Poster #431

Synthetic neuronal promoters that surpass synapsin in the central nervous system

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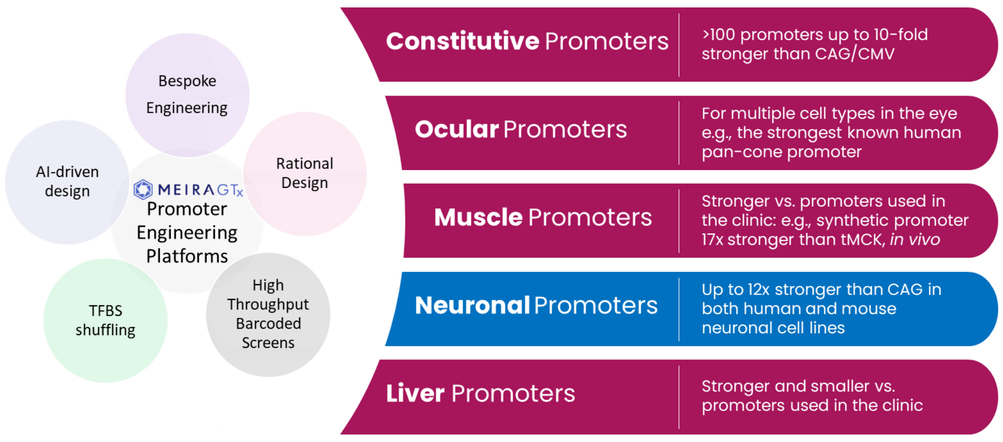
ABSTRACT

The promoter is an essential *cis*-regulatory element in any DNA-based gene therapy. It directly controls gene transcription and thereby therapeutic protein expression. In the context of genetic medicines, stronger promoter activity may enable a lower vector dosage to achieve therapeutic effect, reducing safety risks associated with high vector dosages, as well as reducing manufacturing costs. In addition to strength, a tissue-specific promoter may further attenuate off-target effects and improve safety outcomes. Two well-known neuron-specific promoters are the human synapsin (hSyn) and the rat neuron-specific enolase (rNSE) promoters. We rationally engineered hSyn- and rNSE-based promoters to increase potency while maintaining specificity, thereby creating an improved set of strong, durable, and neuron-specific promoters amenable to central nervous system (CNS) gene therapy applications. The size of engineered promoters ranged from 1120 to 1460 base pairs. Potency and specificity were assessed *in vitro* and *in vivo*. hSyn and rNSE promoter variants were up to 7.5-fold stronger in transfected N2a cells with minimal loss of cell specificity as assessed by comparing potencies in N2a versus in HEK293T cells. The top promoter candidate had an 8-fold improvement over the parental hSyn in AAV9-transduced primary cortical neurons. Promoter specificity was quantified in mouse tissues after systemic AAV9 delivery. Our top promoter was more specific *in vivo* than CAG, a benchmark constitutive promoter. Together our data identified synthetic promoters with greatly improved potency for driving therapeutic gene expression in the CNS.

Promoter engineering can improve strength, specificity, and safety of gene therapies



Benefits of Promoter Engineering for Gene Therapies

- Precise control of therapeutic gene expression[1]
- Cell-specific expression independent of capsid[2]
- Increased potency potentially decreases immune responses and safety risks[3]
- Promoter kinetics impact durability of gene therapy
- Reducing size without sacrificing strength or specificity allows efficient packaging or larger cargo[4]

