Targeting regulatory RNAs with antisense oligonucleotides enables potentially therapeutically relevant upregulation of gene expression

Bryan J. Matthews, Brynn N. Akerberg, Yuting Liu, Ali Al Abdullatif, Yeliz Yuva-Aydemir, Dale C. Guenther, Cecile Mathieu, Jenna Williams, Salome Manska, Jiaqi Huang, Chelsea Bolmer, Simrin Kapoor, Stephanie Lefebvre, Yuichi Nishi, Andrew M. Masteller, Abeer Almutairy, Eric Coughlin, Gabriel Golczer, Shama Pilanker, Gokul Ramaswami, Preeti Sathe, Cynthia Smith, Scott Waldron, Brianna Unda, Shubh Roy, Yuchun Guo, Evan Cohick, Kevin Xiong, Alla A. Sigova, David A. Bumcrot, Daniel F. Tardiff

CAMP4 Therapeutics Corp., Cambridge, MA, USA

Abstract

Transcription of long noncoding regulatory RNAs (regRNAs), including enhancer RNAs (eRNAs) and promoter associated RNAs (paRNAs), is a universal feature of active gene expression, yet their therapeutic potential remains largely untapped. We have discovered that targeting regRNAs with antisense oligonucleotides (ASOs) can selectively increase gene transcription in cis, leading to potentially therapeutically meaningful increases in disease gene expression. To identify regRNAs, we have developed regRNA Capture-seq, a high throughput profiling method that maps regRNAs with high sensitivity. This approach was applied to primary human hepatocytes to identify thousands of regRNAs transcribed from a panel of ~2,000 selected enhancers and promoters.

We next investigated a regRNA associated with transcription of SYNGAP1, a gene whose haploinsufficiency causes a severe developmental epileptic encephalopathy, known as SYNGAP1 related disorder (SRD). Insufficient levels of SYNGAP1 lead to elevated levels of AMPA receptors at the post-synaptic membrane and increased neuronal activity. Restoring endogenous SYNGAP1 expression represents a potential therapeutic strategy for SRD.

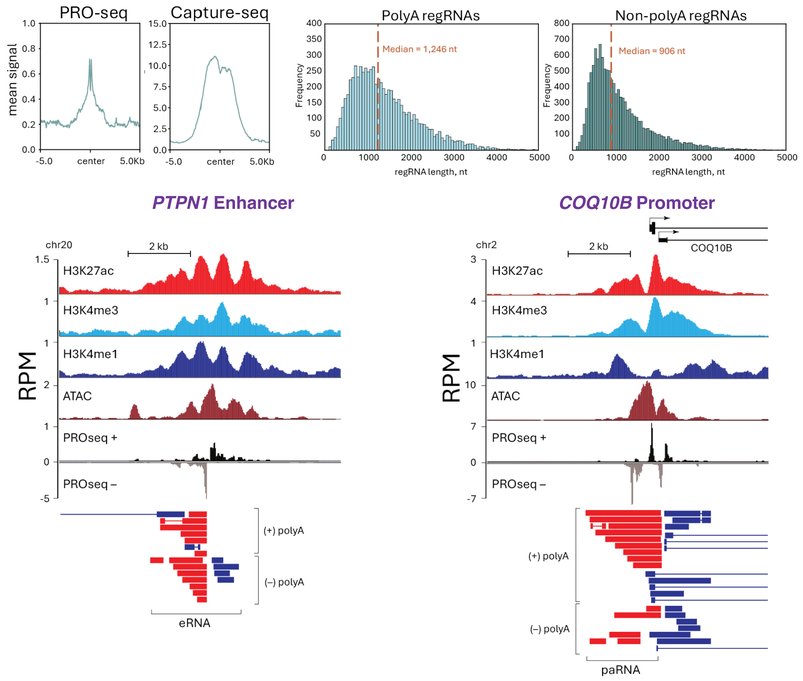
Using regRNA Capture-seq, we characterized SYNGAP1-associated regRNA in multiple human neuronal cell types and brain tissue. ASOs targeting this regRNA induced dose dependent upregulation of the regRNA, SYNGAP1 mRNA, and SynGAP protein across multiple cell types, including patient derived neurons.

