P124

Promoter Engineering Platform at MeiraGTx

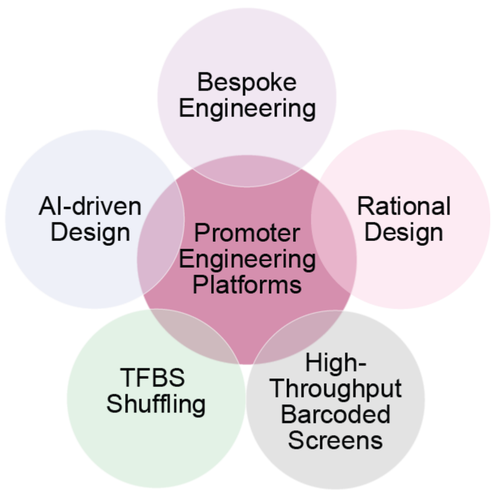
Josefa M. Sullivan[1], Dustin Lee[1], Chenjin Jin[1], Jessica Schmerler[1], Natalia Fabela[1], Enrico Mossotto[1], Edgar Hernandez[1], Kassiani Kytidou[2], Sabine Van der Sanden[2], Mostafa Zamatkesh[2], Diederik Lokhorst[2], Matthew J. During[1], Alexandria Forbes[1], & Ce Feng Liu[1]

[1]MeiraGTx New York [2]MeiraGTx Amsterdam

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

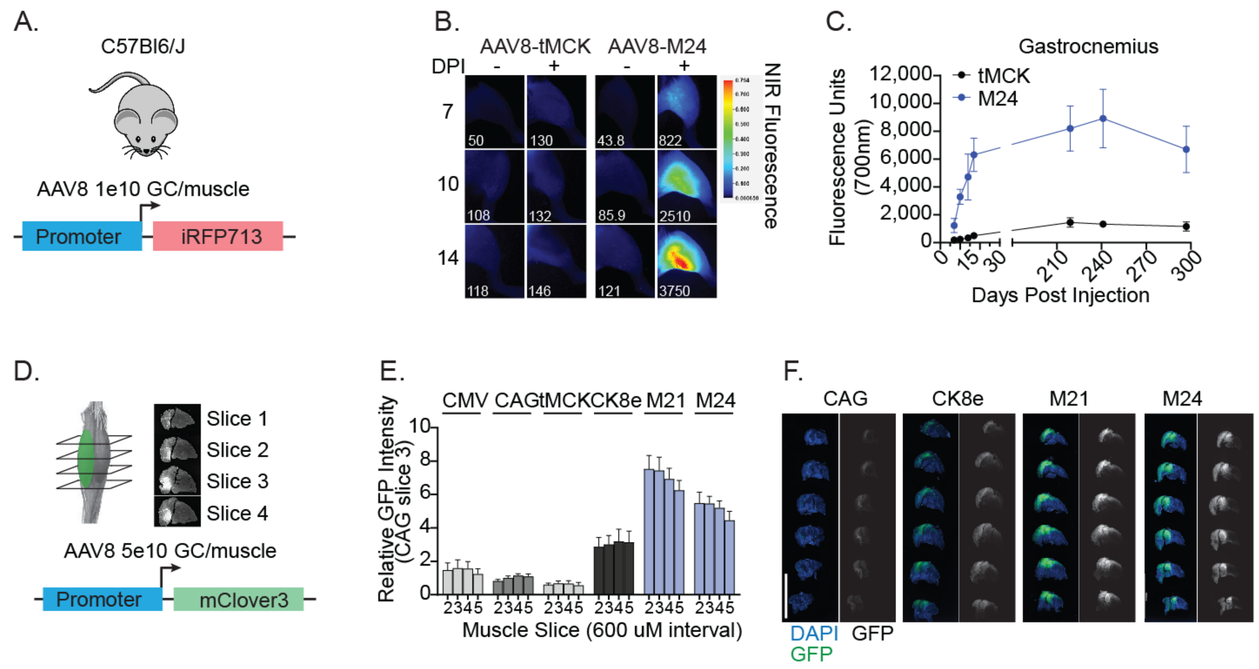
Benefits of Promoter Engineering for Gene Therapies

