Scripps Research

Sculpting Neuronal Gene Expression

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Department of Neuroscience

THE LiPPi LAB

Atlante - Michelangelo Buonarroti
Concept: David Bartel (MIT)

From **Basic** Developmental Neuroscience to **Therapies**

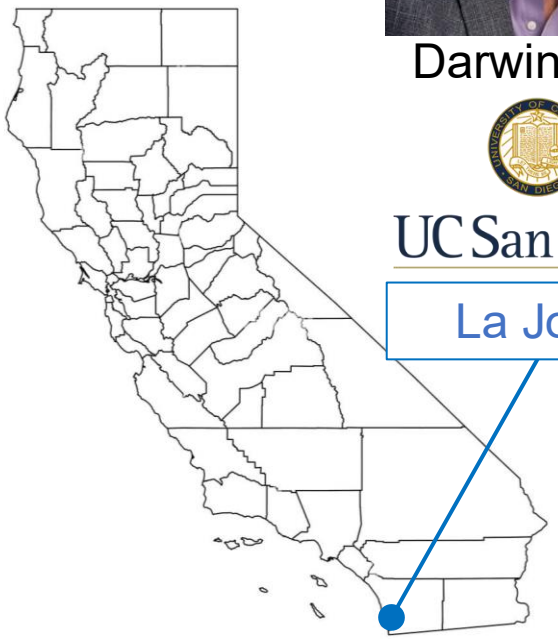
- Fundamentals of **gene expression**: from DNA to microRNAs
- microRNAs helped the **emergence of complex brains**
- Leveraging basic knowledge of microRNA function to **correct neurodevelopmental disorders**

My Journey to **Scripps Research**

Darwin Berg

UC San Diego

La Jolla

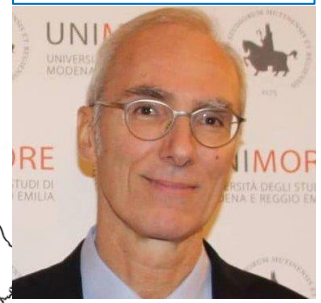


Leicester

Pierluigi Nicotera

MRC Toxicology Unit

Modena



Michele Zoli

UNIVERSITAS STUDIORUM MUTINENSIS ET REGIENSIS 1175

From **Basic** Developmental Neuroscience to **Therapies**

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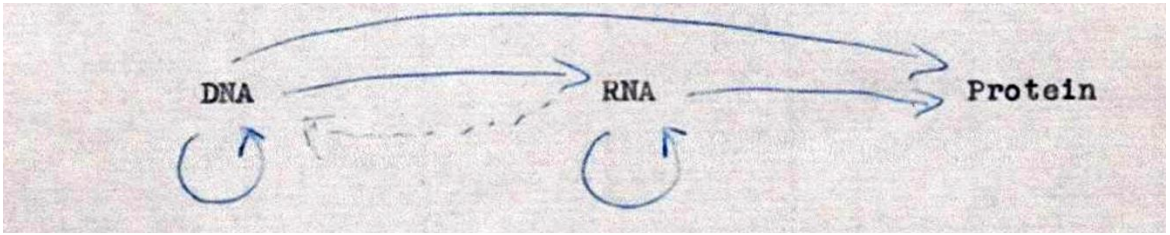
On Protein Synthesis – Society for Experimental Biology Symposium

Crick, Watson, Wilkins
1962 Nobel Prize in Physiology or Medicine

- **The Sequence Hypothesis**: the order of bases in a nucleic acid (DNA or RNA) encodes the sequence of amino acids in a protein
- **The Central Dogma:** once information has passed into a protein structure, it cannot get back out to nucleic acids

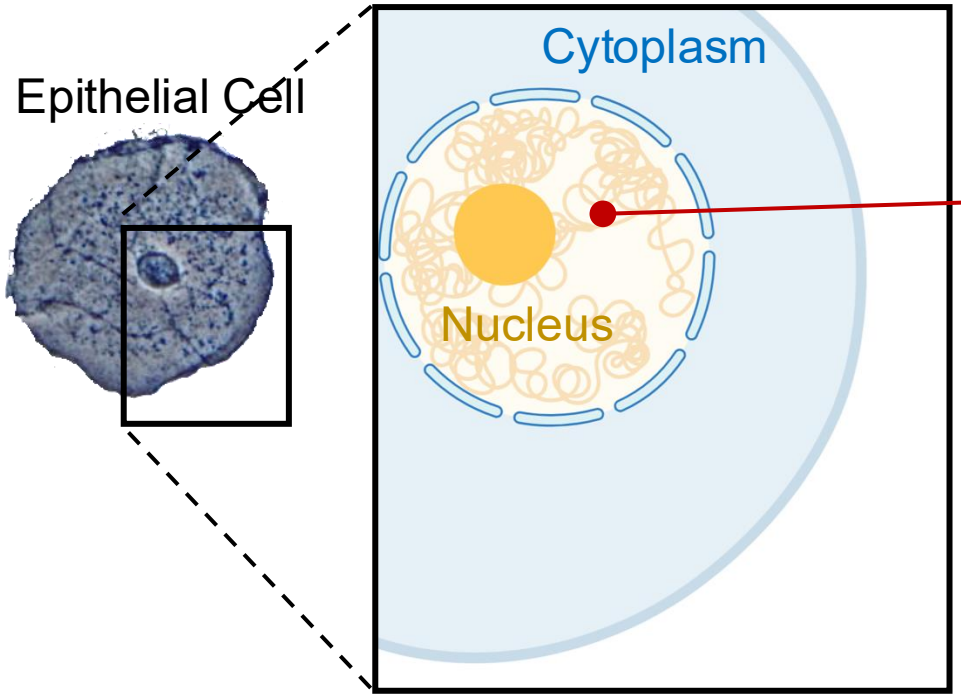
Francis Crick, University College London, 1957



Information transfer in living material

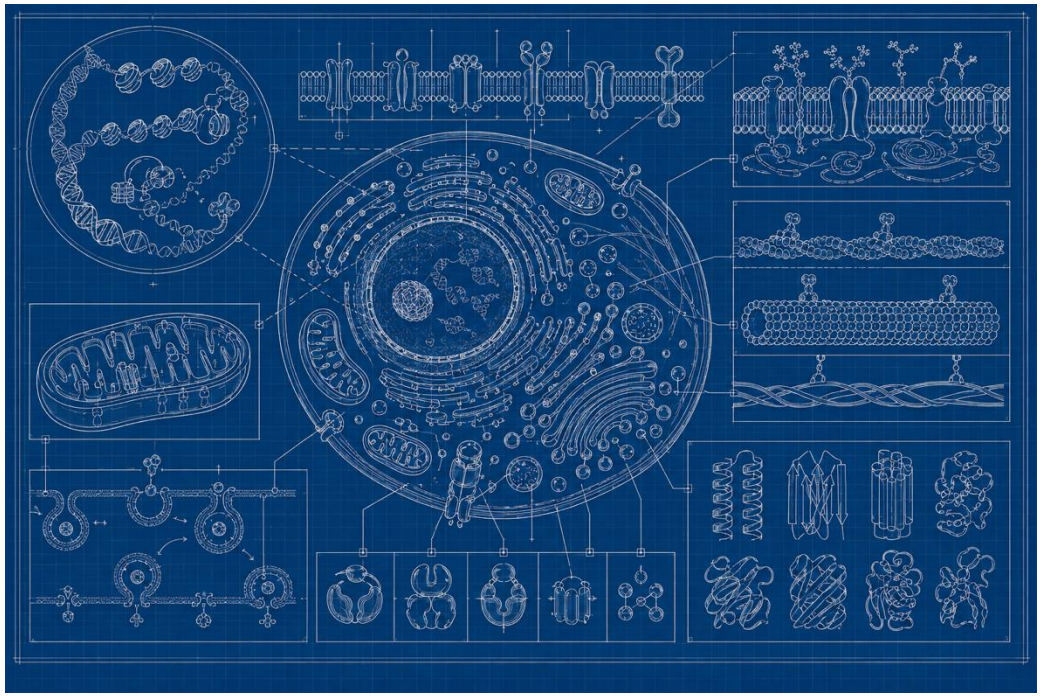
Crick FHC. On protein synthesis. **Symp Soc Exp Biol**. 1958;12:138–163

DNA → RNA → Protein (70 years later)

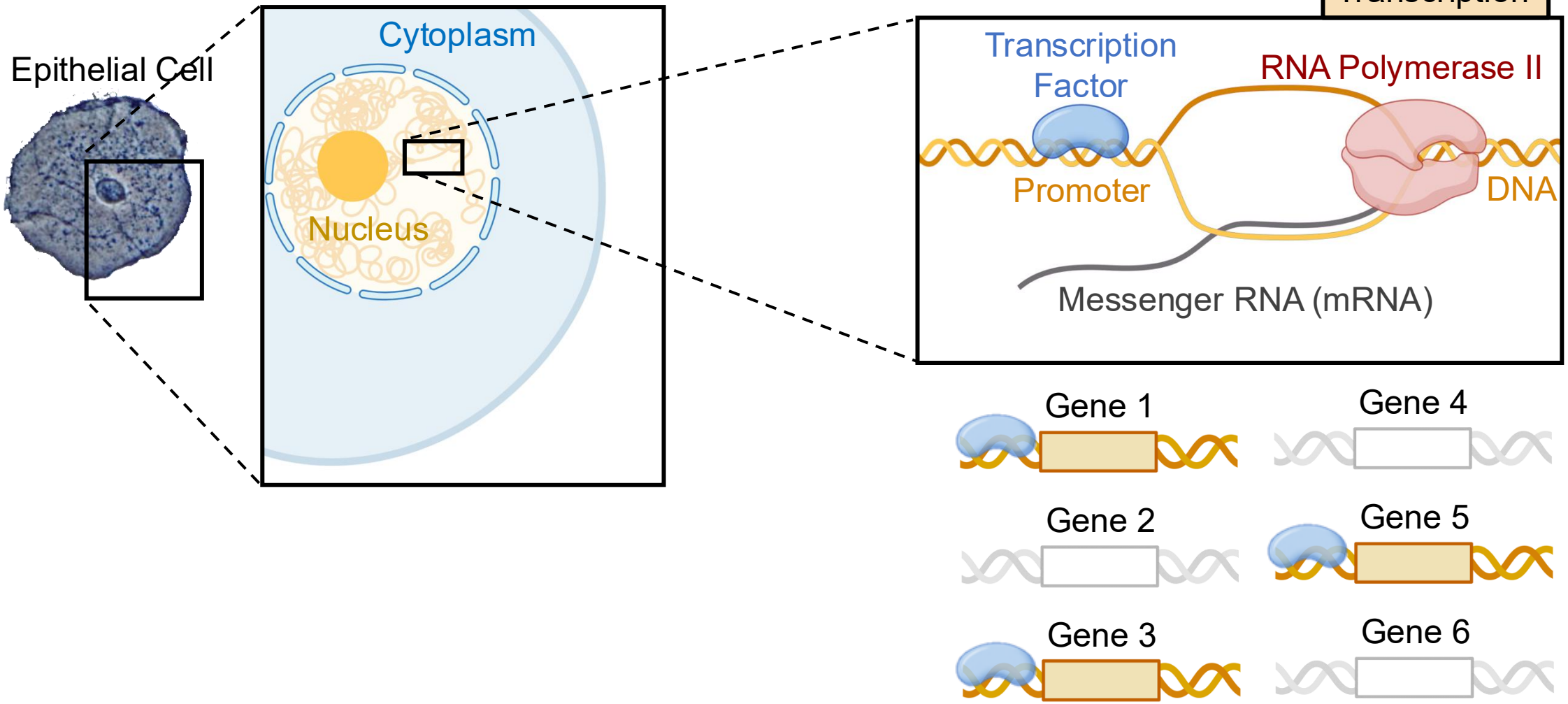


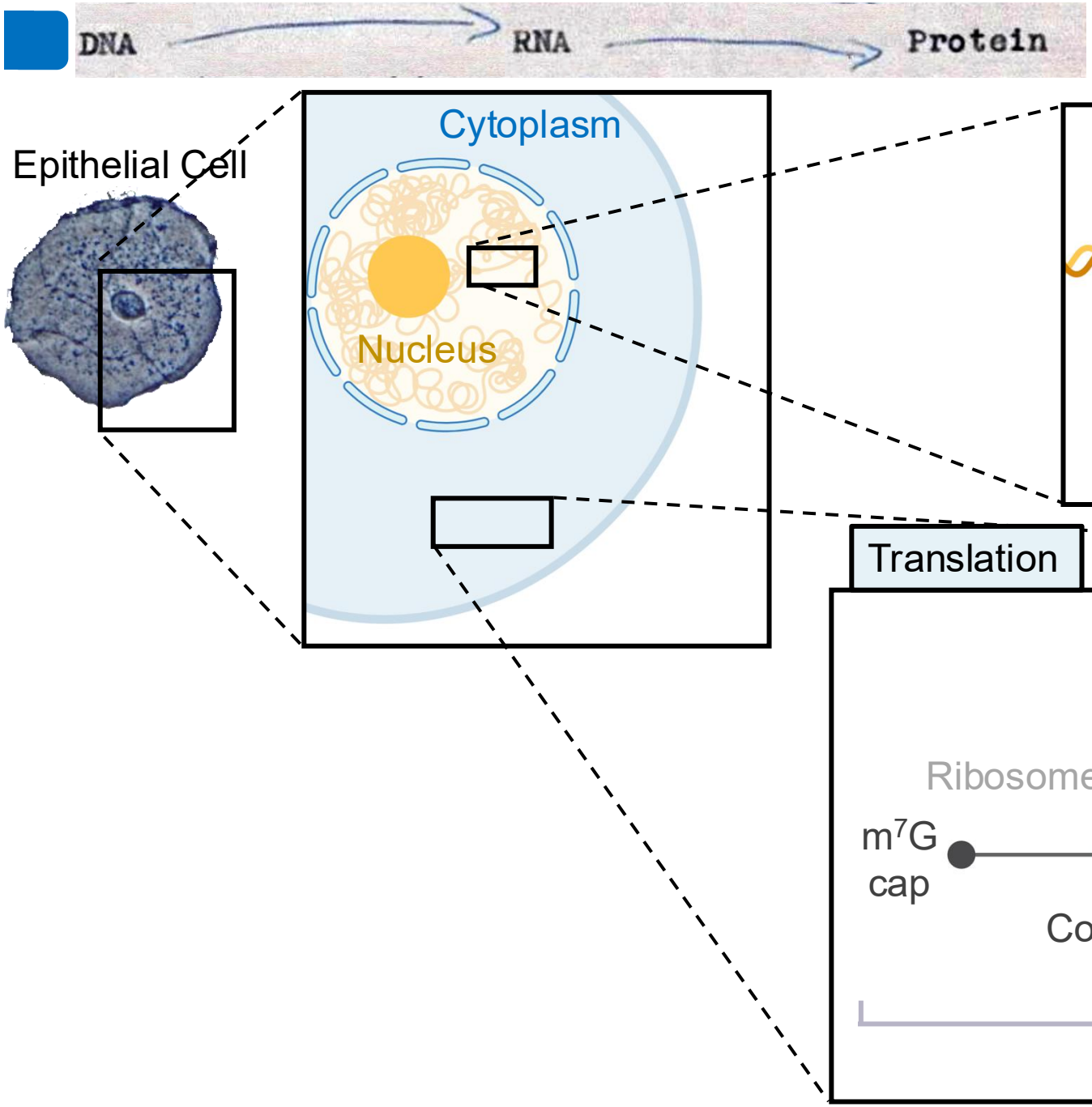
A human cell contains **~20,000** genes

DNA is the blueprint of all cellular structures



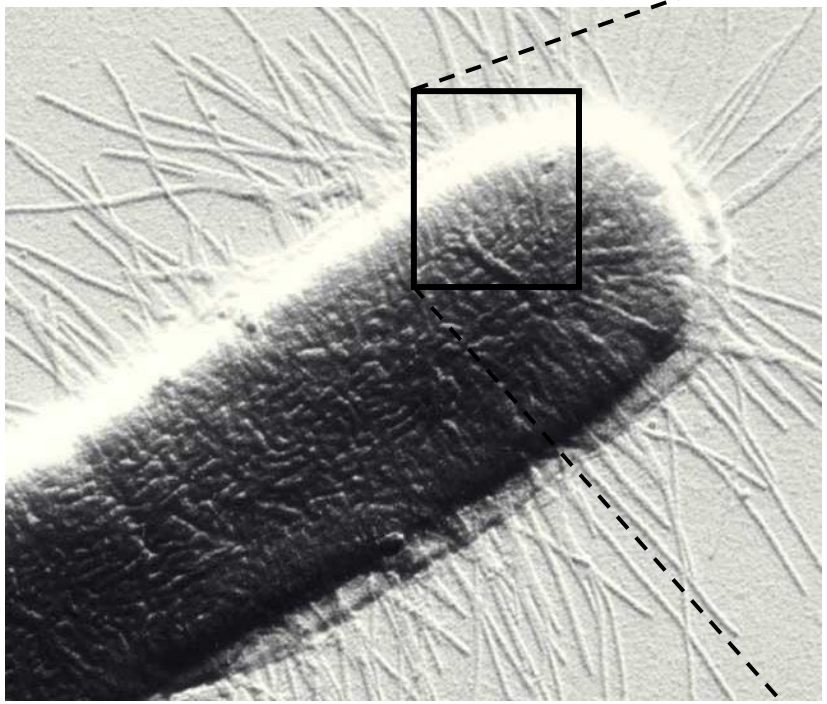
DNA → RNA → Protein (70 years later)




DNA
RNA
Protein
(70 years later)
Epithelial Cell
Cytoplasm
Nucleus
Transcription
Transcription Factor
RNA Polymerase II
Promoter
DNA
Messenger RNA (mRNA)
Translation
Polypeptide
Ribosome
Protein
m7G cap
Coding Sequence (CDS)
Untranslated Region (3'UTR)
polyA
mRNA