Mechanistic studies revealed that CMP-002 treatment enhances transcription of both SYNGAP1 regRNA and mRNA by increasing RNA polymerase II recruitment to the gene's promoter. CMP-002 appears to exhibit steric blocking effects associated with alterations in the target regRNA secondary structure. Together, these findings demonstrate that targeting regRNAs with ASOs can selectively boost endogenous gene expression both in cells and in animals. This work establishes a potentially generalizable therapeutic strategy for correcting haploinsufficiency and highlights regRNAs as actionable targets for ASO-mediated upregulation of gene expression.

Using Capture-seq to catalog regRNA species in cells

Capture-seq identifies full length regRNA species (both polyA+ and polyA-)

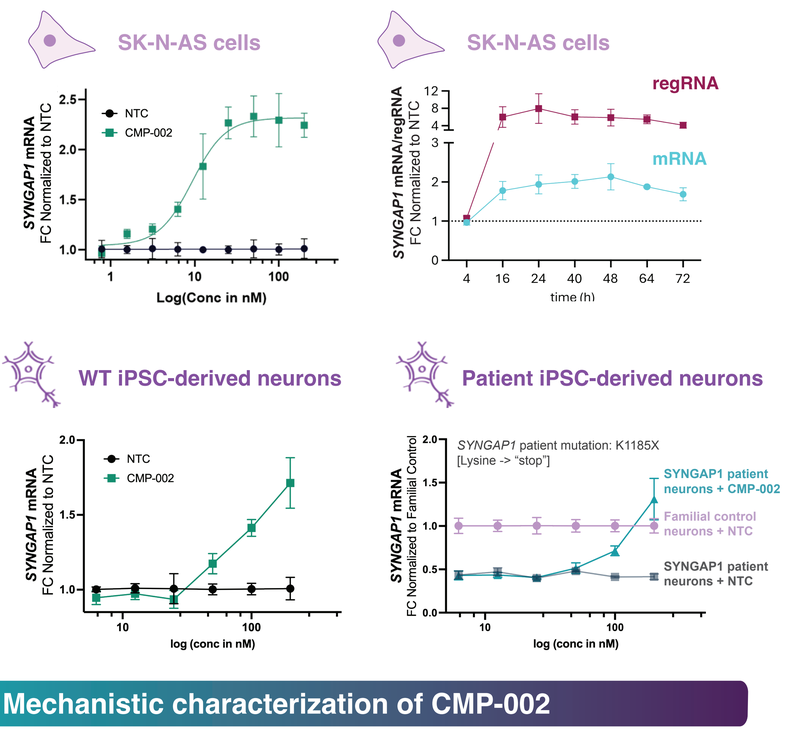
- Capture-seq provides improved transcript coverage relative to PRO-seq
- Polyadenylated regRNAs are longer than non-polyadenylated regRNAs
- regRNAs (+/-PolyA) are produced from enhancer and promoter regions



CMP-002 ASO treatment upregulates regRNA/mRNA

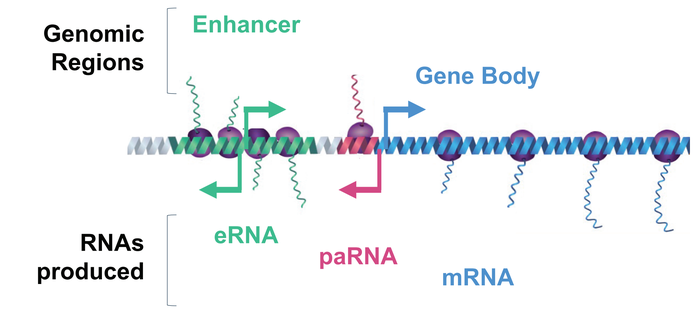
CMP-002 upregulates SYNGAP1 levels in SK-N-AS cells and neurons

- Dose-responsive upregulation observed in the neuroblastoma cell line, SK-N-AS
- Concordant upregulation of SYNGAP1 mRNA/regRNA at 16h post treatment
- Dose-responsive upregulation observed in WT iPSC-derived neurons
- CMP-002 rescues *SYNGAP1* mRNA level in patient iPSC-derived neurons

