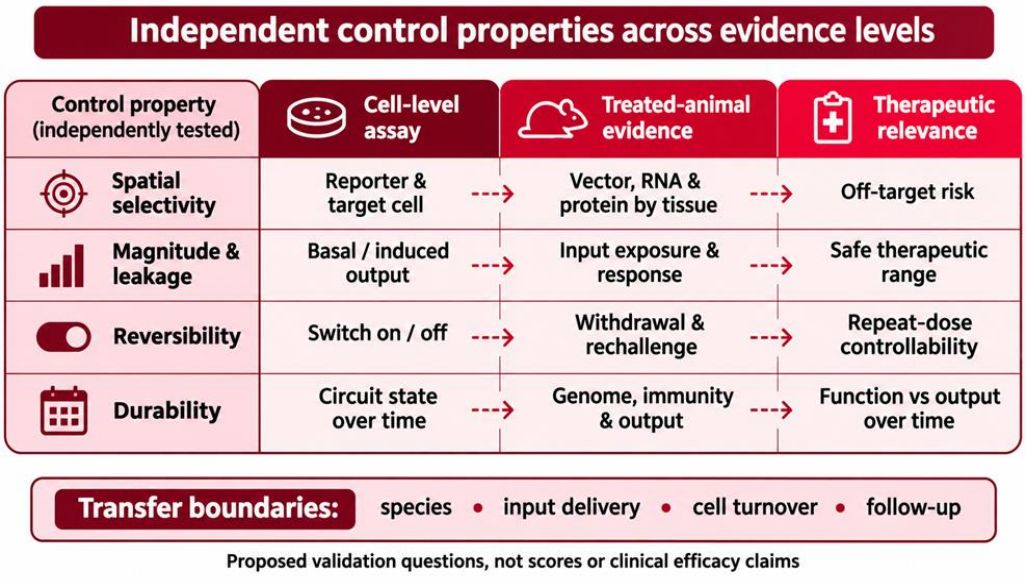


Figure 2. Orthogonal validation of expression-control properties across translational evidence levels

Note. Proposed validation map, not an empirical performance ranking. Rows define independent control properties; columns identify cellular, in vivo and therapeutic-context evidence. Horizontal connectors show questions to be reassessed across evidence levels, not causal proof or clinical validation. All cells are prospective measurements rather than results of a single study.

Results and Discussion

The control options are best compared according to what is being constrained. A cell-restricted promoter can reduce transcription in exposed but transcriptionally unsuitable cells; microRNA response sites can provide another cellular-state-dependent suppression layer; a drug-responsive RNA switch can change transcript availability; and an inducible protein module can act closer to measured output. Each operates at a different mechanistic level, and none alone establishes acceptable therapeutic dose. Their combination may improve selectivity, but it may also create new dependencies on tissue state, regulator availability or inducer penetration. A stacked design should therefore be tested as an integrated cassette, not justified by separately published performance figures.

A further distinction concerns open-loop versus responsive regulation. External dosing can offer an investigator-adjustable input, whereas circuits that sense disease-associated signals may change output as the physiological environment changes. This can potentially reduce the need for repeated manual adjustments, yet feedback may be delayed, noisy or altered by disease progression. Thus a glucose-sensing experiment in diabetic mice is valuable as a demonstration of coupled sensing and response, but does not prove that a closed-loop controller will remain safe after months of varied diet, medication or disease status. Appropriate perturbation and withdrawal studies are still required.

The strongest translational argument joins spatial exposure, circuit-state measurements and functional benefit over a clinically relevant duration. Animal phenotypes cannot validate pharmacological control in humans, and an improved outcome cannot reveal on its own whether benefit arose from precise regulation or simply sufficient expression. More informative comparisons use matched promoter or regulator controls, measured off-target output, and longitudinal readouts that separate the payload's persistence from the vector's fate. Where a control layer fails, that separation should identify the biological cause before the architecture is revised.

This differentiation also changes what a negative result means. If expression drops alongside inflammatory injury but vector genomes remain detectable, the immediate question is whether transcriptional competence or the expressing cell population changed. If the protein remains after transcript suppression, the relevant delay may be payload clearance rather than regulator failure. And if a physiological response persists after expression wanes, benefit may reflect downstream biology rather than persistent switching. Such alternatives require different sampling schedules and are not interchangeable measures of circuit durability.

Limitations

This targeted critical review purposively selected 29 publications rather than attempting comprehensive systematic retrieval. It does not provide a pooled comparison, formal evidence-certainty score or estimate of all relevant trials. Some evidence assignments relied on journal abstracts and accessible sections rather than full inspection of every original result and supplementary figure; those sources support bounded mechanistic statements, not precise cross-study effect estimates. Direct comparisons are also limited by disparate species, target tissues, reporters, delivery routes, induced inputs, timescales and normalization conventions.

There is a second limitation in the proposed synthesis itself: the validation categories organize questions but have not been prospectively tested as a combined regulatory standard. A reassuring short-term cell assay may miss delayed immune

responses, proliferative dilution, variable patient exposure or changes in disease state. Conversely, absence of a clinical trial for a particular switch should not be mistaken for evidence that the underlying mechanism is ineffective. Future evaluations should prioritize within-study controls and transparent reporting of negative as well as positive trajectories.

Conclusion

Programmable expression in gene therapy is a multidimensional control problem, not a single measure of vector targeting or fold induction. Tissue restriction, magnitude, reversibility and durability must each be measured at the molecular and therapeutic levels appropriate to the payload and delivery setting. Promoters, RNA-based detargeting, inducible switches and protein-level regulators offer complementary forms of control, but their value depends on basal output, tissue context, input exposure and biological persistence. The most defensible translational path pairs tissue-resolved expression measurements with withdrawal and rechallenge, longitudinal evidence of circuit integrity, and disease-relevant functional outcomes. These requirements provide a testable design agenda without presuming that promising animal performance already establishes safe, durable human regulation.

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