Proteins are the **Building Blocks** of the Cell

E.Coli



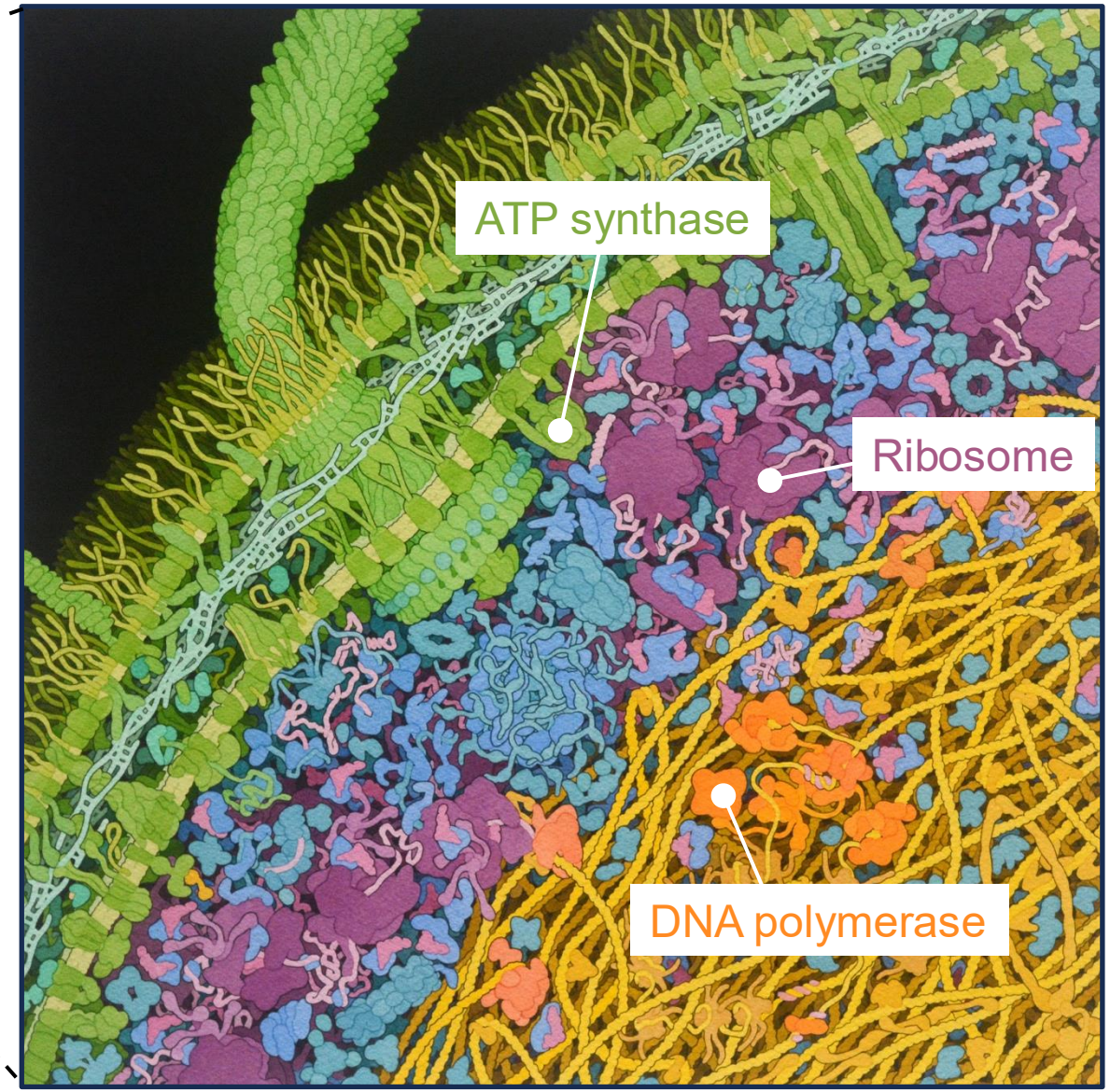


Illustration by **David S. Goodsell**

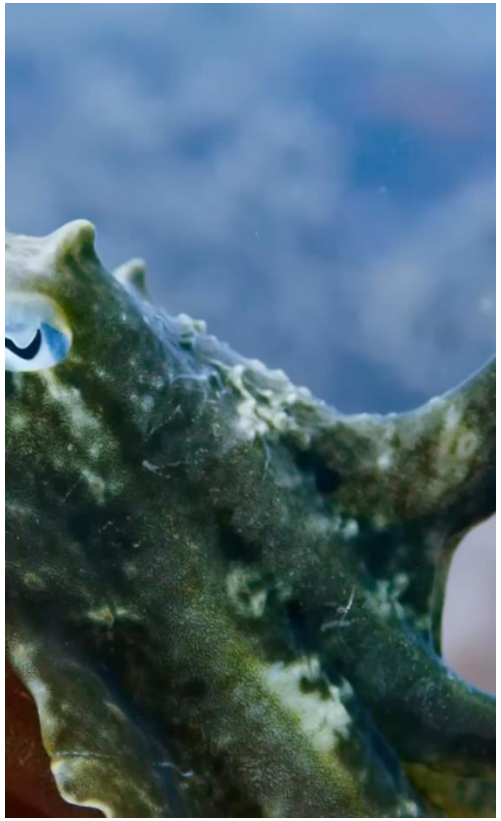
RCSB Protein Data Bank. doi: 10.2210/rcsb_pdb/goodsell-gallery-028

How does Gene Expression Instruct the Emergence of **Biological Complexity**?

Lacrymaria

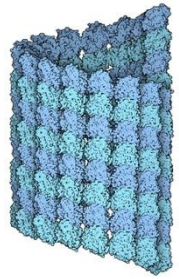
Fungi

Sepia

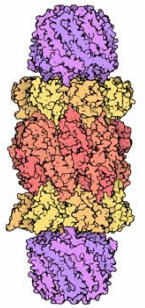


Low complexity → High complexity

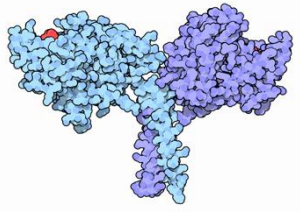
More **proteins?**



Microtubules

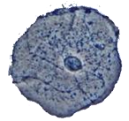


Proteasome



Kinesin

More **cell types** (tissues and organs)



Epithelial Cell

Cardiomyocyte

Erythrocyte

High **complexity**

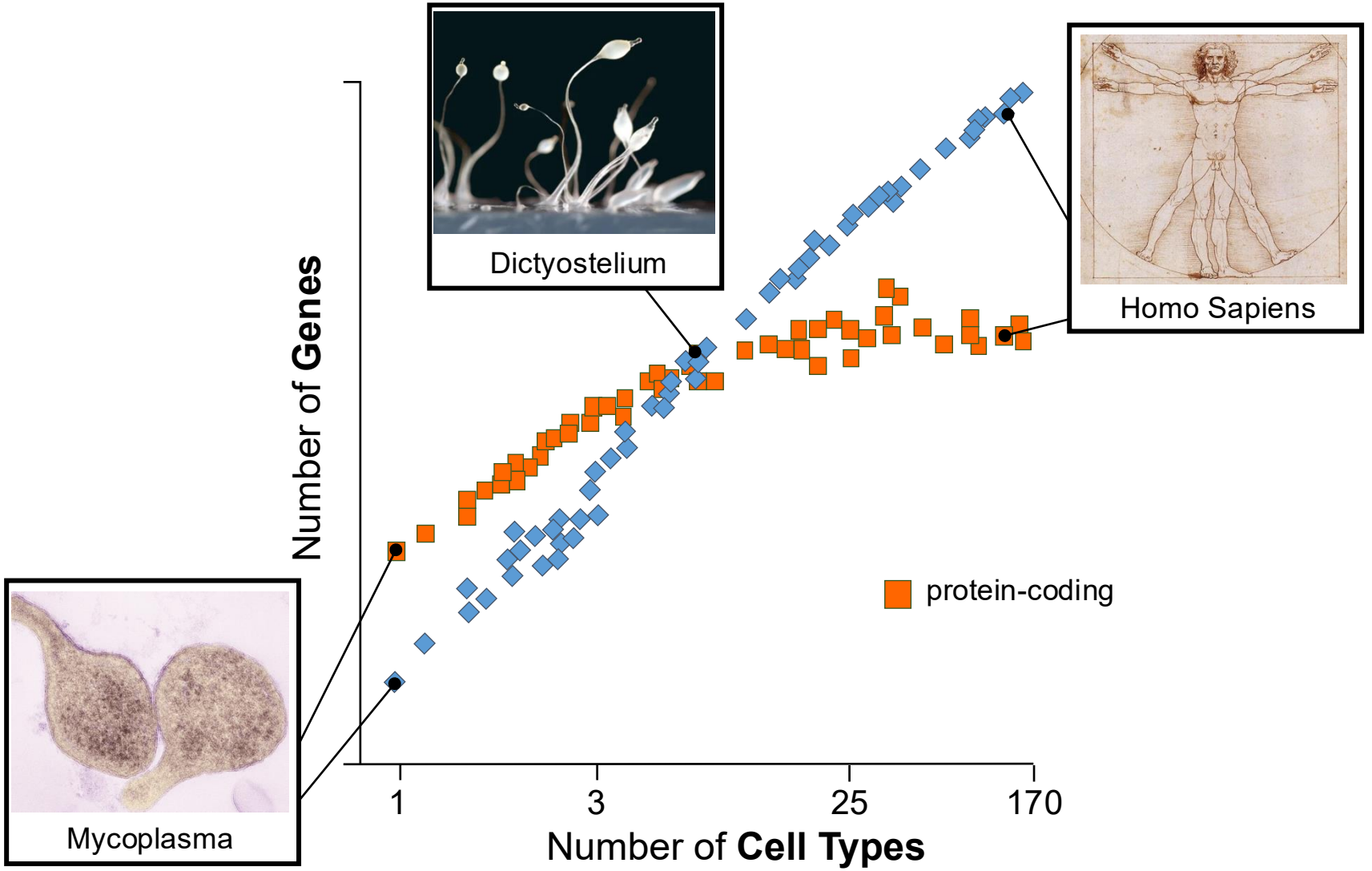
Non-Coding Genes Drove Greater **Biological Complexity**

More **genes?**

More **proteins?**

More **cell types** (tissues and organs)

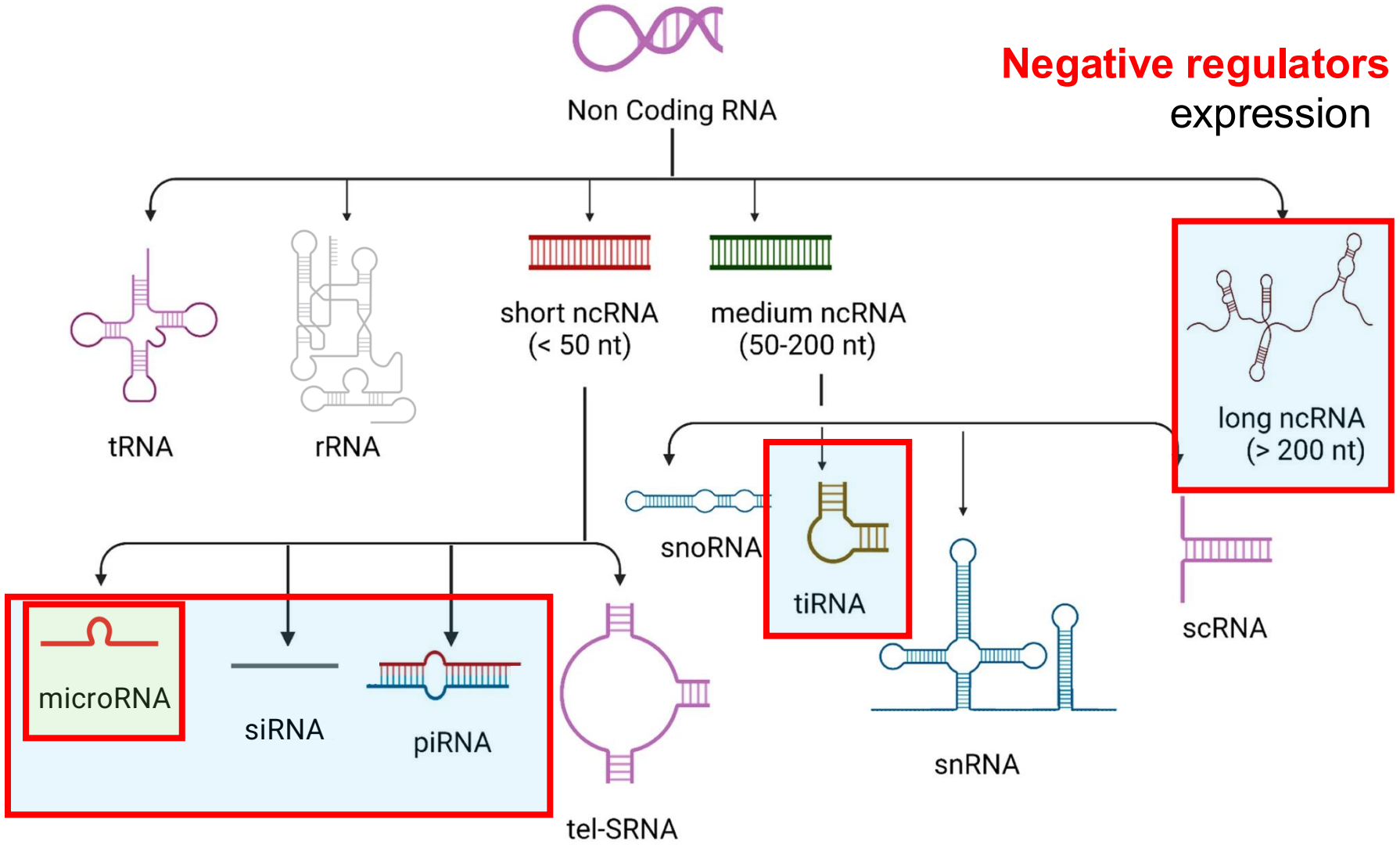
High **complexity**



Adapted from Liu et al. **Cell Cycle** (2013)

Non-Coding RNAs Regulate Gene Expression

Negative regulators of gene expression

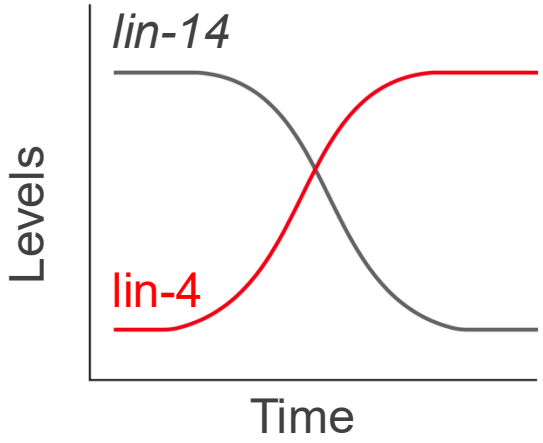


The Discovery of **microRNAs** – a **New Level** of Gene Regulation

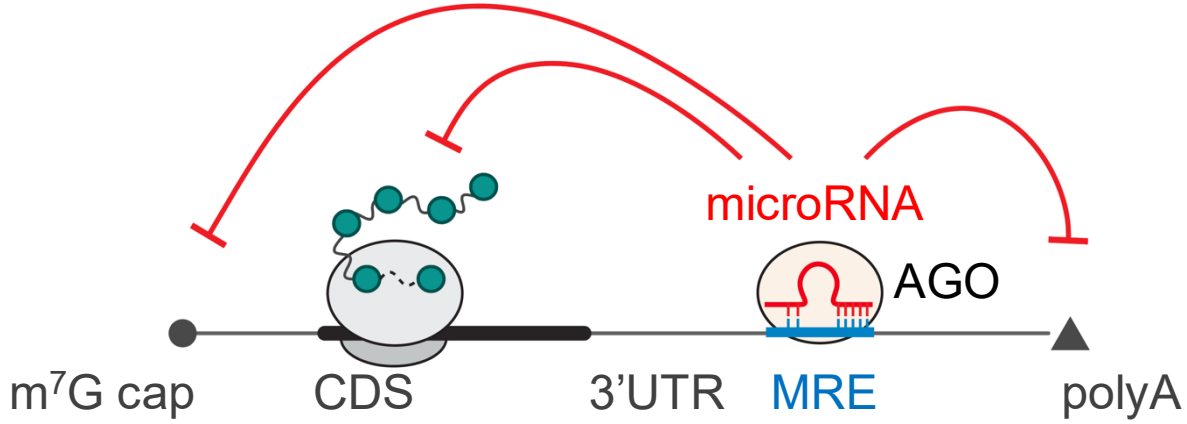
Ambros, Ruvkun
2024 Nobel Prize in
Physiology or Medicine

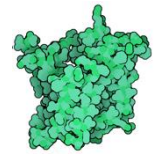
C.Elegans

lin: abnormal lineage



Antisense sequence complementarity





Less
Protein

Lee…Ambros **Cell** (1993); Wightman…Ruvkun **Cell** (1993); Pasquinelli…Ruvkun (2000)
Work from Bartel, Kim, MacRae, Filipowicz, Zamore, Hannon, Carthew, Tuschl, Izaurralde, Burge, Green, Mendel labs

Sculpting Gene Expression to Achieve **Cellular Diversification**

More non-coding **genes?**

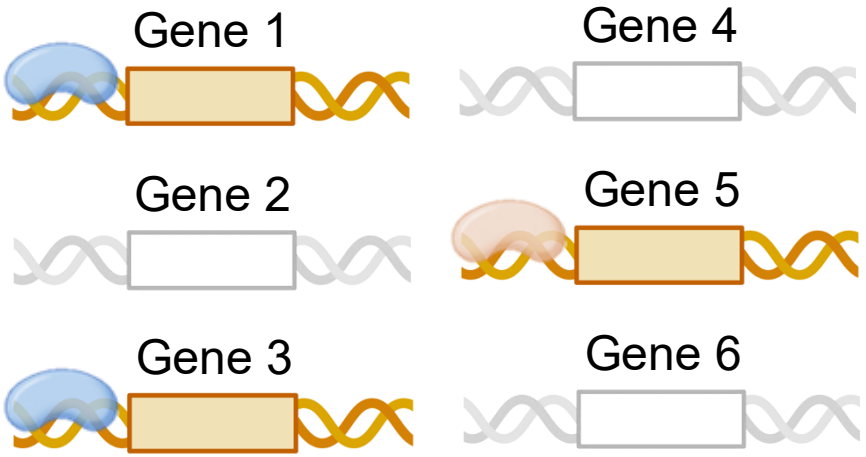
More layers of regulation **of gene expression?**