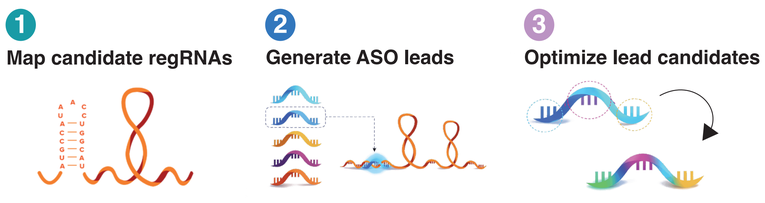


Background

Regulatory RNAs (regRNAs) are a broad class of cis-acting noncoding RNAs



RAP Platform®: Programming medicines targeting regRNAs

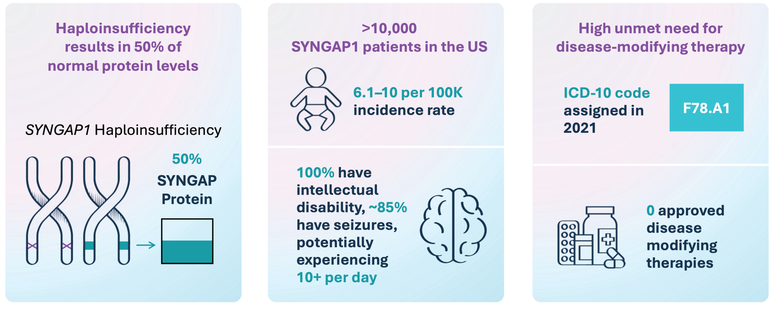


Characteristics of SYNGAP1-related disorder

SYNGAP1 is a haploinsufficiency with patients in need of therapy

- *SYNGAP1* encodes SynGAP, a Ras/Rap GTPase-activating protein that regulates intracellular signaling pathways, AMPA-type glutamate receptor (AMPAR) trafficking, dendritic spine morphology, and synaptic plasticity
- Mutations in SYNGAP1 lead to decreased SYNGAP protein, increased synaptic firing

- **Goal: Increase SynGAP protein levels and improve disease symptoms**

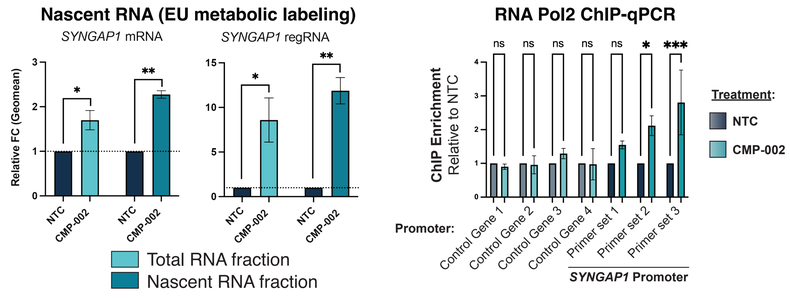


López-Rivera et al., Brain, 2020; Marotta et al., Curr Probl Pediatr Adolesc Health Care, 2024; Holder et al., GeneReviews, 2019; SYNGAP-Related Epilepsy, Epilepsy Foundation (Accessed May 2025); Vlaskamp et al. Neurology, 2019.

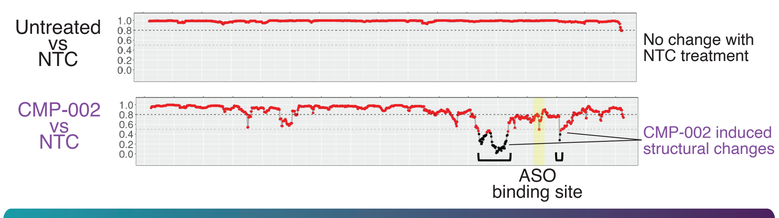
Mechanistic characterization of CMP-002

CMP-002 treatment increases transcription of SYNGAP1 mRNA and regRNA

- CMP-002 increases nascent and steady-state levels of *SYNGAP1* mRNA/regRNA
- CMP-002 increases RNA polymerase II binding at the *SYNGAP1* promoter



