Poster 893

MEIRAGTx

Novel rationally designed promoters surpass CAG in human and mouse models

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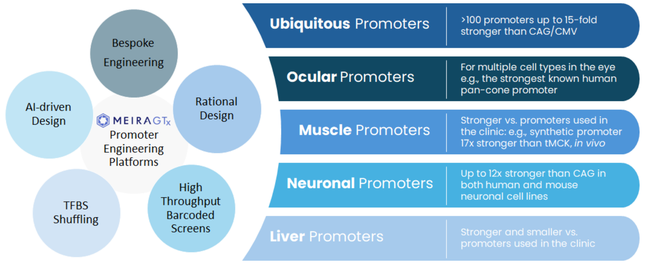
Promoter Engineering to Develop Stronger and Safer Gene Therapies

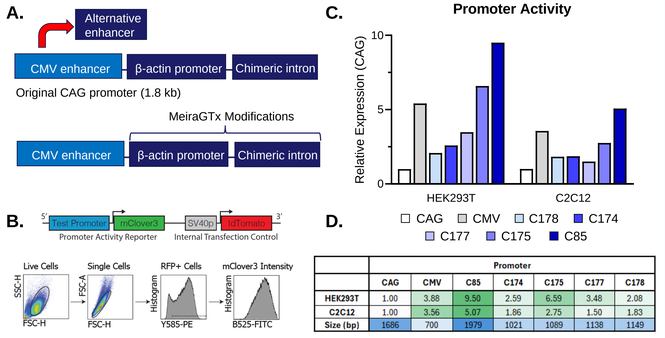
Benefits of Promoter Engineering for Gene Therapies

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



Approximately 50% of gene therapies rely on the CAG or CMV promoters.[2] Due to its size, large transgenes under the control of the CAG promoter cannot be packaged into AAV efficiently, thus limiting its potential for some gene therapy applications.

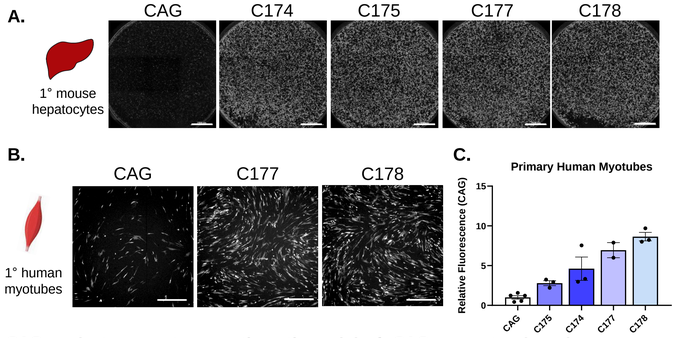
Rationally Designed CAG Variants Increase Expression in Cell Lines



	Promoter						
	CAG	CMV	C85	C174	C175	C177	C178
HEK293T	1.00	3.88	9.50	2.59	6.59	3.48	2.08
C2C12	1.00	3.56	5.07	1.86	2.75	1.50	1.83
Size (bp)	1686	700	1979	1021	1089	1138	1149

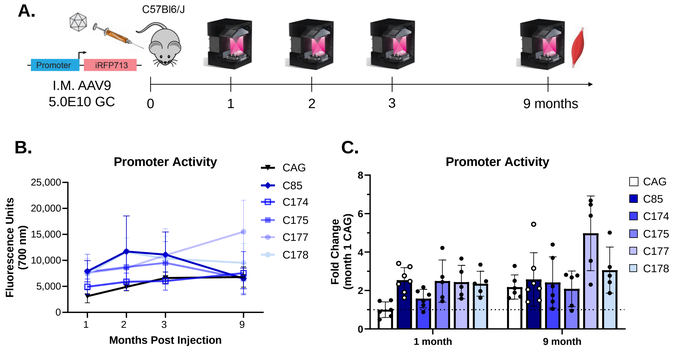
MeiraGTx promoter engineering generates CAG variants that are up to ~10-fold more potent than CAG in cell lines. (A) CAG variants were designed by modifying the promoter, replacing the CMV enhancer, and/or modifying intron regions. **(B)** CAG variant performance was assessed *in vitro* using a fluorescent protein dual reporter plasmid and FACS-based assay. **(C)** Screening in transiently transfected HEK293T and C2C12 cells identified promoters that are up to ~10-fold stronger than the original CAG promoter. **(D)** Heatmap shows size (blue) and activity (green) of CAG variants in various cell lines using our dual-reporter assay.

