Minimal Protein from DNA Mini Circles Provides Therapeutic Benefit in CPS1 Deficiency

Matthew Nitzahn[1,2], Suhail Khoja[2], Jenna Lambert[2], Hiu Man Grisch-Chan[3], Beat Thöny[3], Gerald S. Lipshutz[1,2,4]

[1]Molecular Biology Institute, [2]Surgery, [3]Molecular and Medical Pharmacology, UCLA ; [3]Division of Metabolism and Children's Research Center, University Children's Hospital Zurich

Lab website: https://lipshutzlabucla.com/

Contact: Matt.Nitzahn@gmail.com

Introduction

- Carbamoyl phosphate synthetase 1 (CPS1) catalyzes the first committed step of the urea cycle, eliminating nitrogen waste
- Loss of CPS1 function results in toxic ammonia levels and encephalopathy, often leading to death
- Treatments for CPS1 deficiency are largely ineffective, and donor livers for transplantation are scarce
- AAV-based gene therapy is attractive, and we recently demonstrated its potential in a proof-of-concept study using split AAVs (Nitzahn 2020)
- To avoid expensive, intensive viral approaches, DNA mini circles (MCs) provide an alternative to investigate

Methods

- MCs containing human codon optimized CPS1 (hcoCPS1) driven by the CAG promoter were generated and conjugated to jetPEI-Gal (Polyplus) at nitrogen/phosphate ratio = 8
- Adult female *Cps1*$^{flox/flox}$ mice (Khoja 2018) were injected with AAV-Cre alone (control) or AAV-Cre + MCs (treated) at 1.6-2.4mg/kg MC in 4-5 doses
- Mice were weighed daily and given n-carglumic acid (200mg/L) in drinking water.
- Plasma was collected at regular intervals and at humane euthanasia time points from sick mice
- Livers were collected immediately after euthanasia for analysis

Results

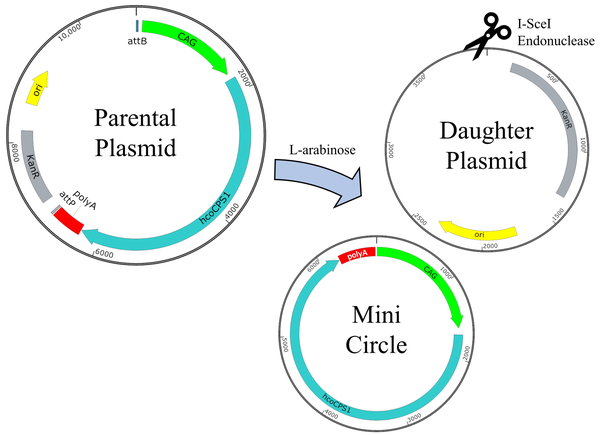


Figure 1. Schematic of MC generation. A parental plasmid containing the transgenic cassette flanked by attB and attP recombination sites is generated and propagated in the specialized *E. coli* strain from Kay 2010. Induction of øC31-integrase with L-arabinose recombines the attB/P sites, forming the MC and daughter plasmid. I-SceI sites in the daughter are recognized and mediate its digestion, yielding pure MCs.

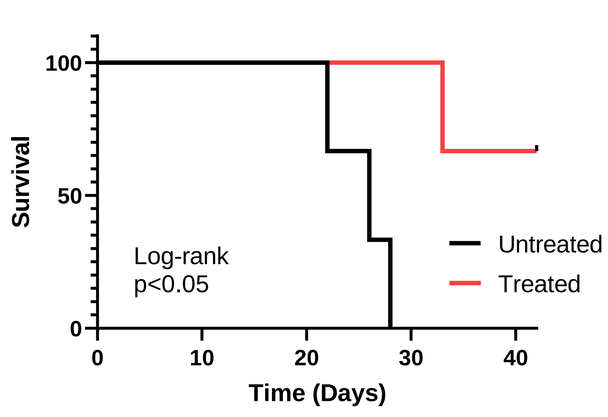


Figure 2. Untreated mice perish while MC-treated survive. MC-treated mice had significantly longer survival than their untreated counterparts ($p < 0.05$). The treated mouse that expired receive the lowest dose (134µg vs. >170µg). n = 3 mice per group

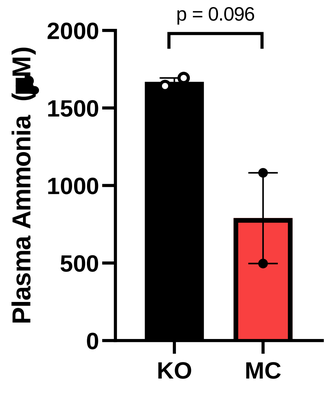


Figure 3. MC-treated mice show reduced plasma ammonia. Mice treated with MCs trend towards reduced plasma ammonia compared to untreated controls. Plasma was not available from the treated mouse that perished. n = 2 per group; bars are mean ± SEM