More **cell types** (tissues and organs)

High **complexity**

Transcription factors

Expansion of the **transcription factor repertoire**



Sculpting Gene Expression to Achieve **Cellular Diversification**

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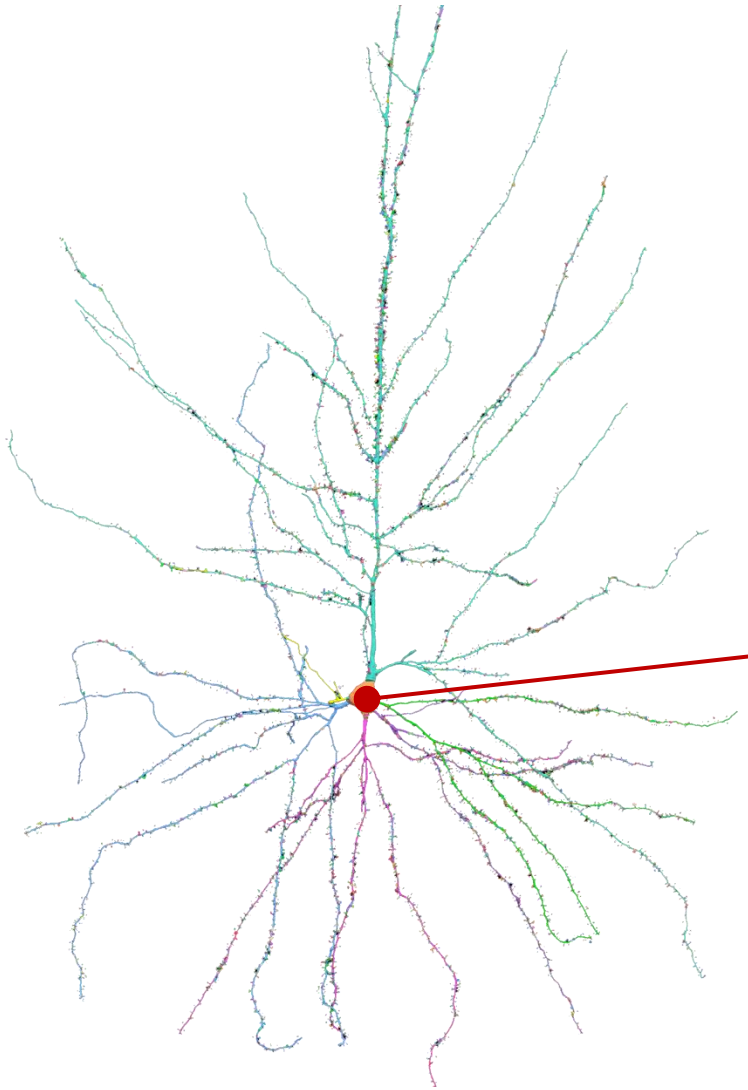
Neurons face Unique **Gene Expression Challenges in Space**

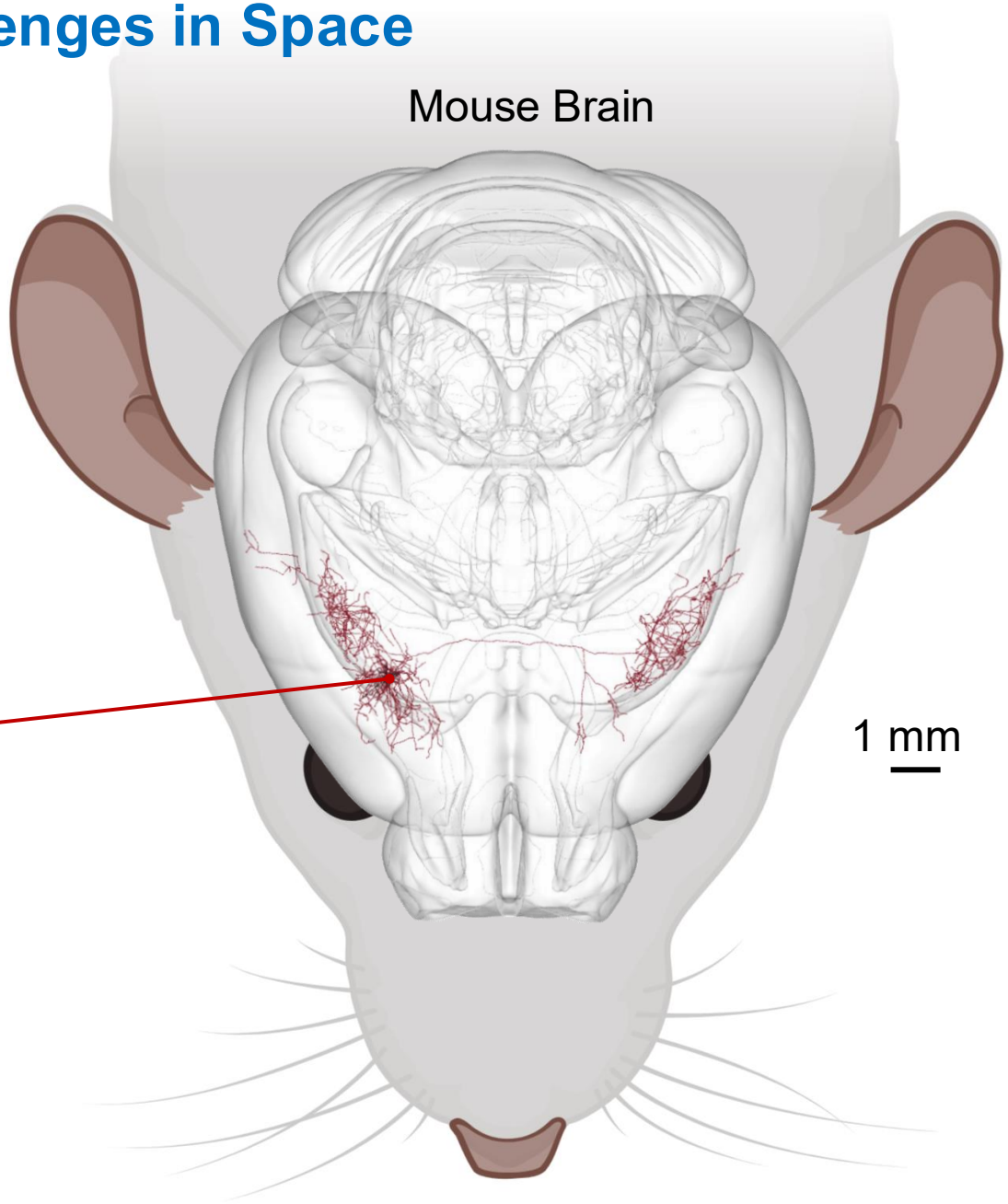
Pyramidal Neuron



Epithelial Cell

100 μm

Mouse Brain



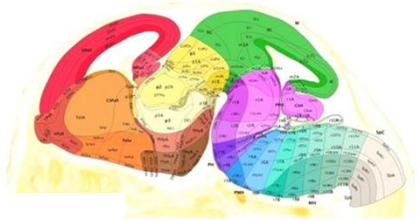
1 mm

MouseLight (2017)

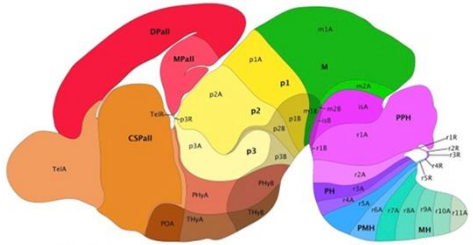
Neurons face Unique **Gene Expression Challenges in Time**

E14.5:

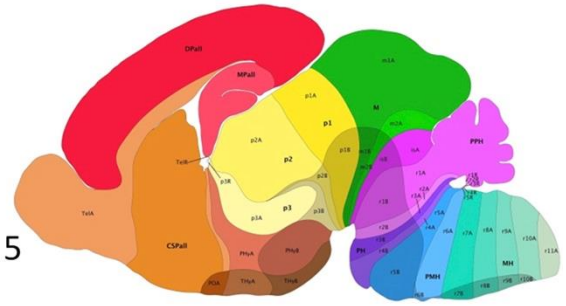
~20,000 Neuronal precursors



E15.5
Level 10

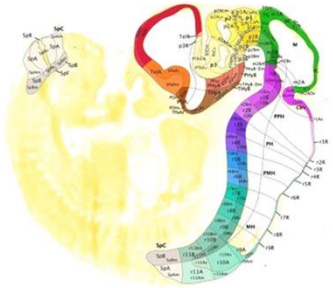


E18.5
Level 5



P4
Level 5

Cx IC
Hip Cb
Put Rt
OB POA



E11.5
Level 9

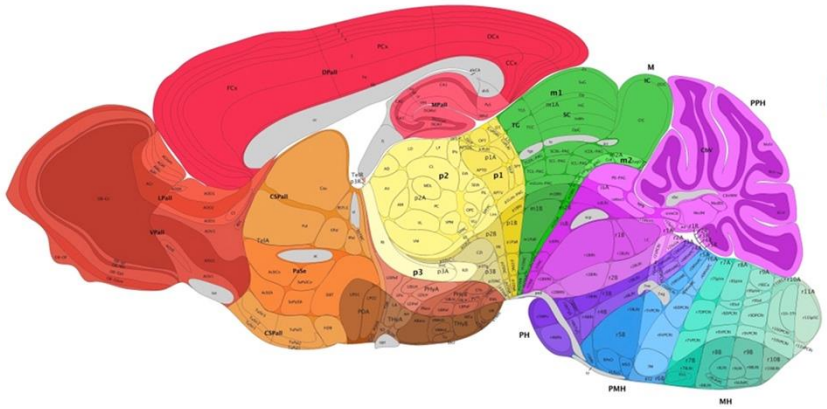
Adult:

~35M cells

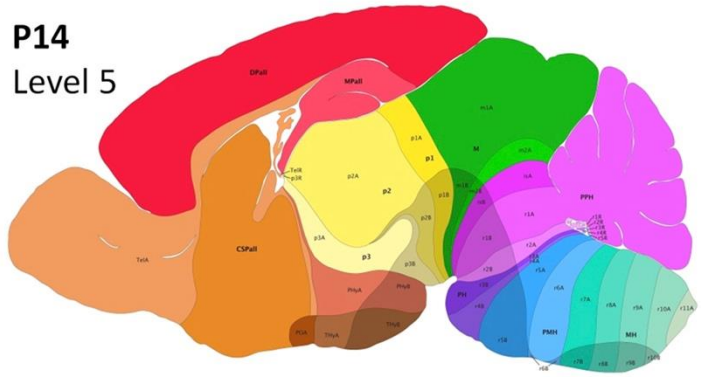
5,300 cell types (34 broad classes)

100B-1T synapses

P56
Level 13

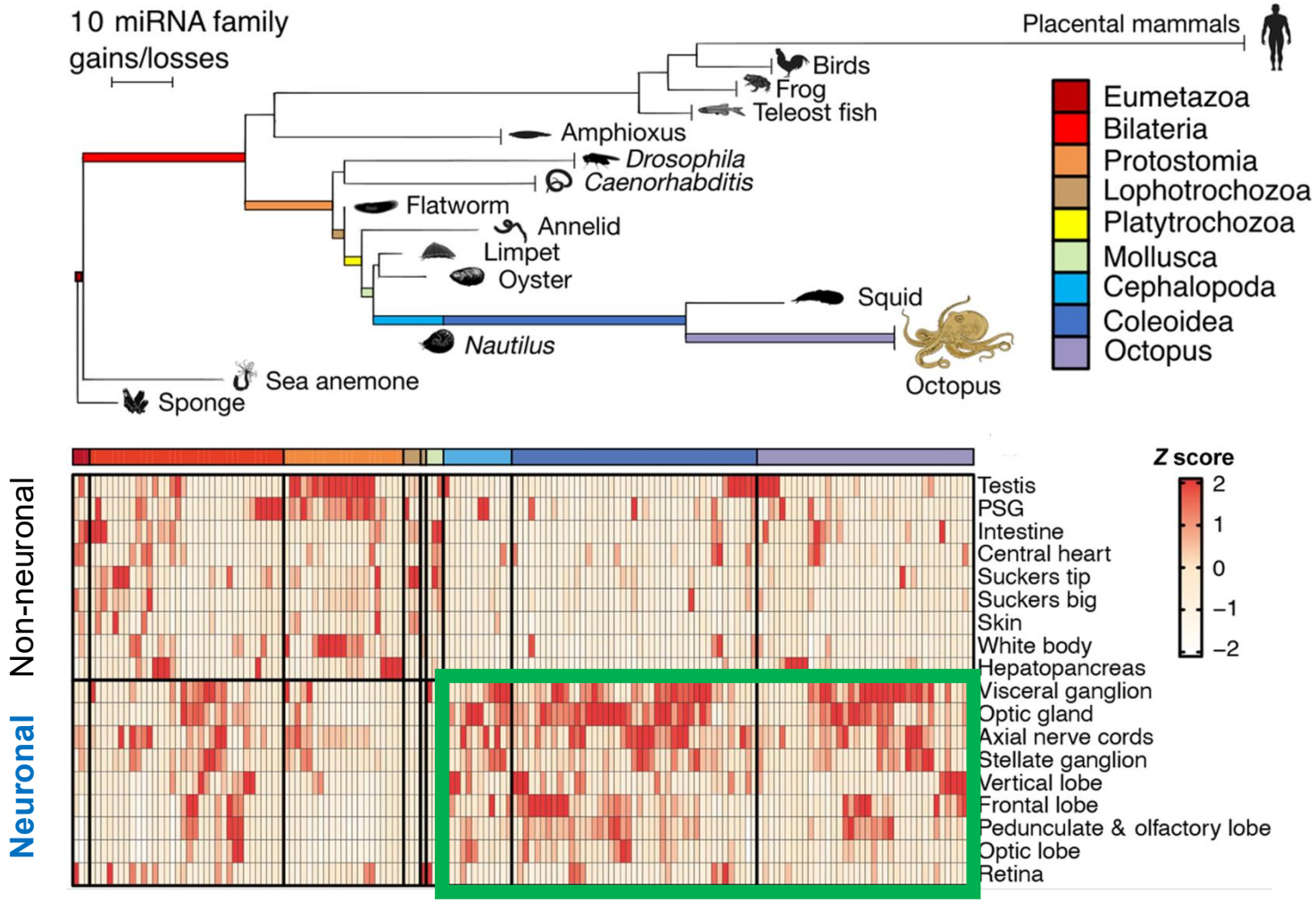


P14
Level 5



Thompson et al. **Neuron** (2014)

Expansion of the **Neuronal microRNA Repertoire**



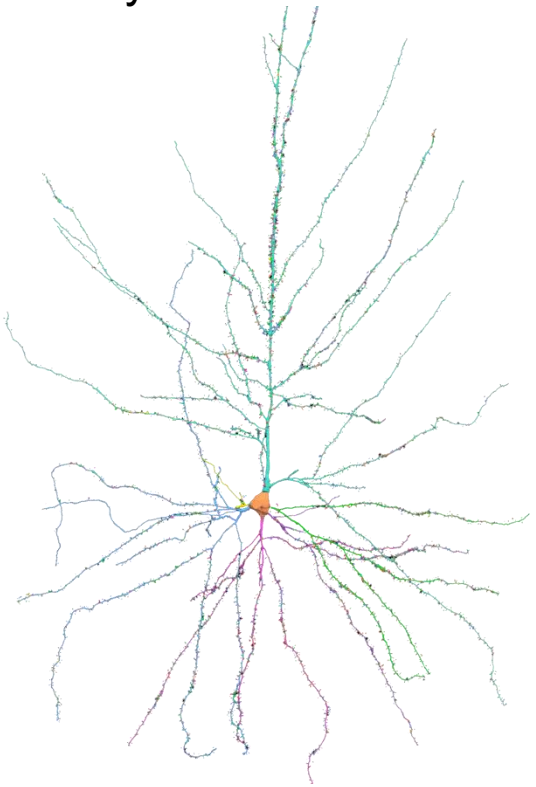
Hypothesis: microRNAs helped the **emergence** of complex brains

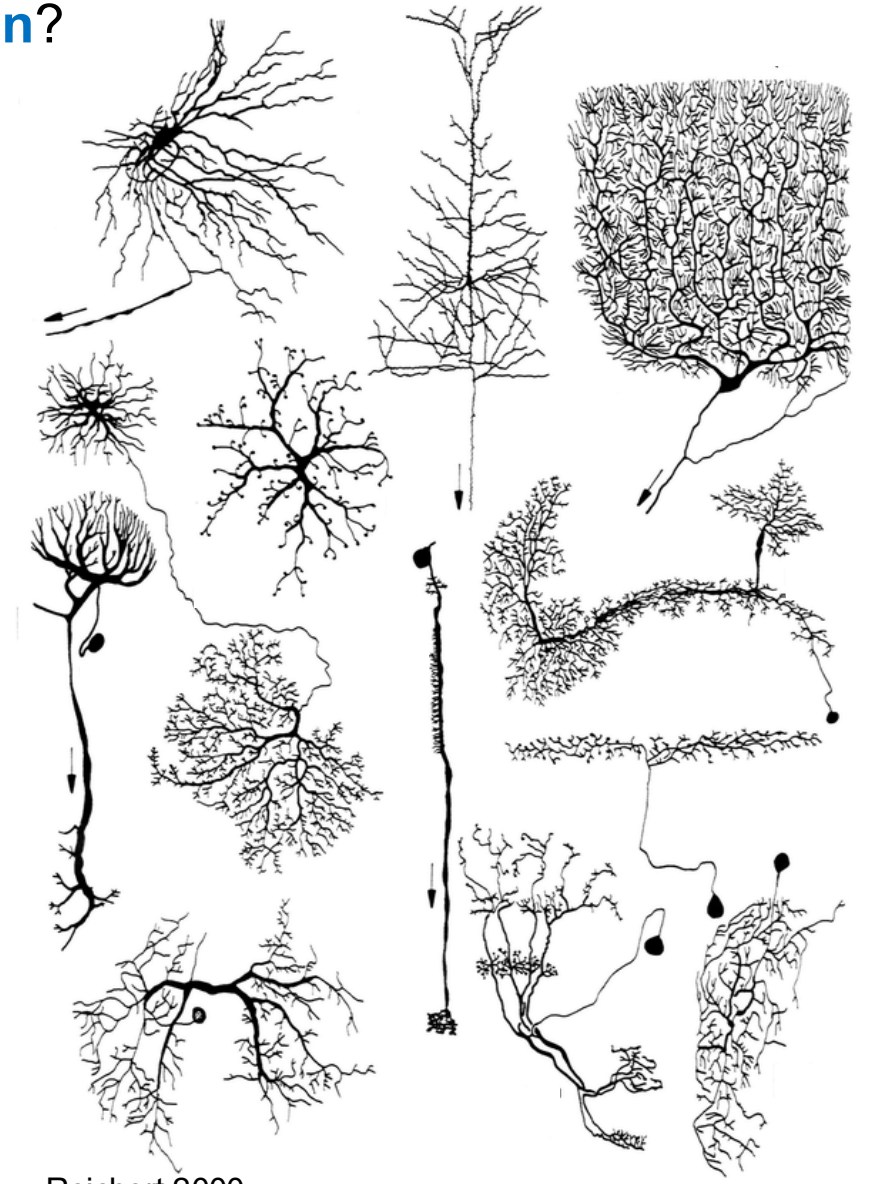
Zolotarov… Rajewsky **Sci Adv** (2022)

Hypothesis: microRNAs helped the **emergence** of complex brains

- Are microRNAs driving **neuronal diversification**?