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



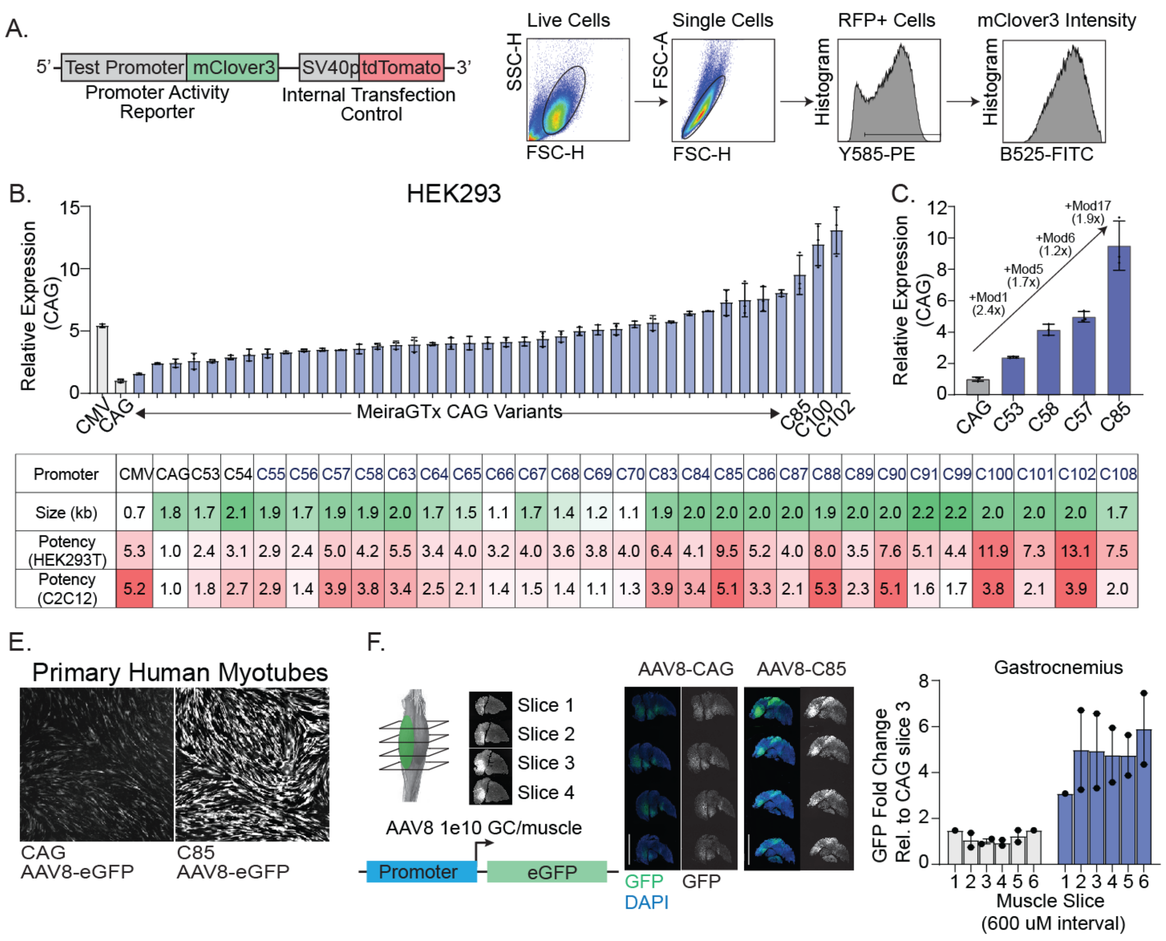
We have built libraries of synthetic promoters and promoter elements available for viral vector optimization. As these regulatory elements are context-dependent, different elements may be most appropriate in different gene sequence and cellular contexts.

MGTx promoters are highly potent and durable in the mouse muscle



(A) AAV8-iRFP713 under control of tMCK or M24 promoter was injected intramuscular to male C57Bl6 mice at 8 weeks of age and imaged using the LICOR Pearl. (B) Representative imaging data shows uninjected (-) and injected (+) hindlimbs for AAV8-tMCK and AAV8-M24. (C) Quantification of fluorescence shows M24 has at least 7-fold higher expression than tMCK that is maintained out to 300 days post injection. (D) AAV8-eGFP was injected into the gastrocnemius of male C57Bl6 mice at 8 weeks of age under the control of CMV, CAG, TMCK, CK8e, and two strong MGTx muscle specific promoters M21 and M24. After 4 weeks, the muscle was harvested and sectioned for native eGFP fluorescence. (E) Quantification and (F) Representative images, scalebar 10 mm, show MGTx promoters M21 and M24 are stronger than CAG or CK8e in the mouse gastrocnemius