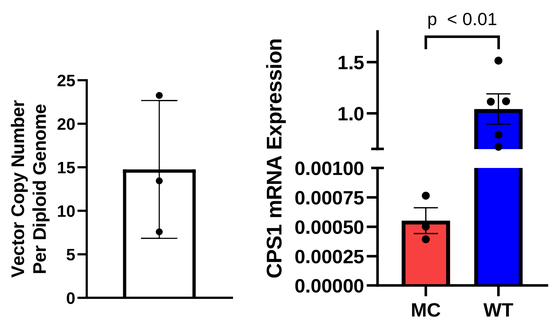
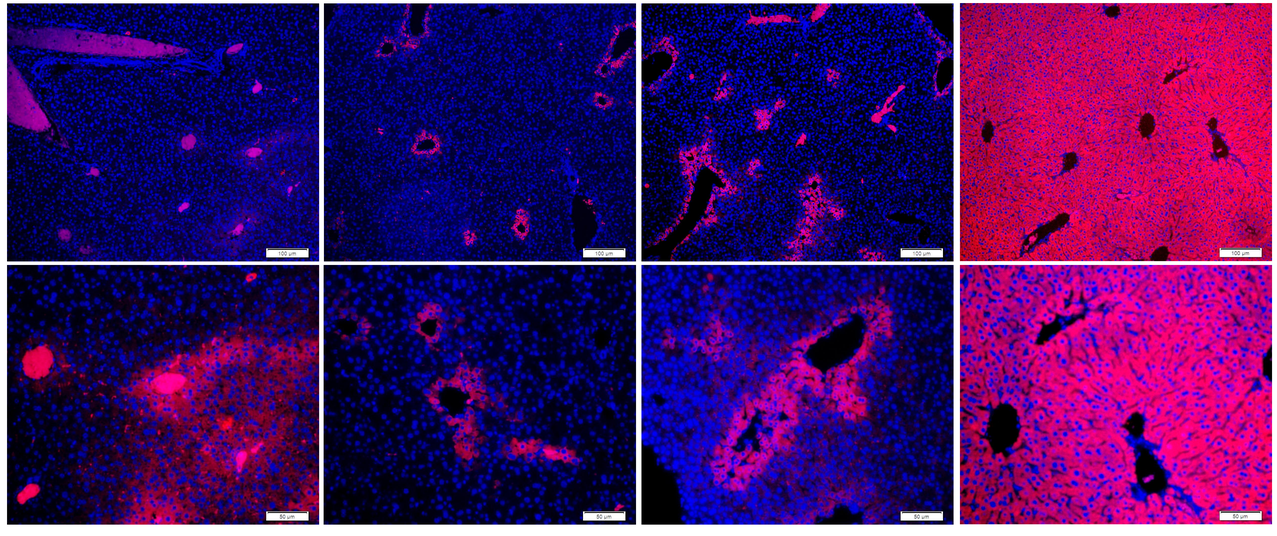


Figure 4. MC-treated mice have extremely low CPS1 expression. Total DNA was extracted and the vector copy number determined in MC-treated mice (left); the ~14 value corresponds to the mouse that perished. Total RNA was also analyzed and compared to wild type mice (right). Treated mice show barely detectable levels of CPS1 expression, despite 2 of 3 showing no outward signs of poor health at the time to euthanasia. The highest expression was found in the mouse that perished. n = 3 treated, 5 wild type.



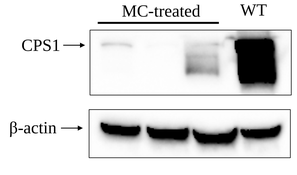


Figure 5. CPS1 protein expression and distribution in MC-treated mice. Top) Immunohistochemistry in mice treated with MCs (left 3 columns) shows bright, perivascular CPS1 expression with little distributed throughout the liver parenchyma. Wild type mice (far right column) have extensive, pan-hepatic CPS1 expression. The treated mouse that perished is on the far left. **Bottom left)** Western blot of MC-treated mice compared to wild type (WT). The treated mouse that perished is in lane 1. Protein levels are far below wild type levels in all treated mice.

Conclusions

- MCs expressing hcoCPS1 are sufficient to extend lifespan and reduce plasma ammonia in Cps1-deficient mice
- MC-treated mice have low gene and protein expression
- MC-derived protein is mainly localized to the perivasculature

Future Directions

- Increase the number of mice to confirm that observed trends are statistically significant
- Expand treatment to include male mice, which may have differential response to therapeutics (Khoja 2018)
- Optimize dosing and administration strategies to maximize therapeutic benefits

References

Kay, M. A *et al*. *Nat Biotechnol* **28**, 1287–1289 (2010).

Khoja, S. *et al. Molecular Genetics and Metabolism* **124**, 243–253 (2018).

Nitzahn, M. *et al. Molecular Therapy.* In press (2020).

Funding & Acknowledgments

Funding for this work was provided by the UCLA Whitcome Pre-Doctoral Fellowship and R21NS091654 to GSL.

The MC bacteria and empty parental plasmid were generous gifts from HMGC and BT.