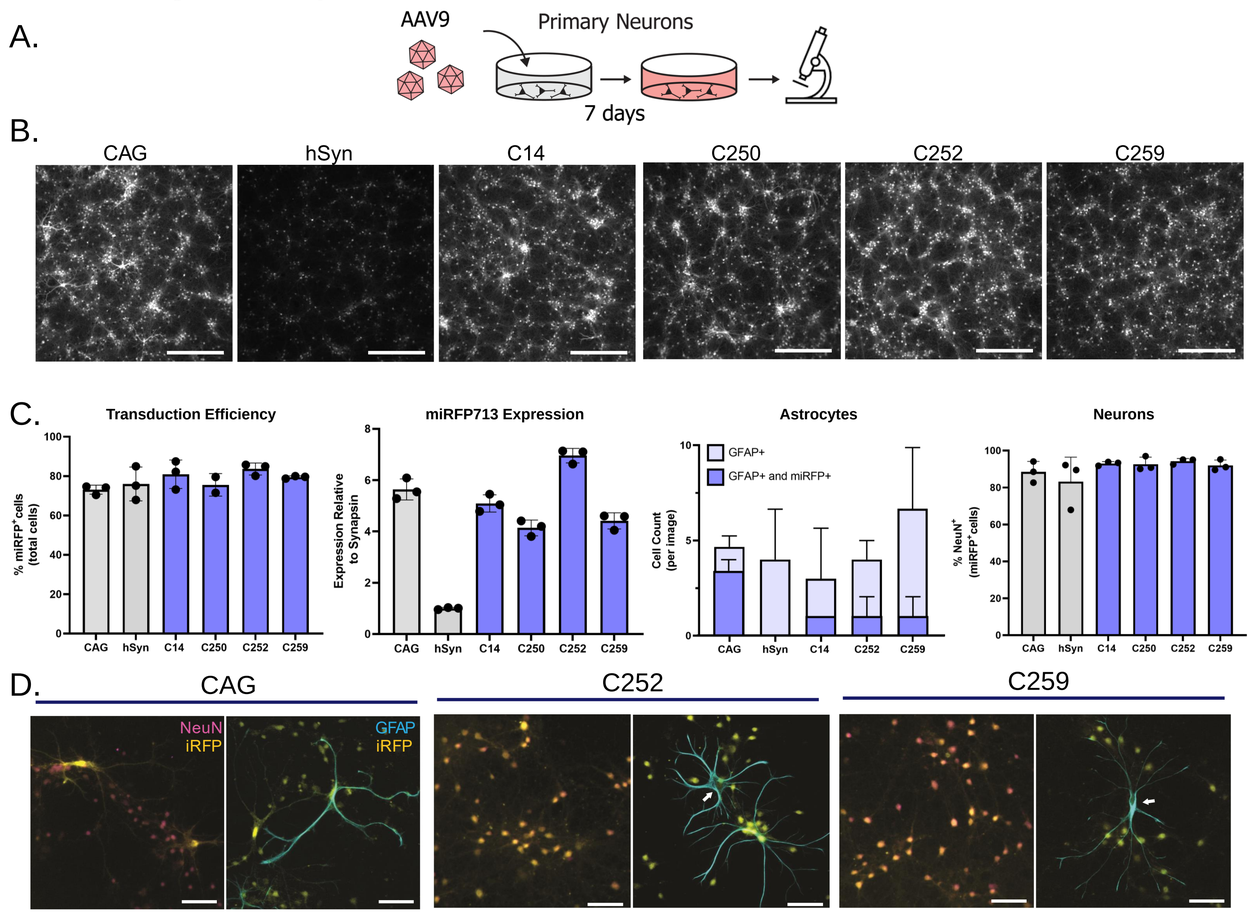
We have built libraries of synthetic promoters and *cis*-regulatory elements available for viral vector optimization. These libraries have a broad range of size, cellular specificity, and potency which can be used in a diverse range of therapeutic applications

Promoters for Central Nervous System Gene Therapies

Two of the most common neuronal promoters are derived from the human Synapsin 1 gene and from the rat Neuron-Specific Enolase gene[5,6]. These promoters provide moderate but highly specific expression for neurons in the CNS. Creating a stronger neuronal promoter would allow for higher expression of therapeutic transgenes at lower viral doses. Lower dosing can prevent serious adverse events such as dorsal root ganglion toxicity. Here we design variants of the hSyn1 and rNSE promoters that are stronger than the parent while maintaining specificity. Our engineered promoters can be multiplexed with MeiraGTx's novel riboswitch technology which together allow for complete temporal and spatial control of expression to make safer gene therapies for the central nervous system.

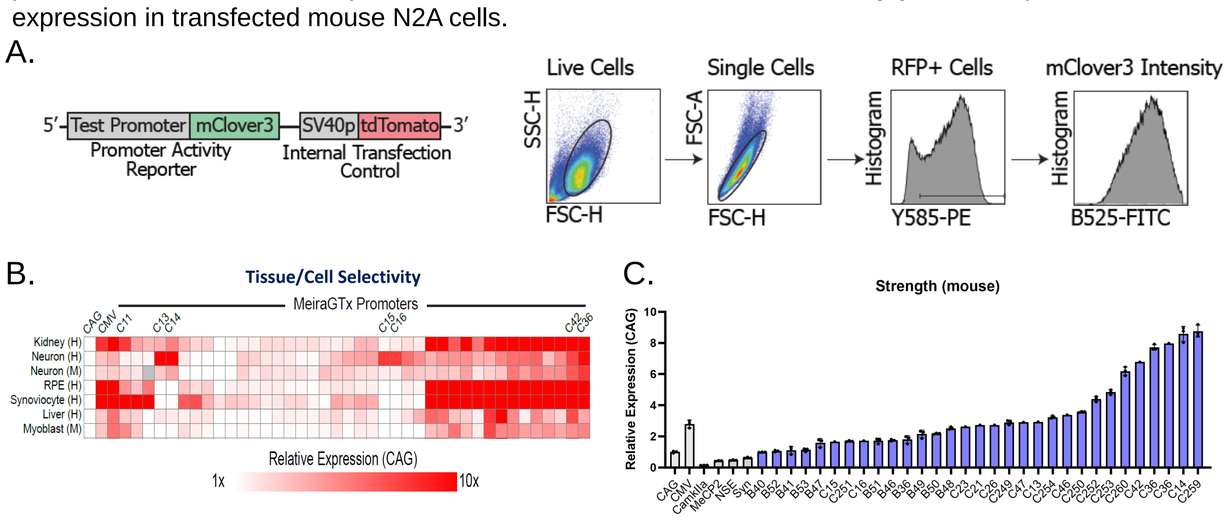
MGTx neuronal promoters drive high expression in primary cortical neurons