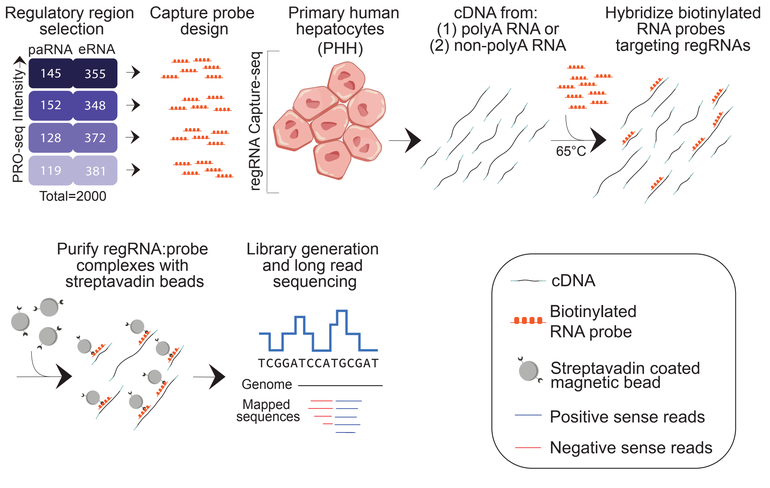
Impact of CMP-002 on RNA structure (as measured by SHAPE-MaP)



Cataloging regRNAs using Capture-seq

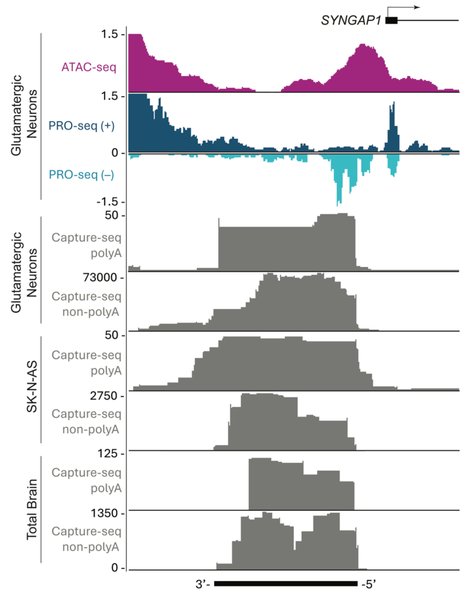
Identifying regRNAs transcribed from 2,000 enhancers in human hepatocytes

- Regulatory regions were stratified into 4 subgroups based on level of PRO-seq signal
- Designed four sets of ~31,000 biotinylated RNA probes for each of the 4 subgroups
- Long-read sequencing was utilized to capture full-length regRNA species



Using Capture-seq to identify SYNGAP1 regRNAs

Capture of SYNGAP1 regRNA in human neuronal cells and brain tissue



- regRNA is divergently transcribed from the promoter of the longest *SYNGAP1* mRNA isoform

- Both polyadenylated and non-polyadenylated *SYNGAP1* regRNA species are identified in all profiled cells and brain tissue

- Non-polyadenylated *SYNGAP1* regRNA species are significantly more abundant than polyadenylated species

- *SYNGAP1* regRNA is conserved across neuronal cell types and brain tissue

Takeaways

regRNA Capture-seq

Developed regRNA Capture-seq and created catalog of regRNAs in hepatocytes

Capture-seq provides improved transcript coverage relative to PRO-seq

Majority of regRNAs are unspliced and non-polyadenylated

Upregulation of *SYNGAP1*

Identified a regRNA that controls SYNGAP1 expression

Discovered an antisense oligonucleotide CMP-002 that binds regRNA to increase SYNGAP1 levels in preclinical studies

CMP-002 upregulates SYNGAP1 mRNA/regRNA levels in concordant fashion

CMP-002 binding to regRNA alters regRNA structure and increases transcription

This work establishes a potentially generalizable therapeutic strategy for correcting haploinsufficiency and highlights regRNAs as actionable targets for ASO-mediated upregulation of gene expression.