Pyramidal Neuron



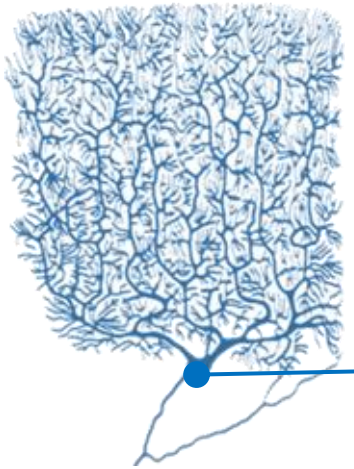


Reichert 2000

Mapping Cell Type-Specific **microRNA-Target Interactions**

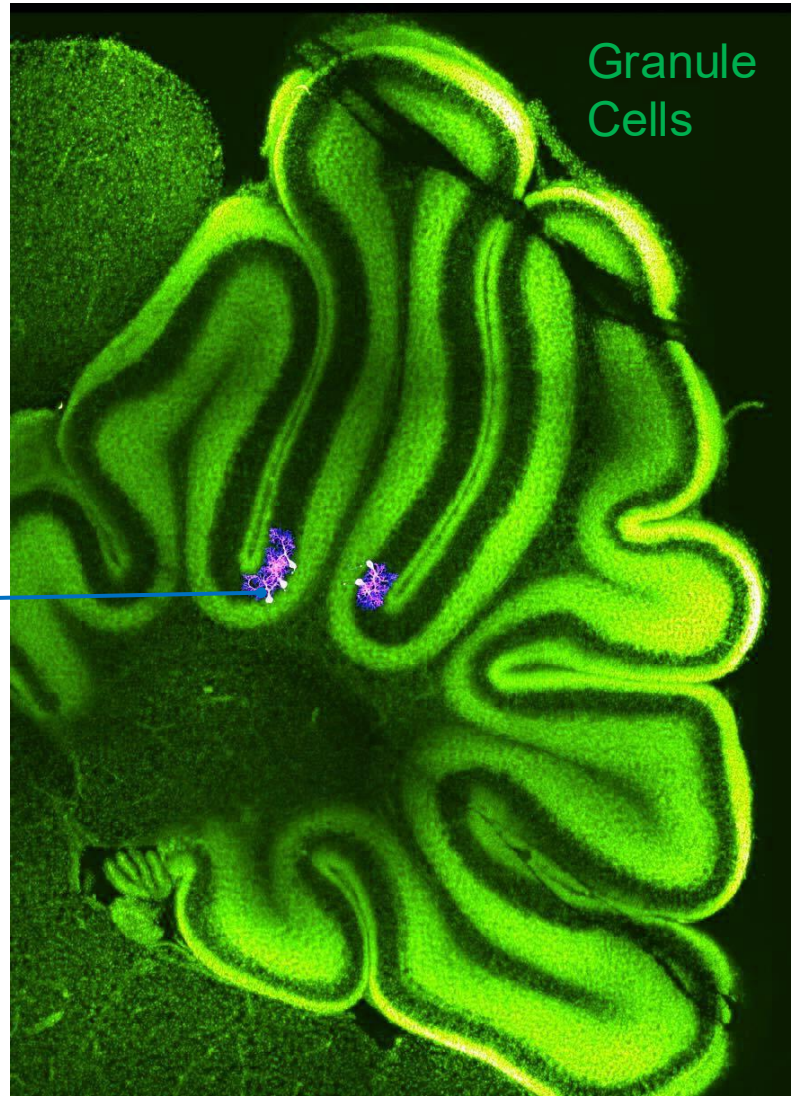
Are microRNAs **important** for these structural difference?

Pyramidal Neuron



Purkinje Cell

~1% of cells



Which **microRNAs** are expressed?
Which **genes** are repressed?

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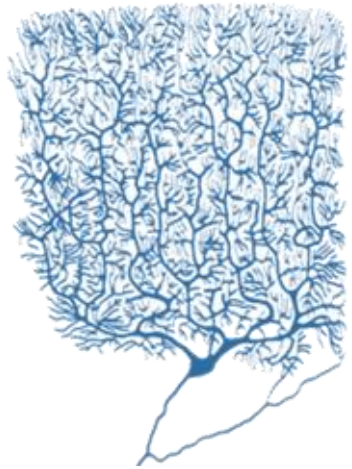
Are microRNAs **important** for these structural difference?

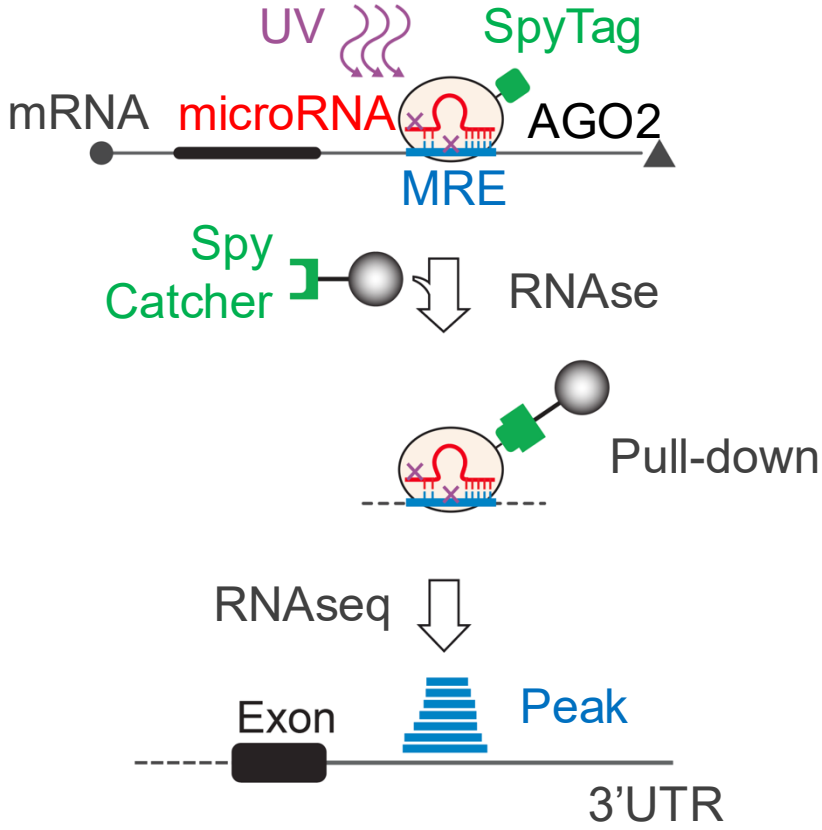
Pyramidal Neuron



Purkinje Cell

Which **microRNAs** are expressed?
Which **genes** are repressed?

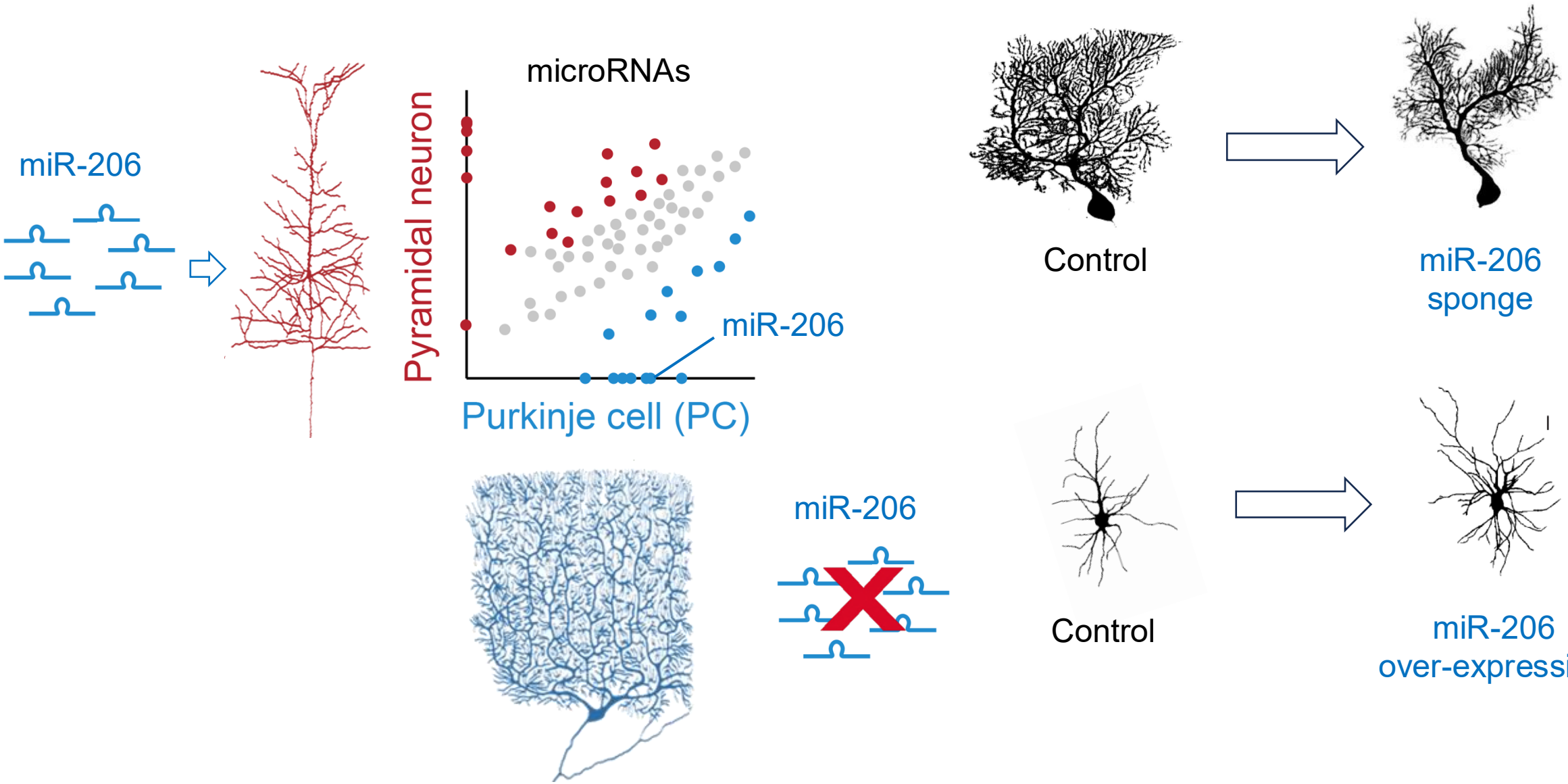
SAPseq



with **Ian MacRae**

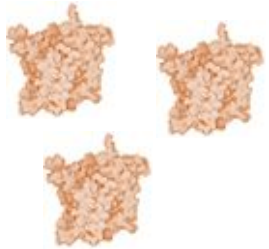
Zolboot*, **Xiao*** et al. **Neuron** (2025)

A **Purkinje Cell-Specific microRNA** Controls Dendritic Arborization

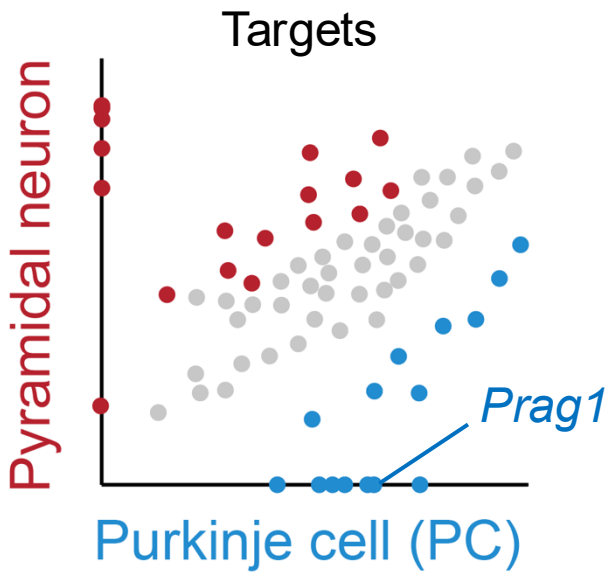


Zolboot*, **Xiao*** et al. **Neuron** (2025)

Purkinje Cell-Specific Targeting Controls Dendritic Arborization

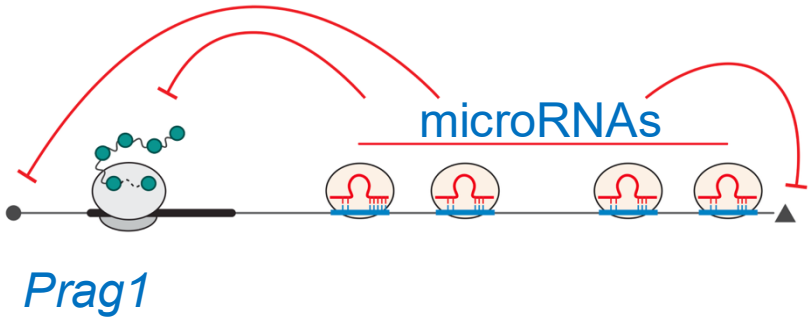


High
PRAG1



Control

PRAG1
Up-regulation



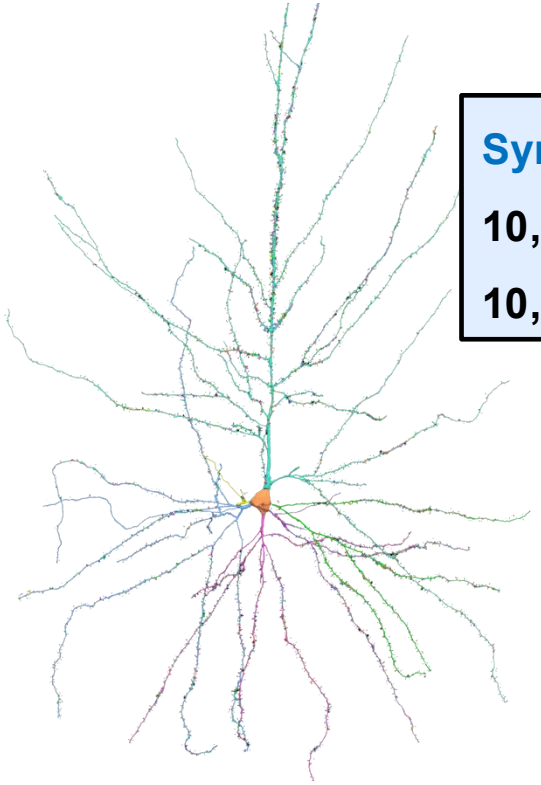
Low
PRAG1

Zolboot*, **Xiao*** et al. **Neuron** (2025)

Hypothesis: microRNAs helped the **emergence** of complex brains

- Are microRNAs driving **neuronal diversification**?
- Are microRNAs instructing **developmental processes** that are increasingly more complex in larger brains? → **Precise synaptic connections**

Pyramidal Neuron



Synapses (1 Neuron):

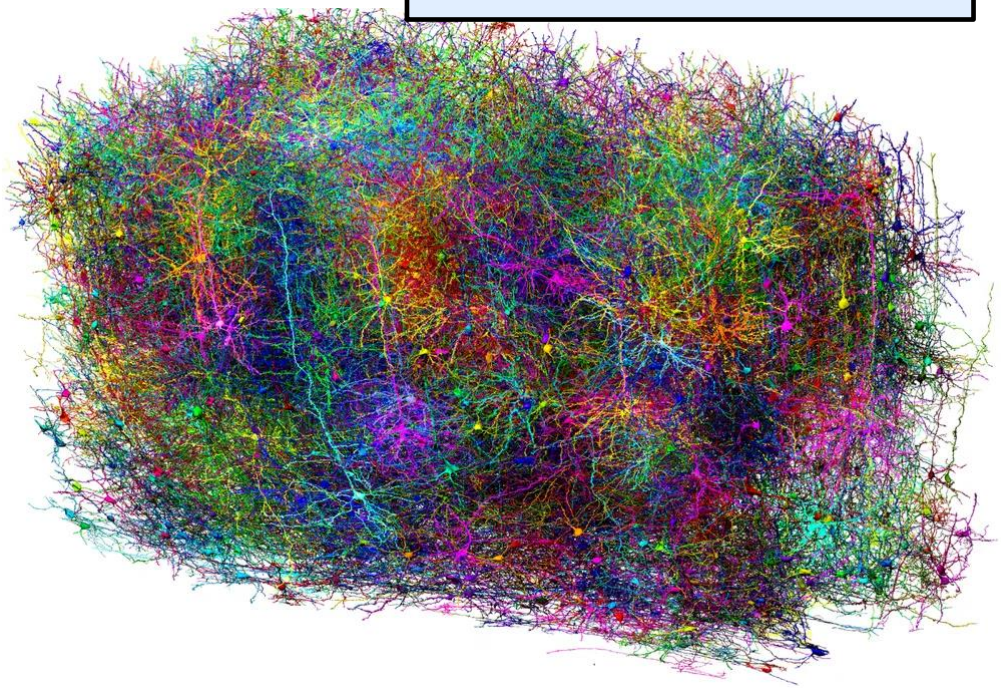
10,000-30,000 Inputs

10,000 Outputs

Finding the right partner:

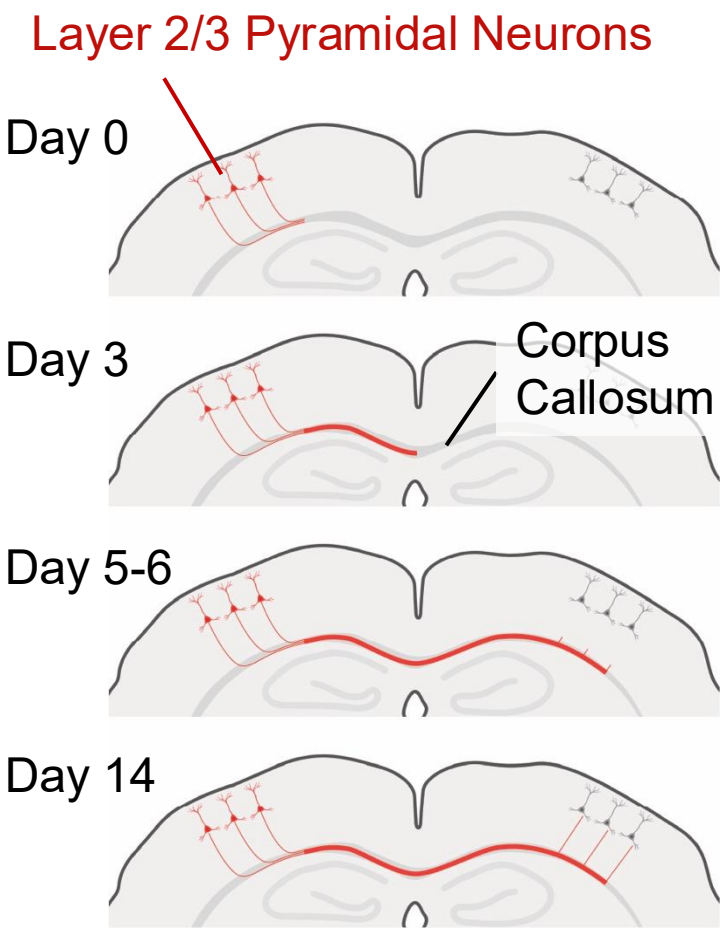
14M Cortical Neurons

⇩ **1000** in Visual Cortex

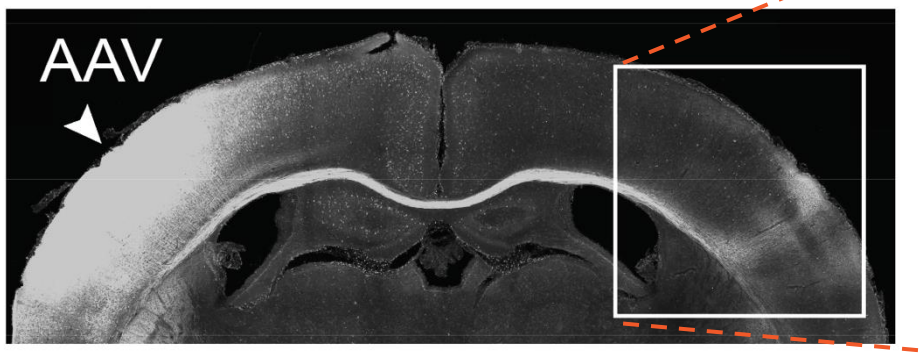


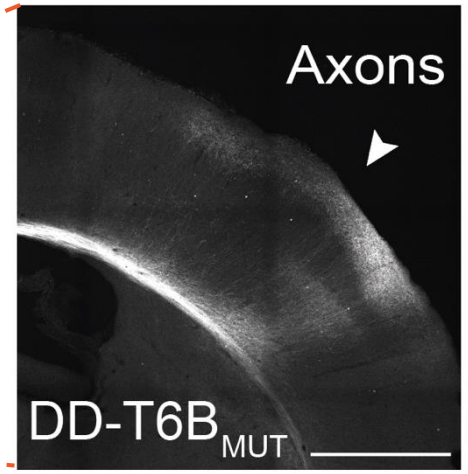
The MICrONS Consortium, **Nature** (2025)

microRNAs Instruct **Contralateral Axon Innervation**

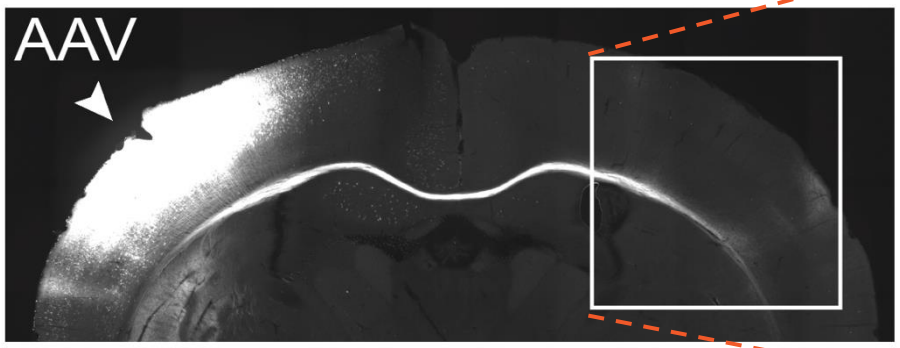


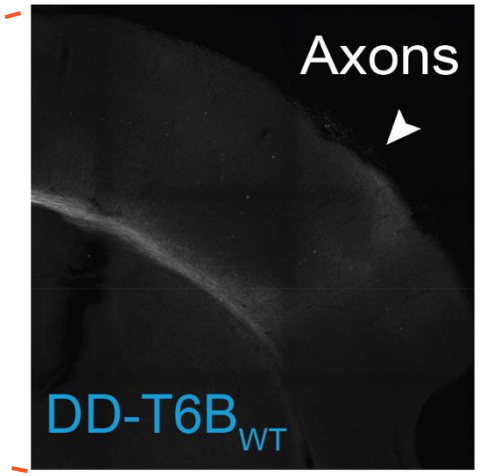
Control





microRNA **loss-of-function** (Day 5-7)

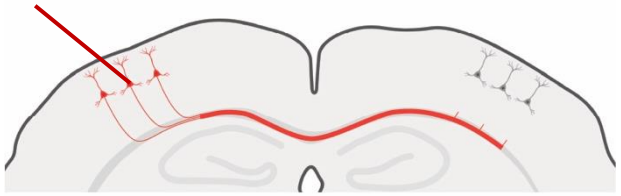




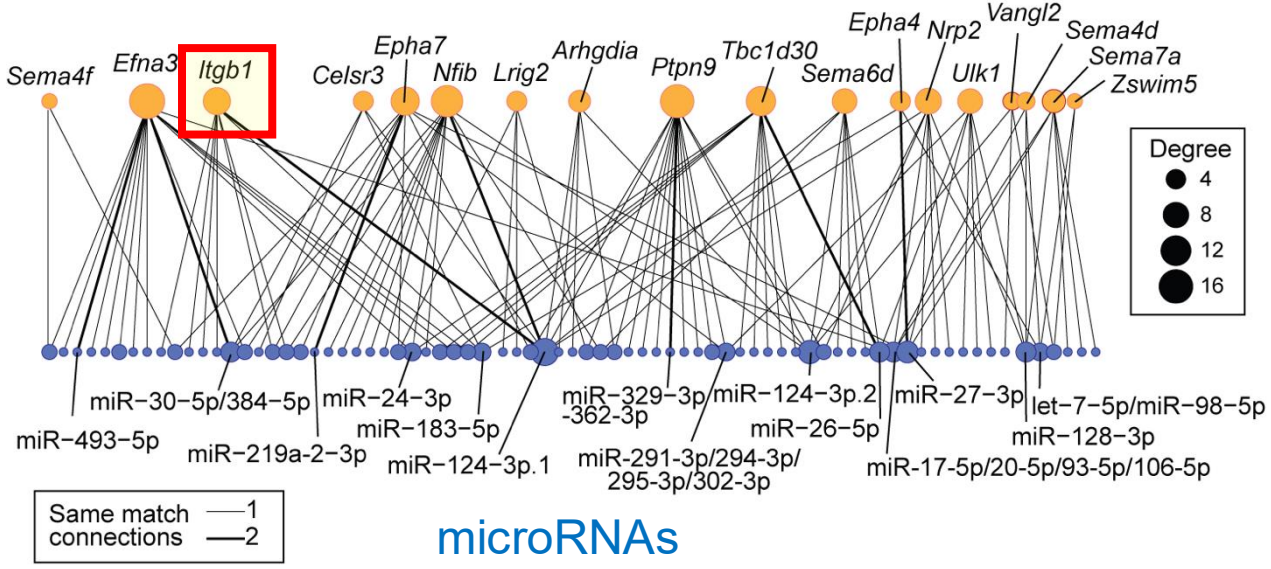
Zampa*, **Sievert*** et al. in preparation

Convergent Repression of *Itgb1* Instructs **Contralateral Axon Innervation**

Map microRNA-target interactions
from Pyramidal Neurons at Day 5

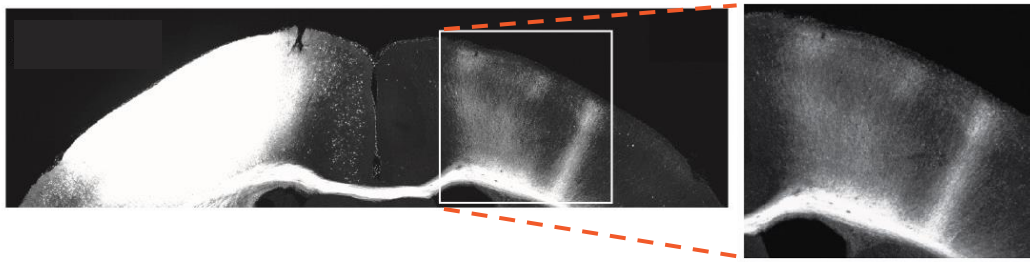


Axon Development Strong Targets

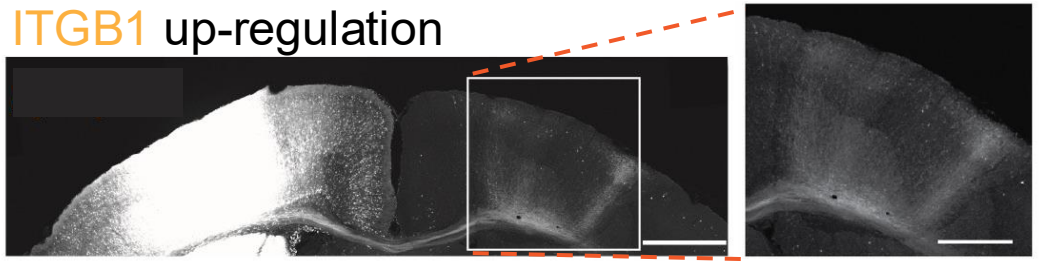


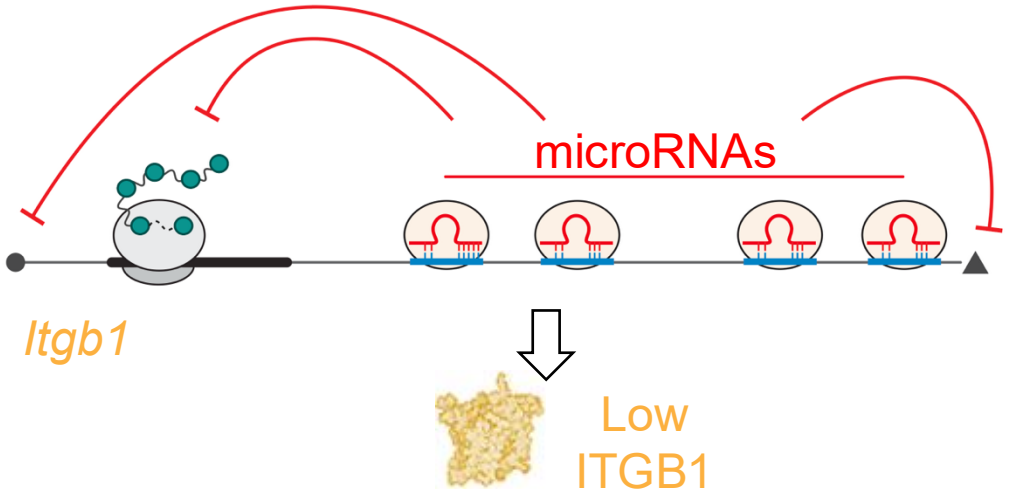
microRNAs

Control



ITGB1 up-regulation

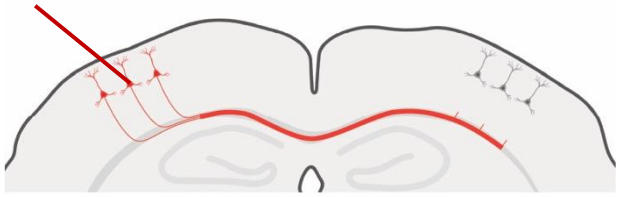




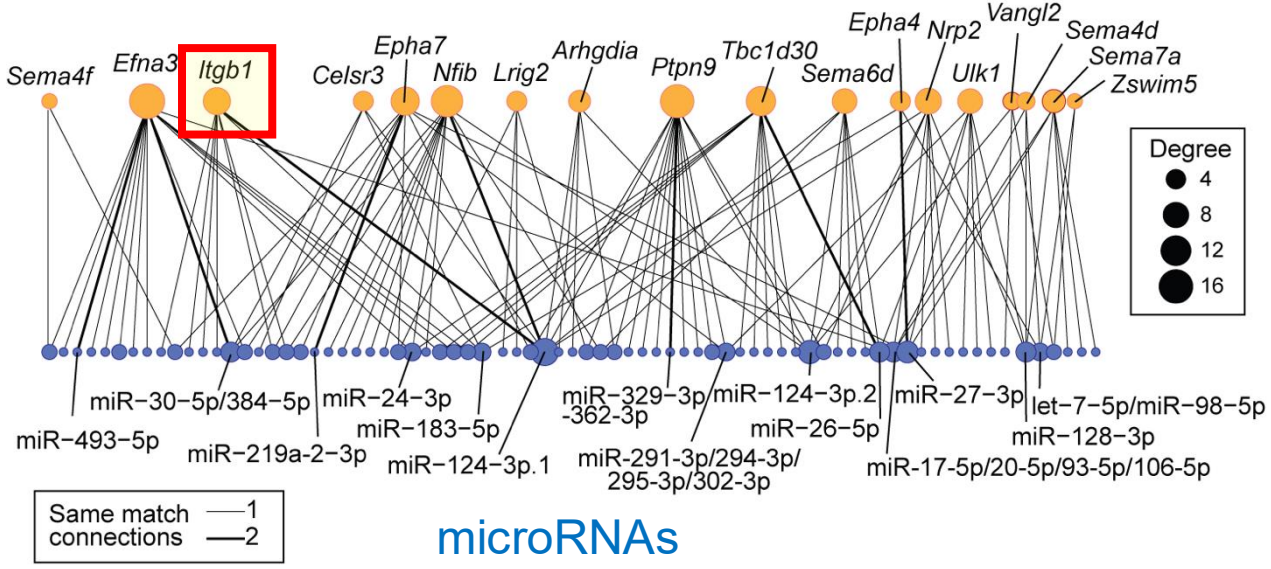
Zampa*, **Sievert*** et al. in preparation

Convergent Repression of *Itgb1* Instructs **Contralateral Axon Innervation**

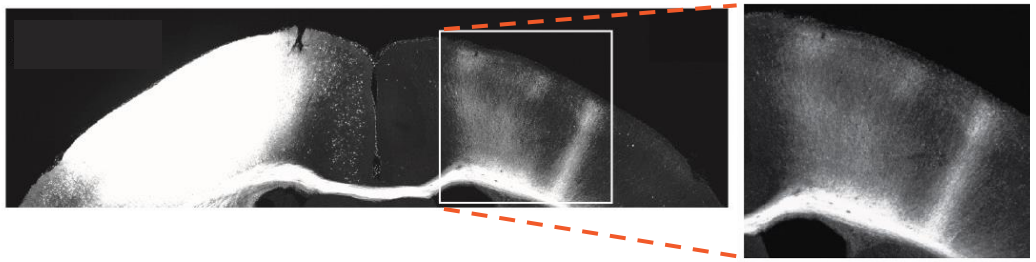
Map microRNA-target interactions
from Pyramidal Neurons at Day 5