Expression and specificity of MGTx promoters in primary neuronal culture. (A). On day 4 *in vitro*, primary mouse cortical neurons were transduced at 50,000 multiplicity of infection (MOI) with AAV9-miRFP713 driven by candidate promoters. **(B).** After 7 days, neurons were imaged to assess miRFP713 expression. Scale bar: 200 µm. **(C).** Transduction efficiency is consistent across constructs. At 7 days post infection (DPI), all MGTx promoters lead to higher expression of miRFP713 than synapsin (hSyn) and show fewer miRFP713-positive astrocytes than CAG. **(D).** Representative images of neuronal specificity by immunocytochemistry (miRFP713 in yellow, NeuN in magenta, GFAP in cyan). Arrows indicate miRFP713-negative astrocytes. Scale bar: 50 µm



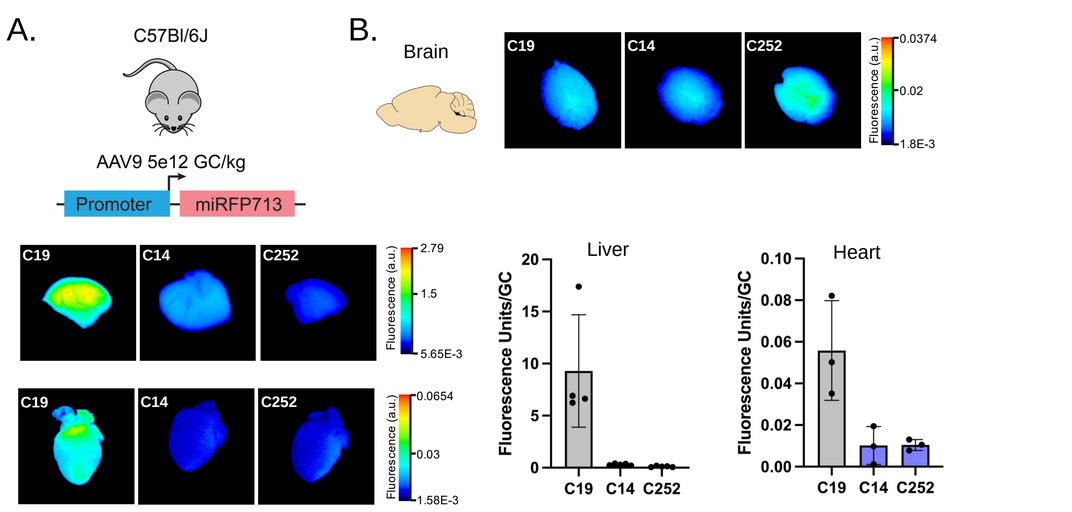
CNS promoter overview

Development and screening of a promoter libraries *in vitro*. (A) We used a FACS-based assay to screen promoter activity *in vitro*. Promoter candidates are cloned upstream of mClover3 (GFP) in a dual-reporter plasmid, which also contains a tdTomato (RFP) expression cassette that is used as an internal transfection control. Promoter activity is quantified as a ratio of the mean fluorescence intensity of mClover3 and tdTomato in single, live tdTomato+ cells. **(B)** Relative expression of MGTx ubiquitous promoters show cell selectivity across human and mouse cell lines *in vitro*. **(C)** Selected promoter expression in transfected mouse N2A cells.



MGTx neuronal promoters are highly specific *in vivo*

Expression in peripheral mouse organs. (A) Male C57Bl/6J mice were injected intravenously with 5e12 GC/kg AAV9-miRFP713 driven by candidate promoters. After 4 weeks, fluorescence was measured for each organ *ex vivo* and genomes harvested for qPCR to quantify transduction. **(B**) Representative images of *ex vivo* fluorescence of whole brain showing higher expression driven by MGTx promoters compared to CAG. **(C)** Peripheral tissue expression normalized to viral genomes shows low off-target expression in the mouse liver and heart driven by MGTx promoters.



Expression and specificity of neuronal promoters *in vitro*

Rational design of neuronal promoters achieves variants stronger than CAG while maintaining original specificity. (A) Relative expression of promoters in mouse N2A (blue), human HEK293T (gray) and human HuH7 (yellow) cells using the dual reporter assay. **(B)** Distribution of mClover3 fluorescence in tdTomato+ N2A cells. **(C)** Designed promoters are all smaller and stronger than CAG. **(D)** Promoter variants are more specific than CAG or CMV, but not as specific as the original promoters.

Conclusions

- hSyn and rNSE promoter variants were up to 7.5-fold stronger in transfected N2a cells with minimal loss of cell specificity as assessed by comparing potencies in N2a versus in HEK293T cells.
- The top promoter candidate had a 7-fold improvement over the parental hSyn in AAV9-transduced primary cortical neurons.
- Our top promoter was more specific *in vivo* than CAG, a benchmark constitutive promoter.
- Together our data identified synthetic promoters with greatly improved potency for driving therapeutic gene expression in the CNS.

Future Work

- Quantify expression and cellular specificity in the mouse CNS *in vivo*
- Verify promoter potency in human iPSC-derived models
- Examine promoter kinetics using long-term bioimaging of the mouse CNS

References

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