CAG Variants are Potent in Complex Human and Mouse *in vitro* Models



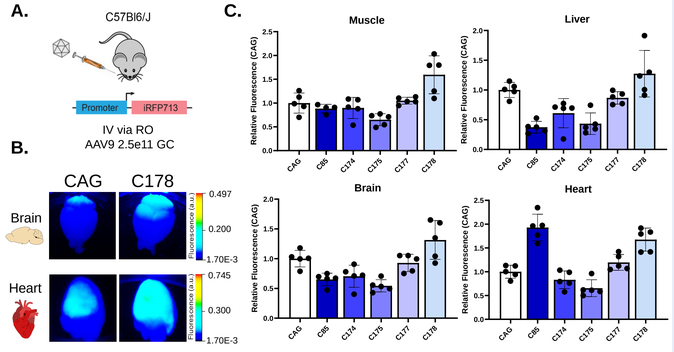
CAG variants are stronger than the original CAG promoter in primary mouse hepatocytes and primary human myotubes. (A) Representative images of primary mouse hepatocytes 7 days after transduction with AAV8-miRFP713 driven by candidate CAG promoters at 10,000 multiplicity of infection. Scale bar: 2 mm. CAG variants drive strong expression compared to the original CAG. **(B)** Representative images of primary human myotubes transduced at 200,000 multiplicity of infection with AAV8-miRFP713 driven by candidate CAG promoters. Scale bar: 1mm. **(C)** 21 days post transduction, MeiraGTx promoters show up to 8-fold higher relative fluorescence compared to the original CAG.

CAG Variants Exhibit Durable Expression in the Mouse Muscle Following Direct Injection



CAG variant promoters show durable expression in the mouse muscle. (A) Male C57Bl/6J mice were injected intra-muscularly (I.M.) into the gastrocnemius with 5e10 GC AAV9-miRFP713 driven by rationally designed CAG promoters. Fluorescence was measured *in vivo* at one, two, three-, and six-months post injection. **(B)** Quantification of miRFP713 signal shows strong and durable expression driven by CAG variants up to nine months after injection. **(C)** CAG variants are up to ~2.5-fold stronger than the original CAG in the mouse muscle nine months after injection while being ~600 base pairs shorter in size.

Optimized CAG Variants Drive High Expression *in vivo* Following Systemic Delivery



Expression of CAG variants in peripheral mouse organs. (A) Male C57Bl/6J mice were injected retro-orbitally (RO) with 2.5e11 GC AAV9-mirRFP713 driven by rationally designed CAG promoters. After 4 weeks, fluorescence was measure for each organ *ex vivo*. **(B)** Representative images of *ex vivo* fluorescence of whole brain and heart show higher expression driven by MeiraGTx CAG variants compared to the original CAG promoter. **(C)** MeiraGTx optimized promoters show constitutive expression in the brain, liver, heart, and muscle after systemic delivery.

Conclusions

- MeiraGTx has robust promoter engineering methods which yield potent and durable promoters *in vitro* and *in vivo*, regardless of species.
- By modifying various elements of the original CAG promoter, we generated several variants which are as strong, or stronger and shorter in length than the original CAG.
- MeiraGTx CAG variants drive up to ~10-fold higher expression in human and mouse cancer cell lines and up to ~8-fold higher expression in primary human myotubes and primary mouse hepatocytes
- When tested in the mouse muscle, our CAG variants show up to ~2.5-fold higher expression nine months after direct injection and show stronger expression than the original CAG in the mouse liver, brain, and heart following systemic injection
- These smaller, more potent CAG variants could be advantageous for the design of gene therapies for the muscle and central nervous system where transgenes are typically large, and packaging constraints are of major concern.

References

1. Wang et al. Adeno-associated virus vector as a platform for gene therapy delivery. *Nat Rev Drug Discov*. 2019 May;18(5):358-378.
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4. Wu et al. Effect of genome size on AAV vector packaging. *Mol Ther*. 2010 Jan; 18(1): 80–86.