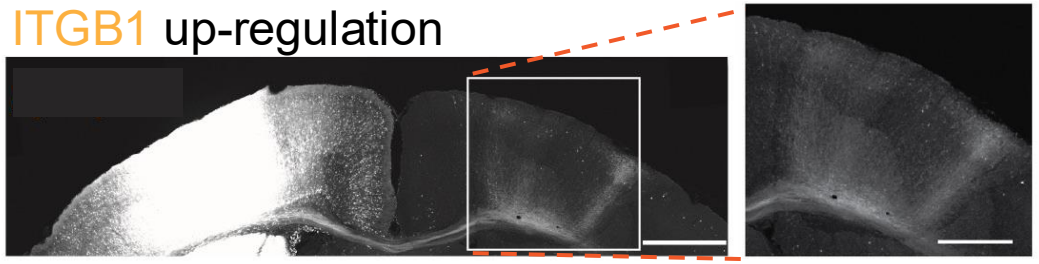
Axon Development Strong Targets

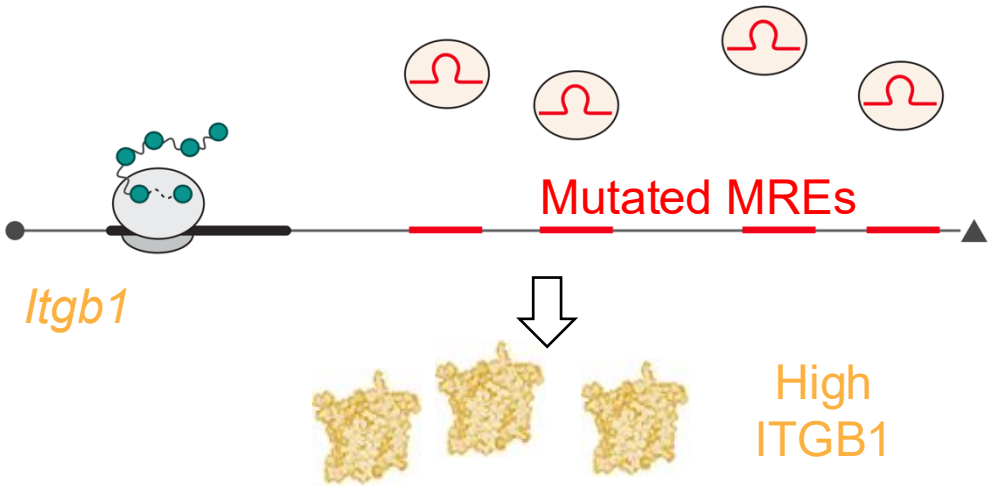


Control



ITGB1 up-regulation



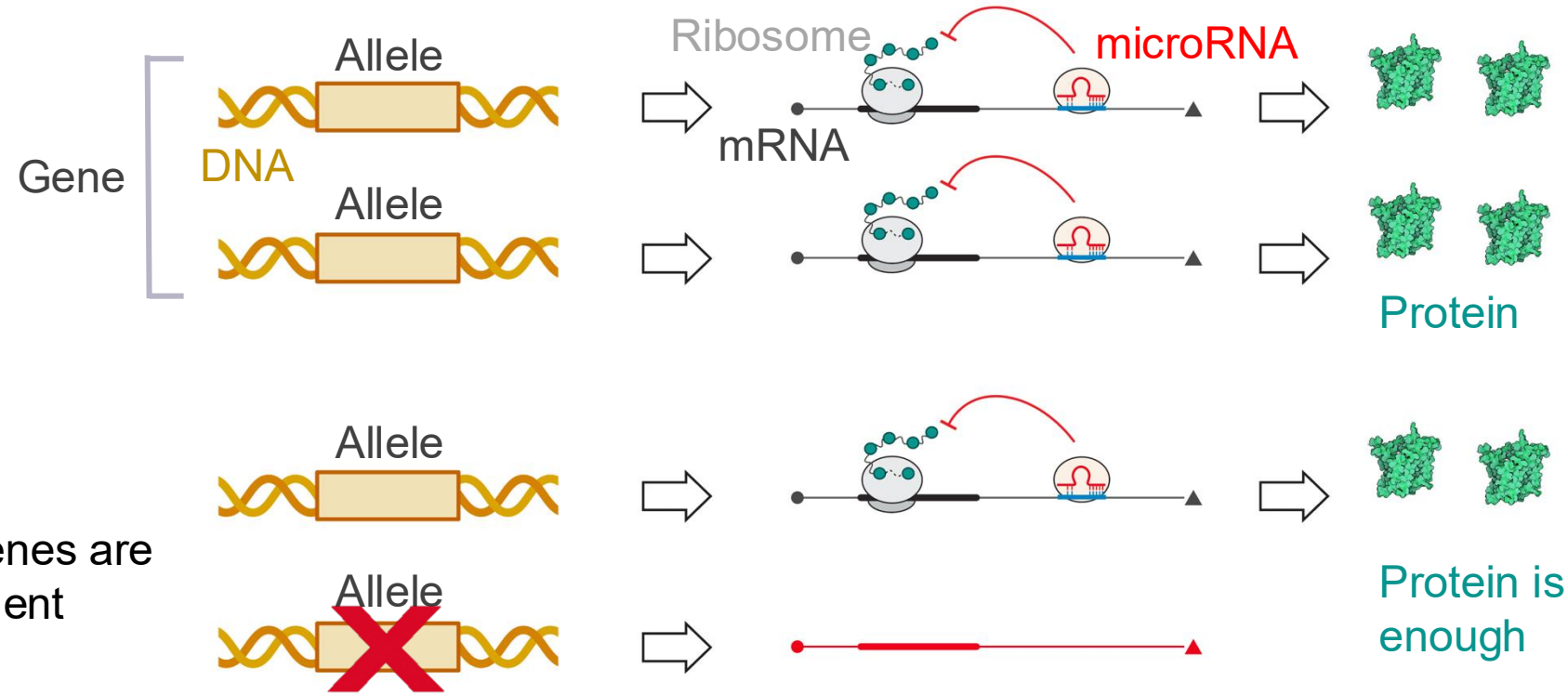


Zampa*, **Sievert*** et al. in preparation

From **Basic** Developmental Neuroscience to **Therapies**

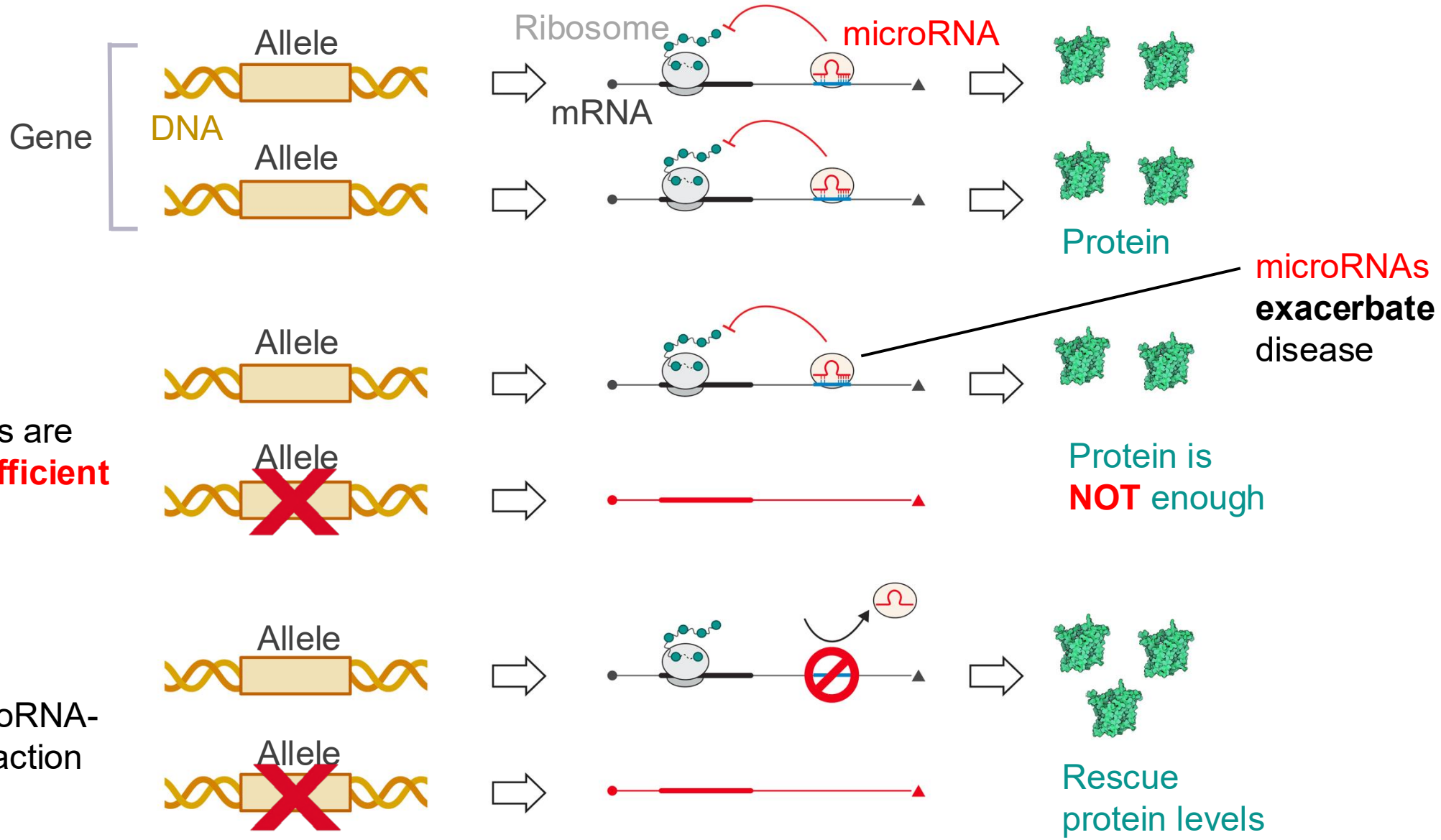
- Fundamentals of **gene expression**: from DNA to microRNAs
- microRNAs helped the **emergence of complex brains**
- Leveraging basic knowledge of microRNA function to **correct neurodevelopmental disorders**

Gene Augmentation for Therapy – **Haploinsufficiency-related Disorders**



~19,000 genes are haplosufficient

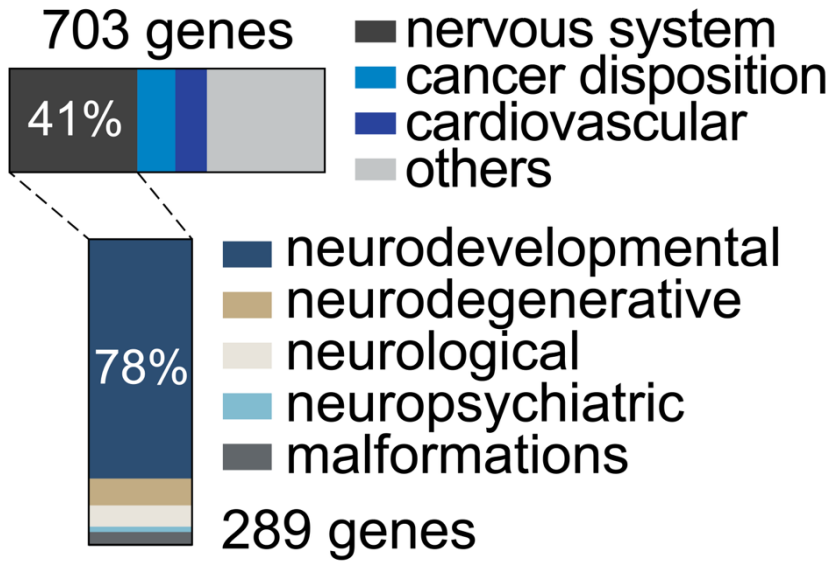
Gene Augmentation for Therapy – **Haploinsufficiency-related Disorders**



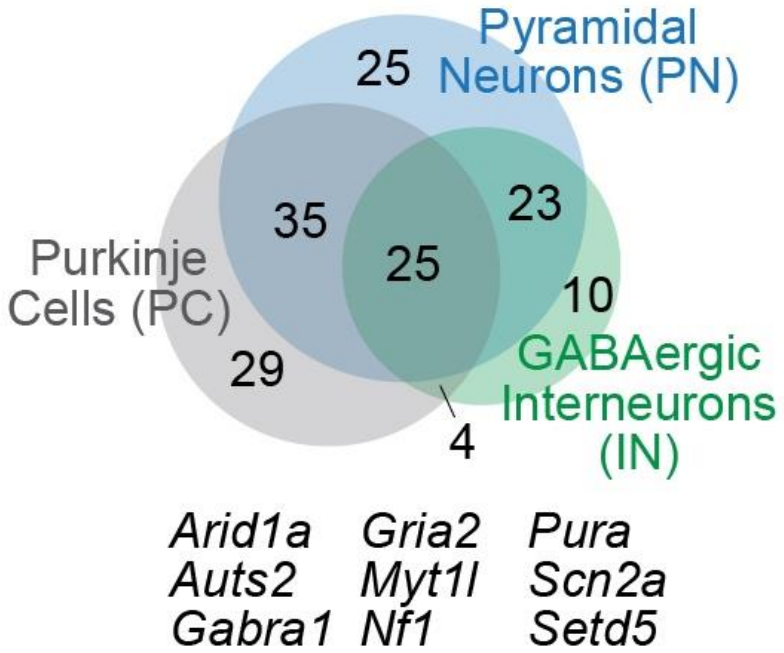
~700 genes are **haploinsufficient**

Block microRNA-target interaction

Neurodevelopmental Disease (NDD) Genes are **microRNA Targets**



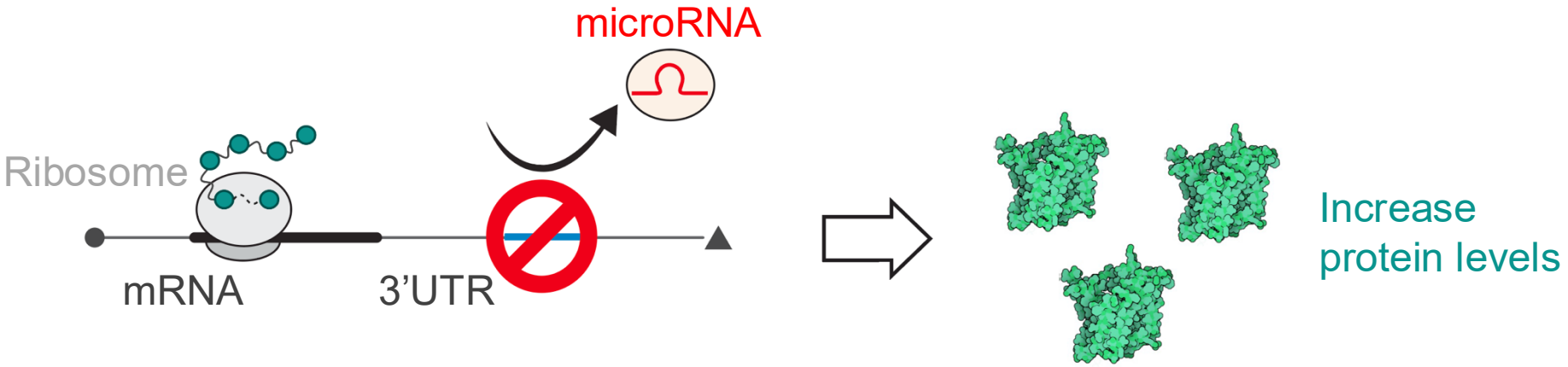
NDD Genes targeted by microRNAs



- Affect **200,000 births per year**
- Majority have **no disease-modifying treatments**

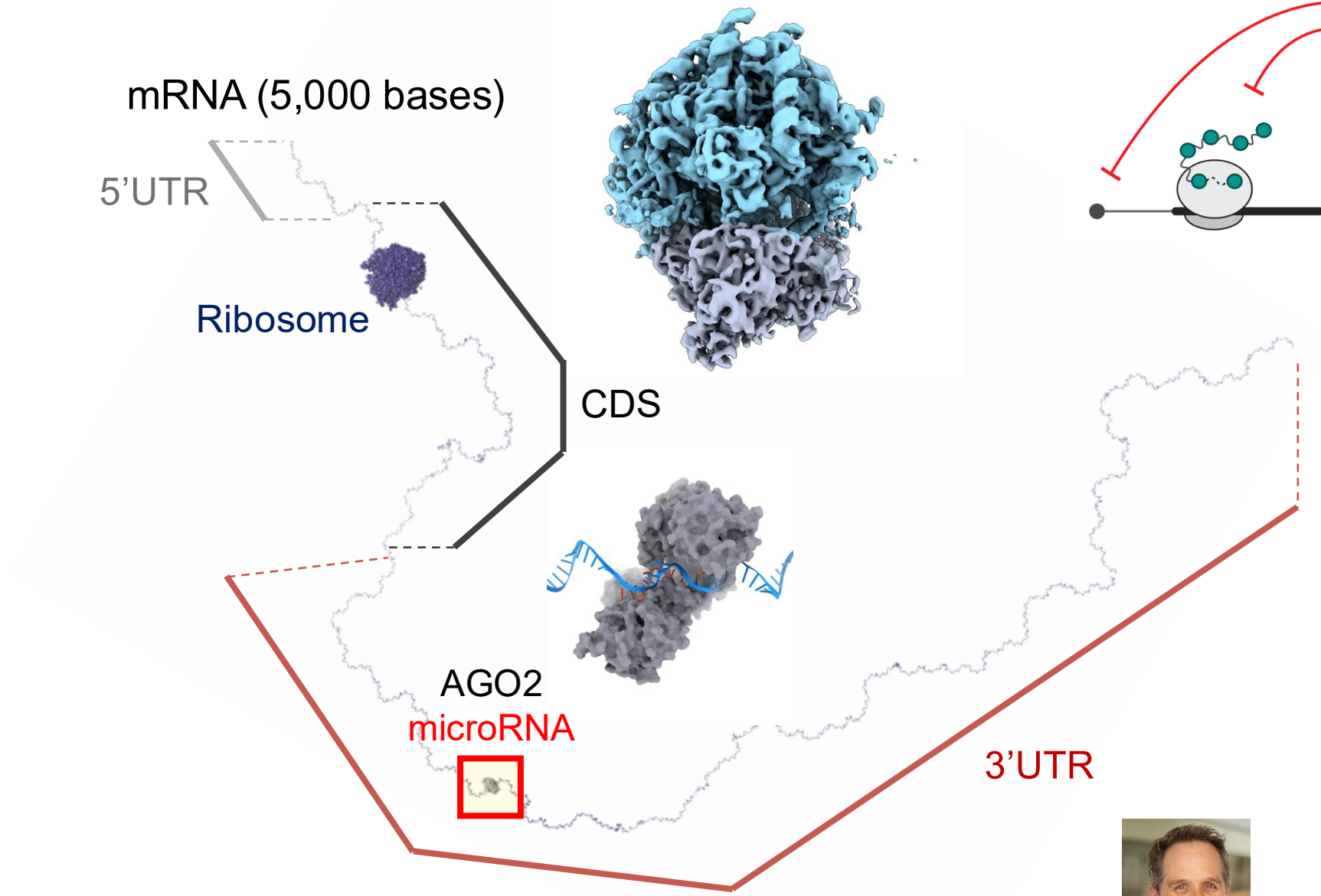
Zolboot*, **Ghanem*** et al. in preparation