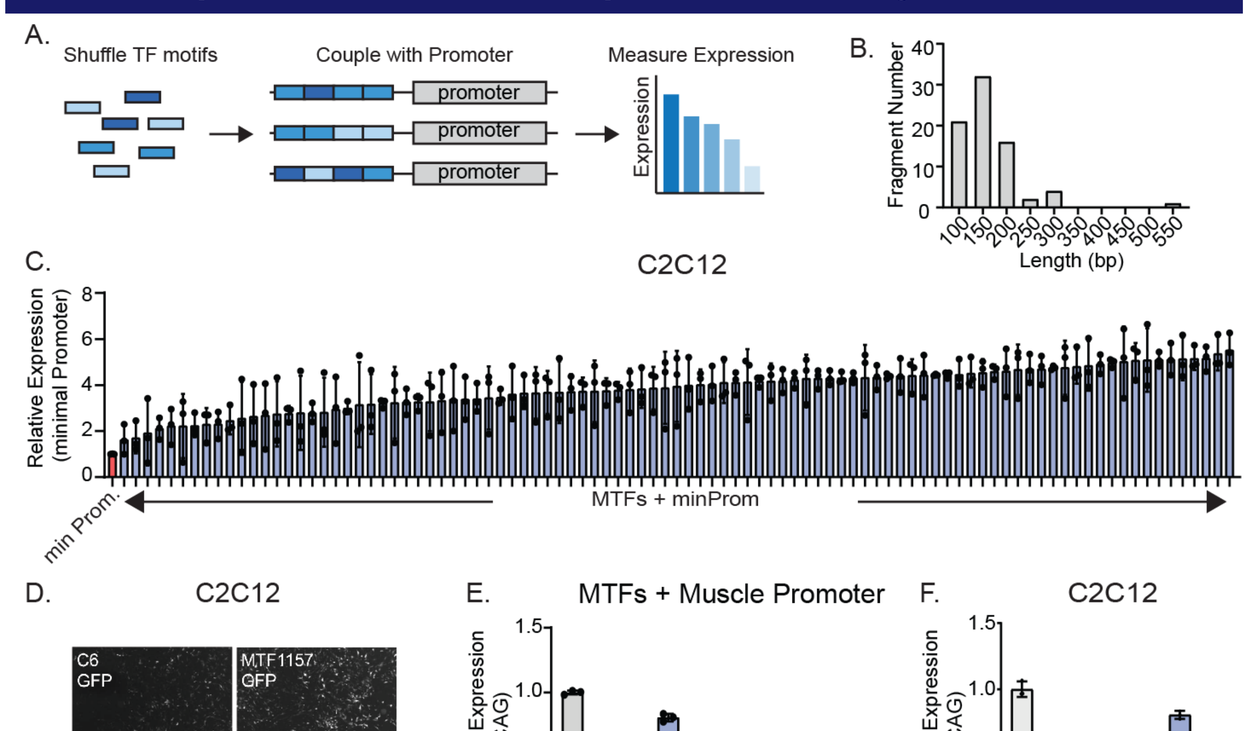
Designing smaller or more potent CAG promoter variants



Promoter	CMV	CAG	C53	C54	C55	C56	C57	C58	C63	C64	C65	C66	C67	C68	C69	C70	C83	C84	C85	C86	C87	C88	C89	C90	C91	C99	C100	C101	C102	C108
Size (kb)	0.7	1.8	1.7	2.1	1.9	1.7	1.9	1.9	2.0	1.7	1.5	1.1	1.7	1.4	1.2	1.1	1.9	2.0	2.0	2.0	2.0	1.9	2.0	2.0	2.2	2.2	2.0	2.0	2.0	1.7
Potency (HEK293T)	5.3	1.0	2.4	3.1	2.9	2.4	5.0	4.2	5.5	3.4	4.0	3.2	4.0	3.6	3.8	4.0	6.4	4.1	9.5	5.2	4.0	8.0	3.5	7.6	5.1	4.4	11.9	7.3	13.1	7.5
Potency (C2C12)	5.2	1.0	1.8	2.7	2.9	1.4	3.9	3.8	3.4	2.5	2.1	1.4	1.5	1.4	1.1	1.3	3.9	3.4	5.1	3.3	2.1	5.3	2.3	5.1	1.6	1.7	3.8	2.1	3.9	2.0