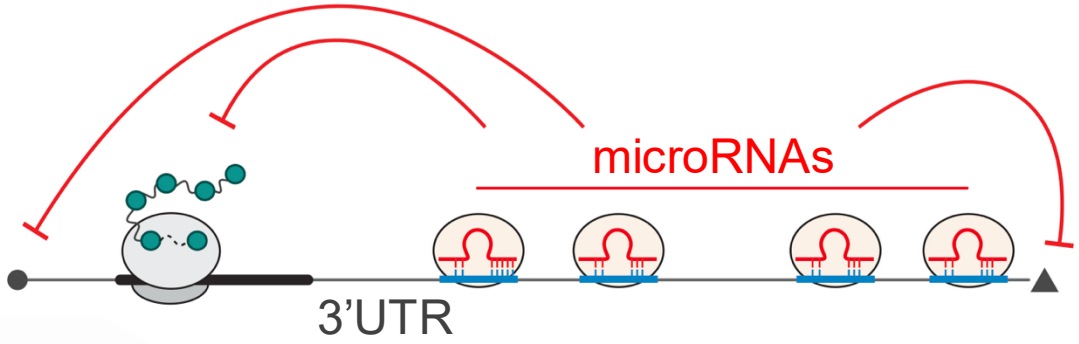
Functional Validation of microRNA-Target Interactions



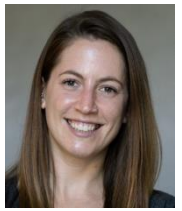
Zolboot*, **Ghanem*** et al. in preparation

The **Scale** of Gene Expression Regulation in **Mammalian Neurons**

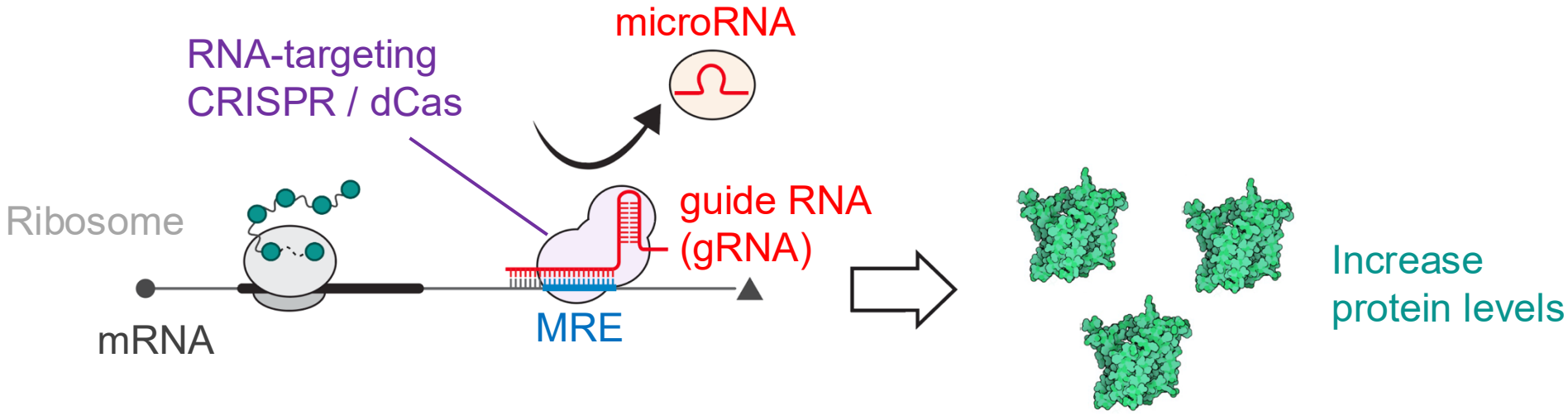




- Longer 3'UTR (up to 20kb)
- Higher density of MREs
- More microRNAs

Images from **MacRae**  , **Grotjahn**  , **Williamson**  labs

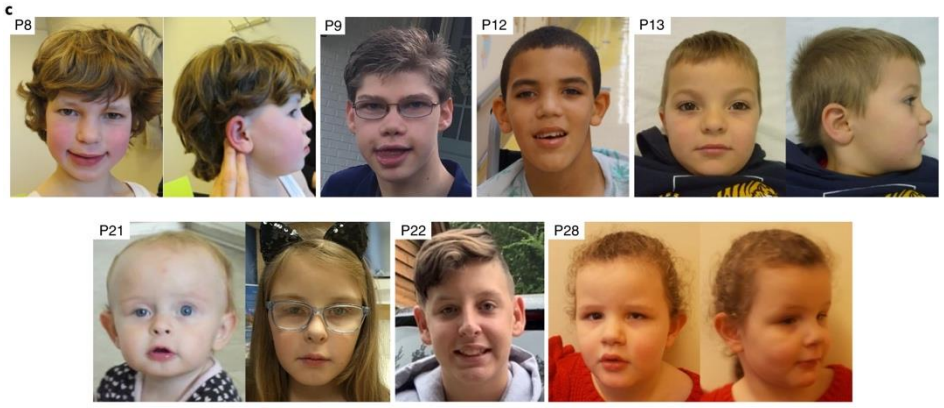
A **CRISPR/dCas** System Blocks microRNA-Target Interactions

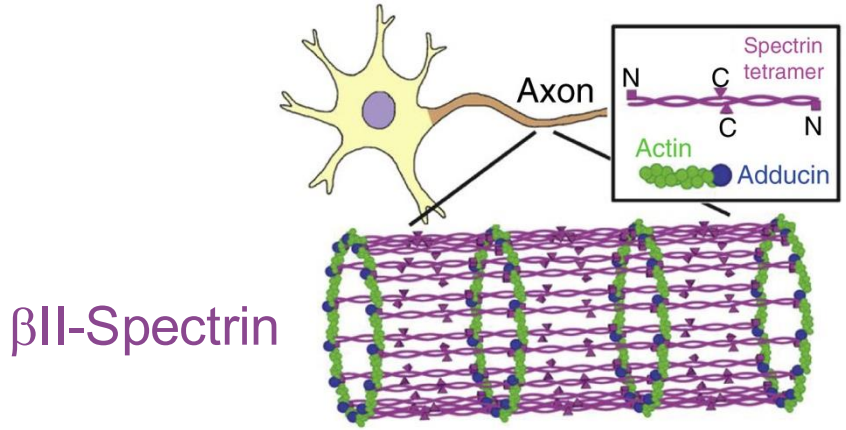


- High **affinity** for RNA
- **Programmable** and highly **specific**
- Paired with high-throughput protein measurements can be used to **scan the entire 3'UTR**

Zolboot*, **Ghanem*** et al. in preparation

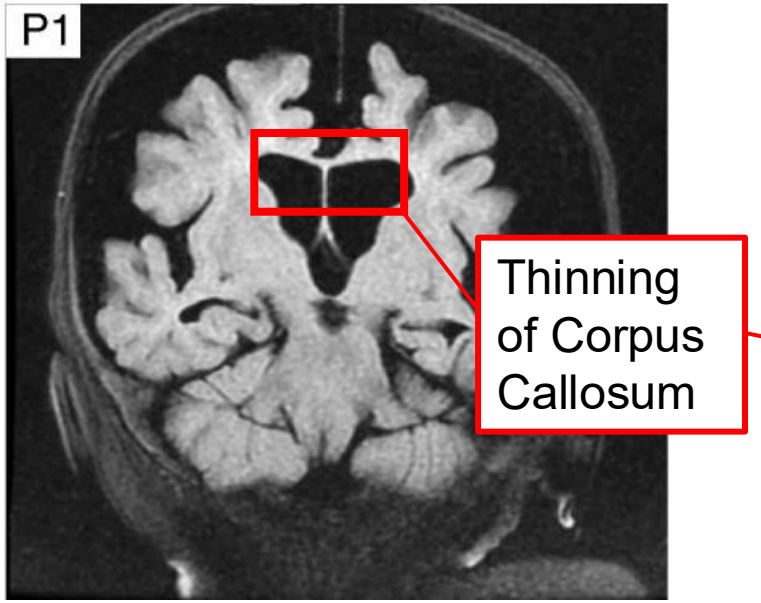
SPTBN1 haploinsufficiency causes a Neurodevelopmental Syndrome



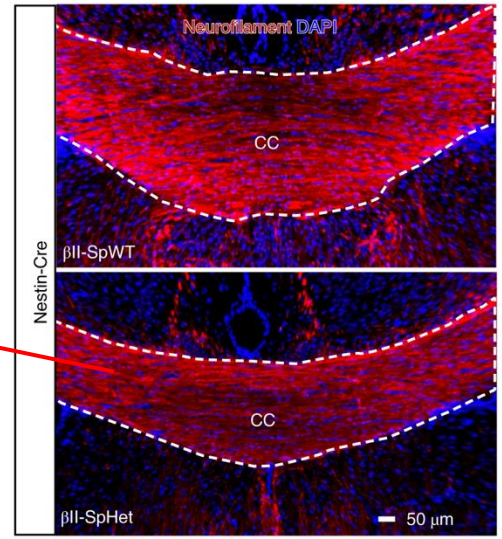


- Developmental delay
- Intellectual disability
- Autistic features
- Seizures
- Dysmorphic facial features

Patient MRI

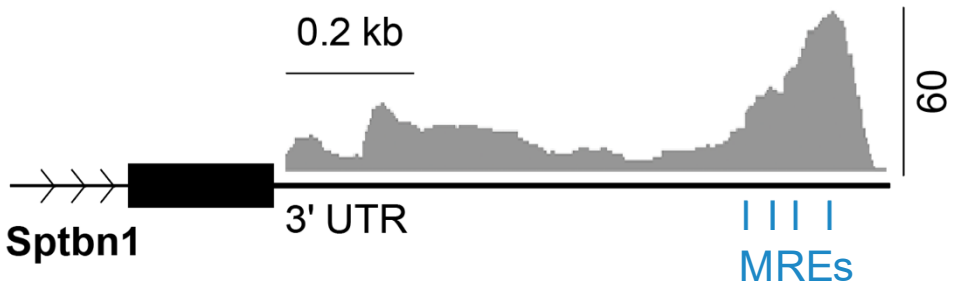


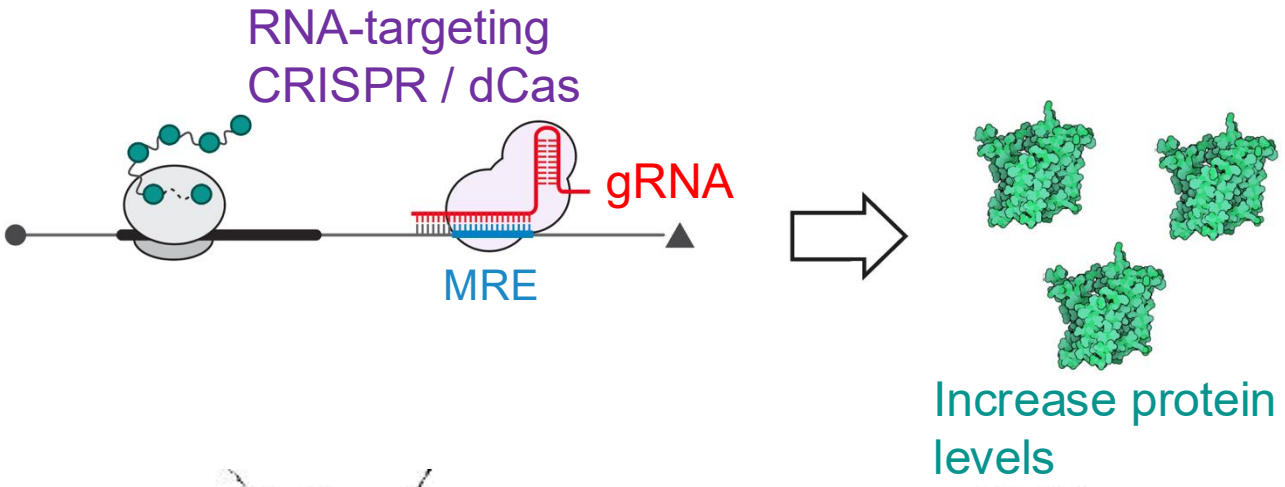
Mouse model Sptbn1 +/-



Cousin et al. **Nat Gen** (2021), Teliska et al. **Curr Bio** (2021)

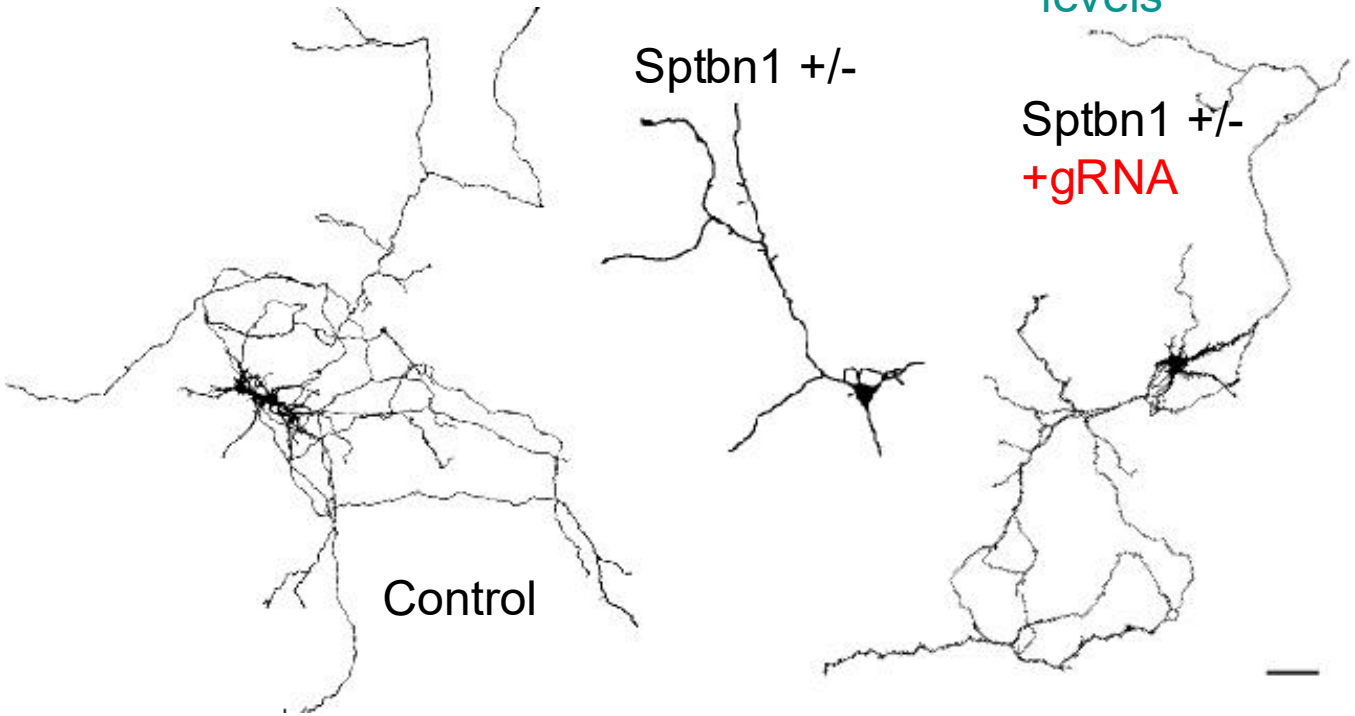
CRISPR/dCas Up-Regulates ***Sptbn1*** and Rescues **Axonal Defects**





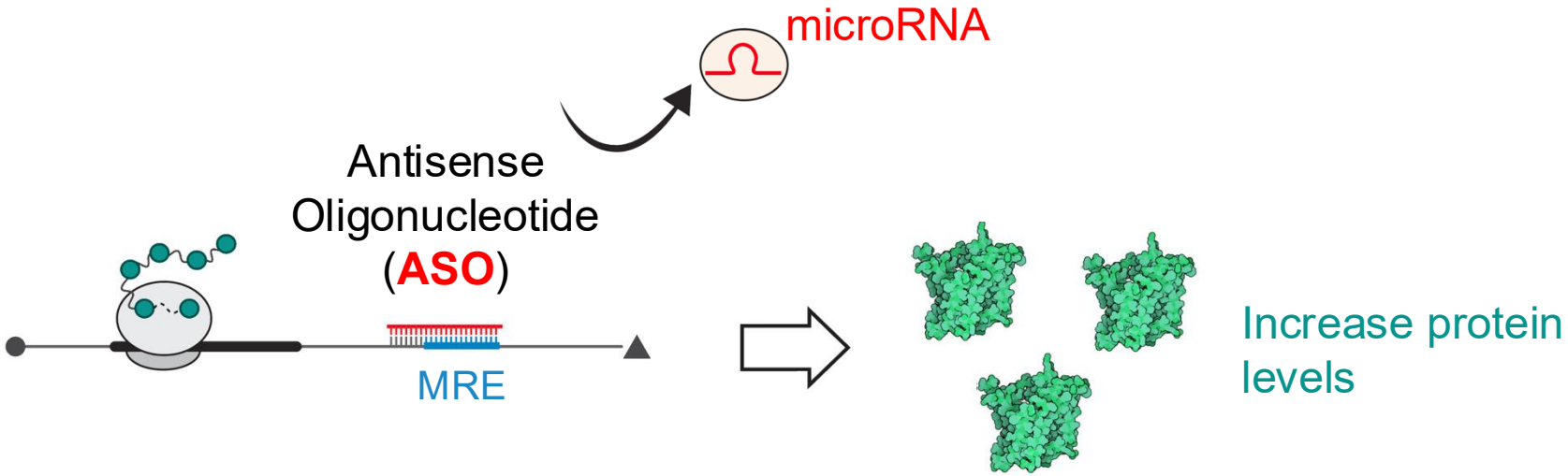
βII-Spectrin

Control | Sptbn1 +/- | Sptbn1 +/- +gRNA

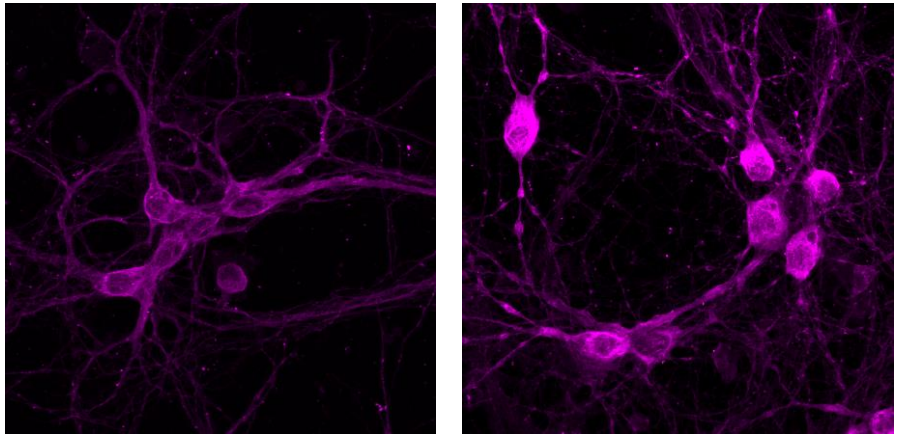


Zolboot*, **Ghanem*** et al. in preparation

ASOs Targeting the Same MRE Induce **Up-regulation**



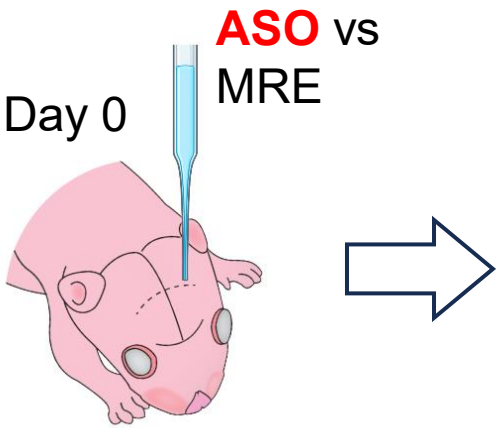
βII-Spectrin

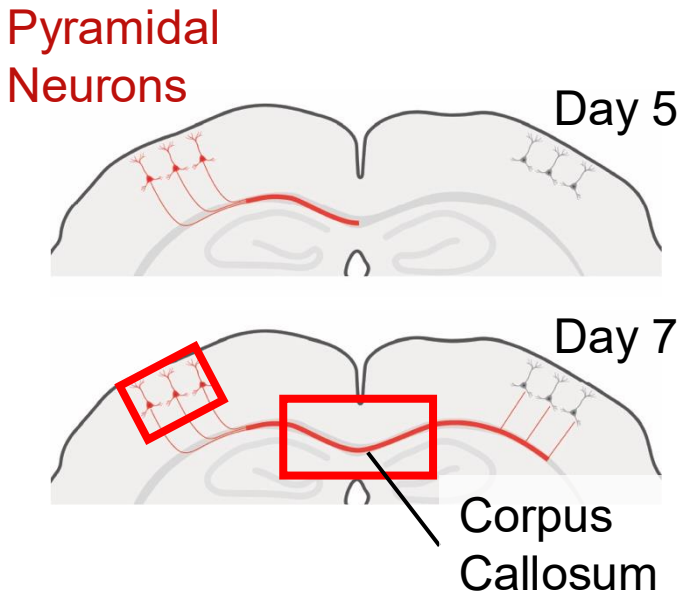


Control ASO

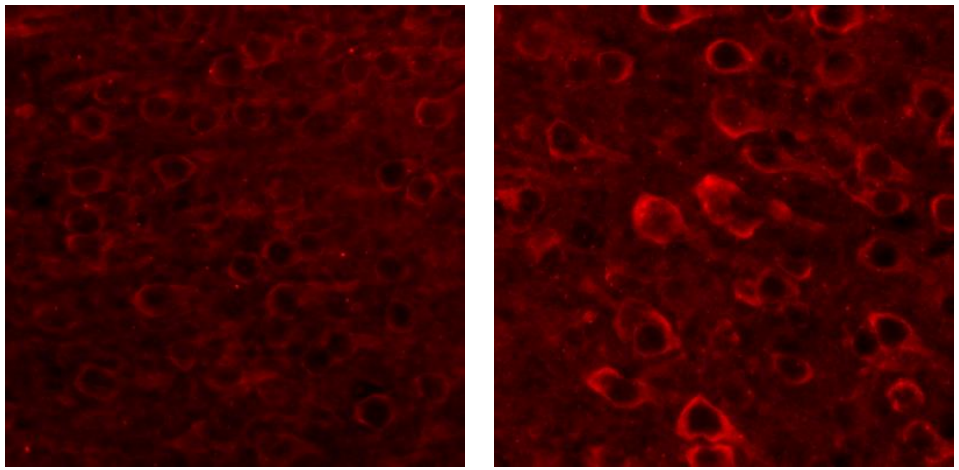
Zolboot*, **Ghanem*** et al. in preparation

ASO Rescues **βII-Spectrin** Levels and Axonal Defects ***in vivo***





βII-Spectrin

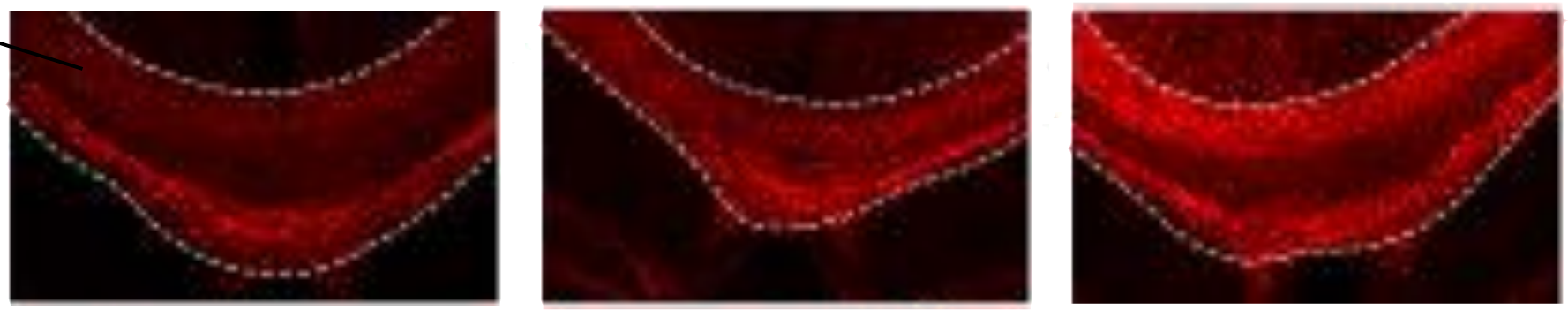


Sptbn1 +/-

Sptbn1 +/-
+ASO

Corpus
Callosum

Neurofilament



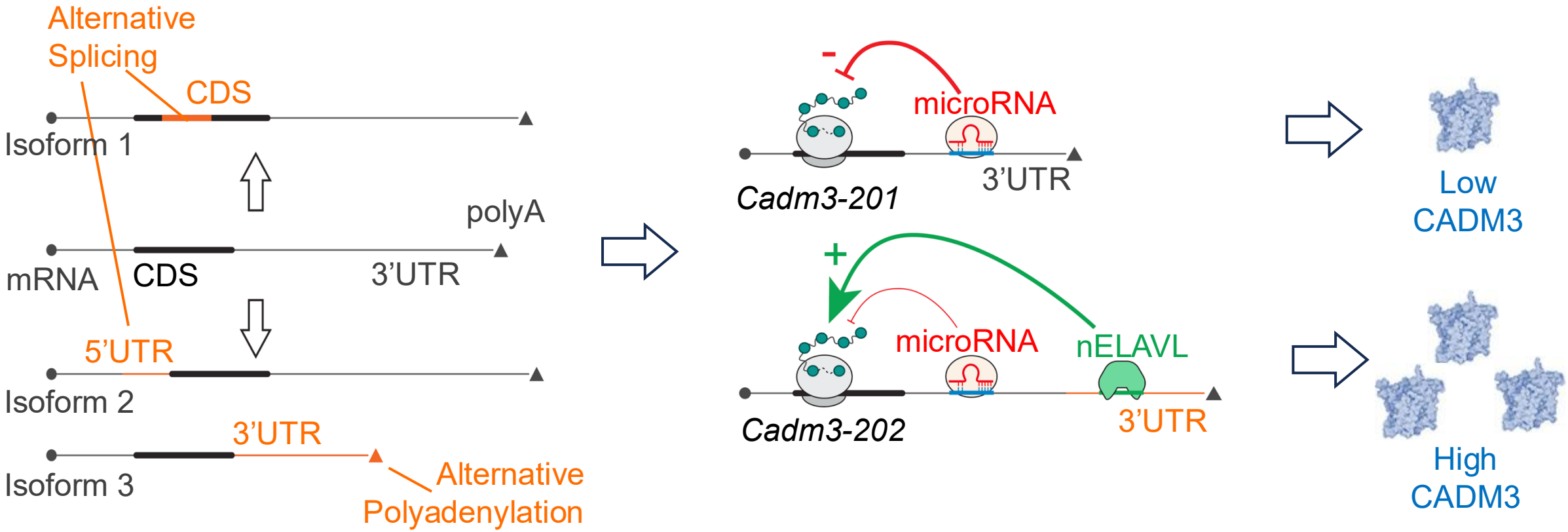
Control

Sptbn1 +/-

Sptbn1 +/-
+ASO

Zolboot*, **Ghanem*** et al. in preparation

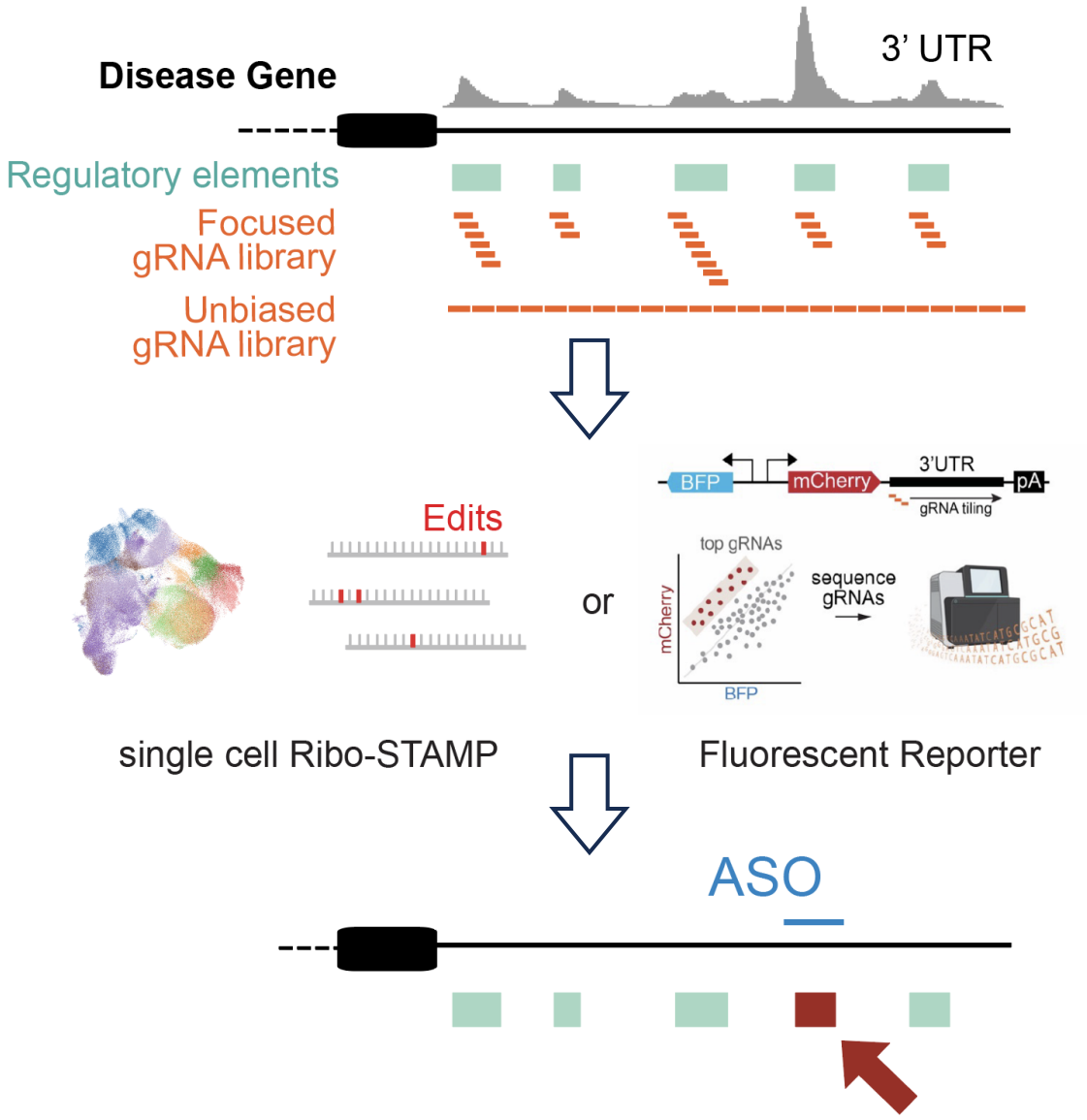
mRNA Isoforms Change the Translation Potential



with **Gene Yeo** (UCSD)

Sison*, **Zampa***, **Kofman*** et al. **Nature** (2026)

A **Flexible Platform** for Gene Augmentation – Unbiased Discovery



Map regulatory elements

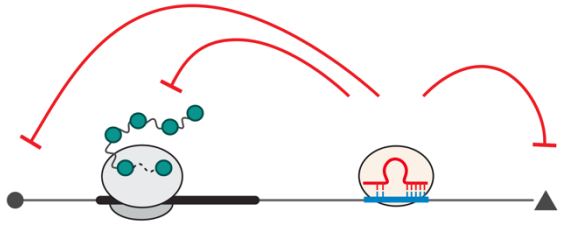
Deploy RNA-targeting CRISPR/dCas

Measure protein levels with high-throughput technologies

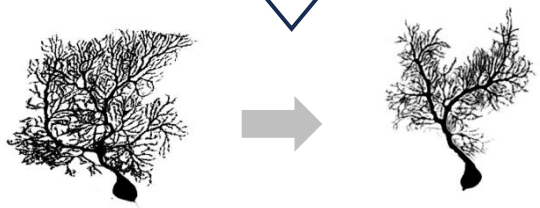
Identify the top regulatory elements to target with ASO

Zolboot*, **Ghanem*** et al. in preparation

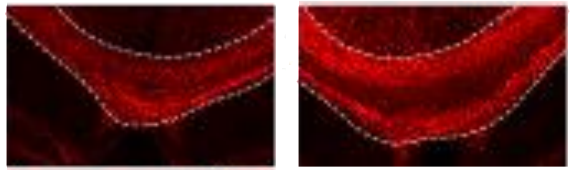
From **Basic** Developmental Neuroscience to **Therapies**



Gene expression



microRNAs drive development



Block microRNA-target interactions to rescue haploinsufficiency

FDA-approved treatments

Scripps Research

ABthrax™
raxibacumab
Anthrax toxin antibody

Bavencio®
avelumab
1st Merkel cell carcinoma tx

Benlysta®
belimumab
1st new lupus drug in 50 yrs

COVID-19 Vaccines
Key SARS-CoV-2 innovations

Cyramza®
ramucirumab
Gastric, liver, lung cancers

ENCELTO™
revakinagene taroretcel-lwey
Implant for MacTel eye disease

Humira®
adalimumab
Arthritis & autoimmune disease

Leustatin®
cladribine, IV
Hairy cell leukemia

Mavenclad®
cladribine, oral
1st oral short-course MS drug

Modeyso™
dordaviprone
Pediatric brain cancer (DMG)

Monoclate®
Factor VIII
Hemophilia treatment

Palynziq®
pegvaliase-pqpz
Enzyme therapy for PKU

Portrazza®
necitumumab
Metastatic lung cancer

Surfaxin®
lucinactant
Infant respiratory distress

Takhzyro®
lanadelumab-flyo
Hereditary angioedema

Trodelvy®
sacituzumab govitecan-hziy
Bladder & breast cancers

Unituxin®
dinutuximab
Pediatric neuroblastoma

Vyndaqel® / Vyndamax®
tafamidis
ATTR heart disease

Zeposia®
ozanimod
Relapsing multiple sclerosis

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Federico Zampa (Ionis)
Andrea Hartzell (Neurocrine)
Andrea Carvelli (Temple U)
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Sawyer Randles
Wil Brothers
Katie Rothamel
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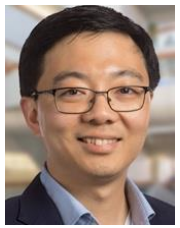
Stowers Lab
Sourish Mukhopadhyay



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Lucio Schiapparelli

Jin Lab
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Xinhe Zheng

Kennedy Lab

Grotjahn Lab
Hamidreza Rahmani

Williamson Lab
Kai Sheng

Ran Lab
Nicole Lynch

Encalada Lab

NIH National Institute of Neurological Disorders and Stroke

NIH National Institute of Mental Health

CIRM CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE

BRAIN RESEARCH THROUGH ADVANCING INNOVATIVE NEUROTECHNOLOGIES BRAIN INITIATIVE

WHITEHALL FOUNDATION SINCE 1937

autism speaks

BACHRACH FAMILY FOUNDATION

AES AMERICAN EPILEPSY SOCIETY

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