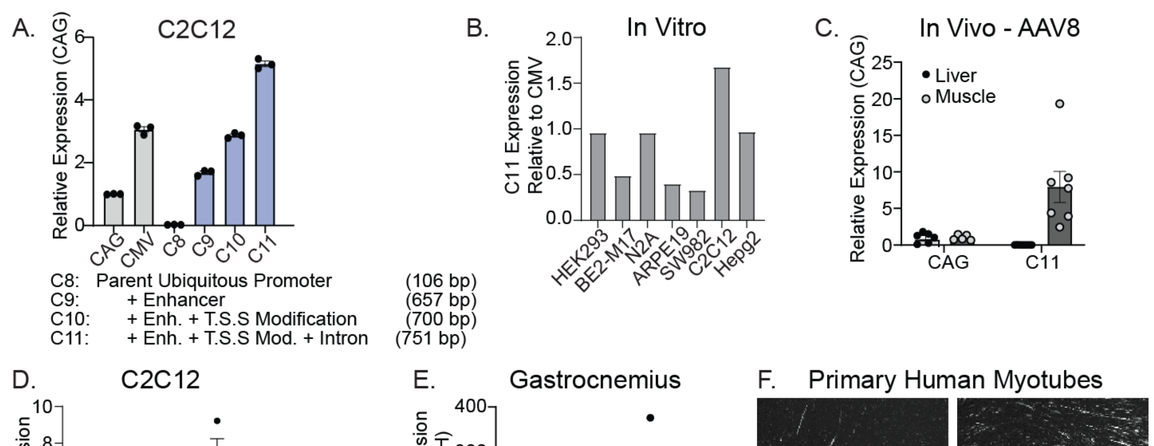
(A) FACS-based assay to screen promoter activity in vitro. (B) Screening of CAG variants in transiently transfected HEK293 cells identifies candidates that are up to 13-fold stronger than the original CAG promoter. (C) Example of the step-wise evolution of one top CAG variant. (D) Heatmap shows size (green) and activity of CAG variants in HEK293 and C2C12 cells in vitro. (E) Transduction of primary human myotubes shows increased expression from MGTx CAG variant, C85. (F) C85 drives stronger expression in vivo as indicated by native eGFP fluorescence in the mouse gastrocnemius. Scale: 5 mm

High-throughput screening of synthetic elements to identify sequences that enhance promoter activity in muscle



(A) TF motifs are randomly ligated together and then cloned upstream of a minimal, ubiquitous promoter. Expression is measured in a microscopy-based assay. (B) Representative sequencing shows majority of synthetic sequences are 200 bp or less. (C) Synthetic fragments increase expression from a minimal promoter up to 8-fold in transfected, mouse C2C12 muscle cell line. (D) Representative image of a top hit identified by the screen. (E) Fragments can be used in combination with other promoters to generate potent, novel muscle promoters. (F) MGTX-M51 is smaller and more potent than gold-standard muscle promoters currently used in the clinic.

Evolution of strong, muscle-specific promoters



(A) Step-wise evolution of a small, constitutive promoter driving expression in transfected C2C12 cells. (B) C11driven expression in various transfected cell lines. (C) Transduction of the liver (i.v.) and muscle (i.m.) with AAV8 in wild-type, 8 week-old male mice shows C11 promoter activity is stronger in the muscle. (D) Step-wise evolution of a muscle-specific promoter (M22 to M24) rather than CAG derived promoter, in transfected C2C12 cells. (E) Western blot of transduced mouse muscle tissue. (F) Transduction of primary human myotubes with AAV8-mClover3-WPRE-SV40pA

Conclusions

- We have generated libraries of multiple novel composite promoters driving strong expression in muscle cell lines that are >>CAG and a subset that are >CMV.
- MeiraGTx CAG variants are 13-fold stronger than the original CAG *in vitro*.
- Evolution of a small, ubiquitous promoter, C11, results in 5-fold higher activity than CAG specifically in mouse muscle.
- Muscle-specific M24 promoter leads to stronger sustained expression than tMCK *in vivo*
- Our high-throughput platforms have yielded several synthetic enhancers which we have used to make new muscle promoters that are stronger and smaller than CK8e and other clinically-relevant promoters.
- These results highlight our diverse platform for promoter engineering at MeiraGTx which we have applied to other tissues and cell-types, including the liver, central nervous system and the salivary gland.

References

1. Wang et al. Nat Rev Drug Discov. 2019 May;18(5):358-378.
2. Au et al. Front Med (Lausanne). 2022 Feb 9;8:809118
3. Cordier et al. Hum Gene Ther. 2001 Jan 20;12(2):205-15
4. Wu et al. Mol Ther. 2010 Jan; 18(1): 80-86.