

# A Neuronal Atlas of RNA-Binding Protein Expression at Single-Cell Resolution

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## Background

The nervous system consists of diverse neuronal subtypes

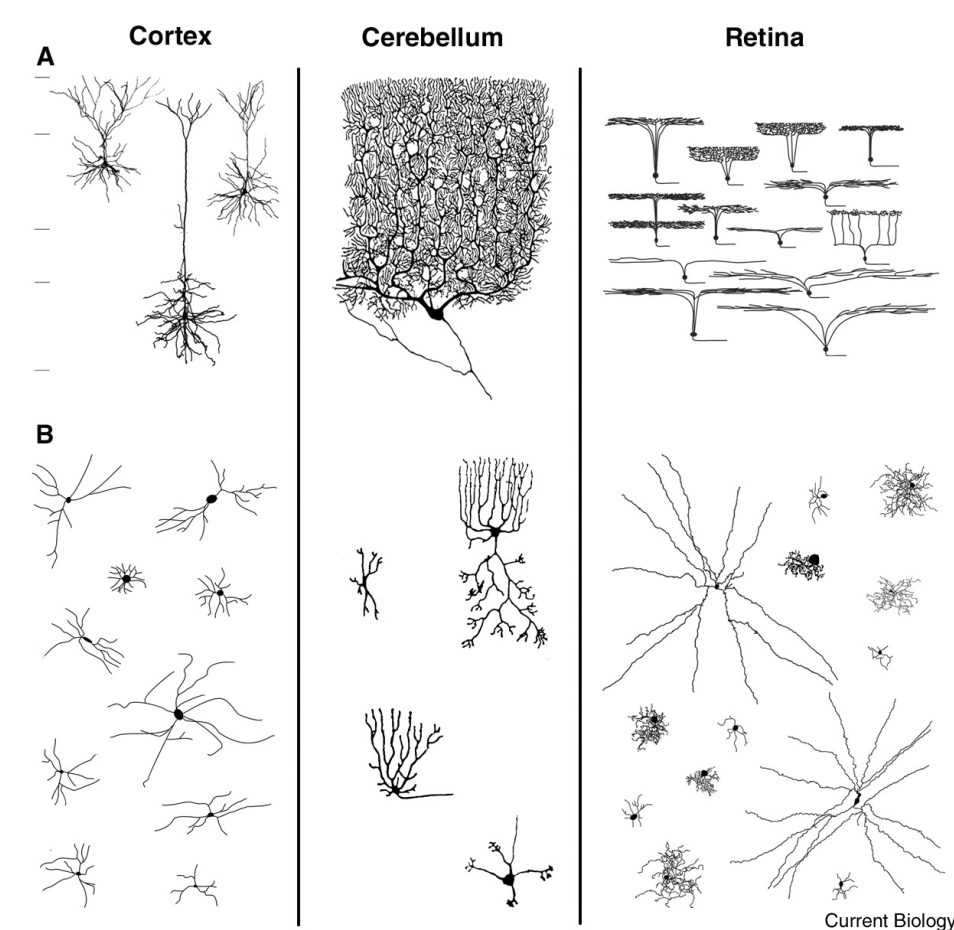


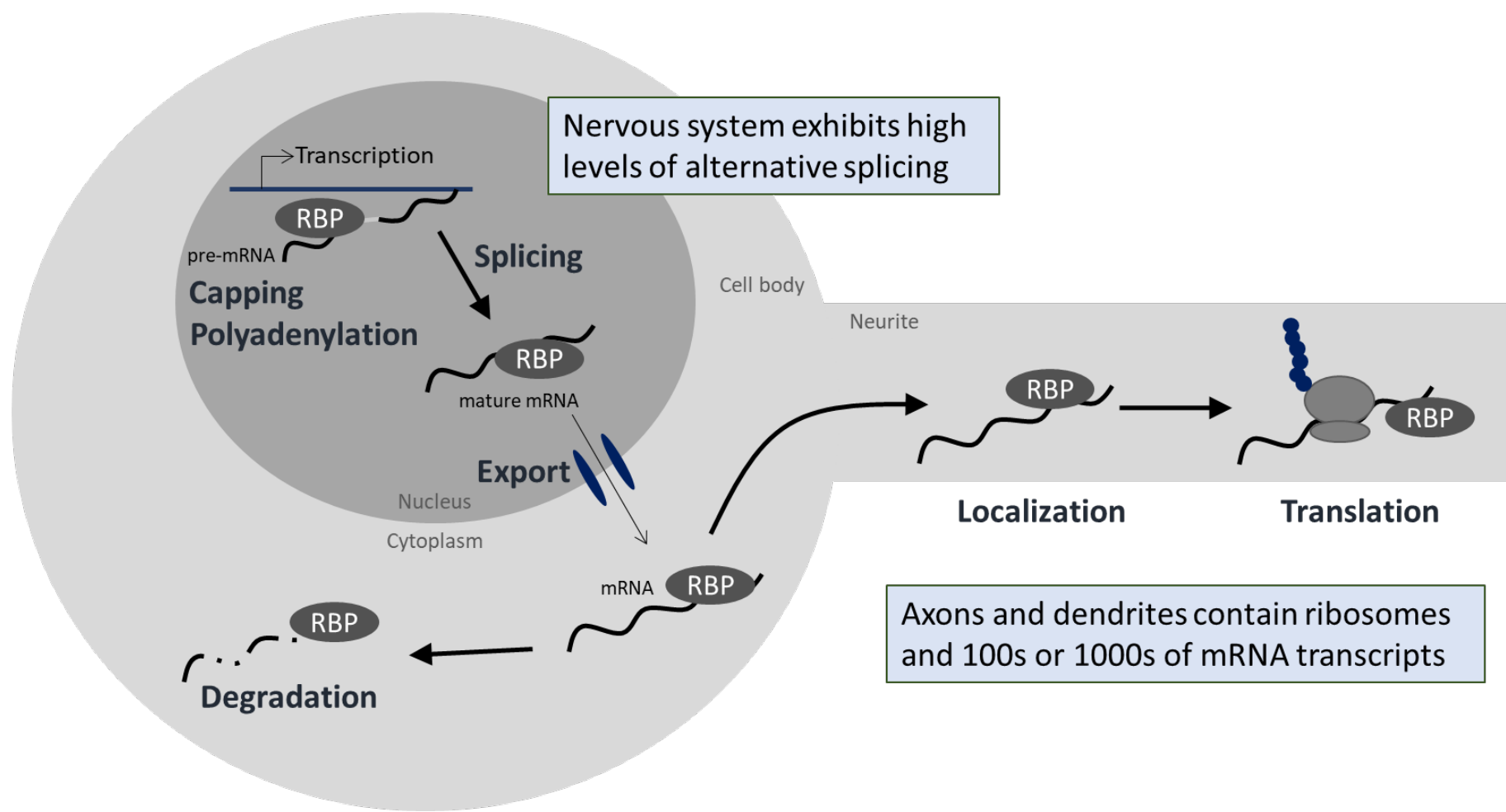
Figure 2, Masland, 2004, *Curr Biol* 14(13):PR497-R500  
Selected types of neurons in three different CNS structures

Distinct neuronal subtypes differ in:

- function
- morphology
- susceptibility to diseases

Differential gene expression is essential for specification and function of neuronal subtypes

RNA-binding proteins are important regulators of neuronal gene expression



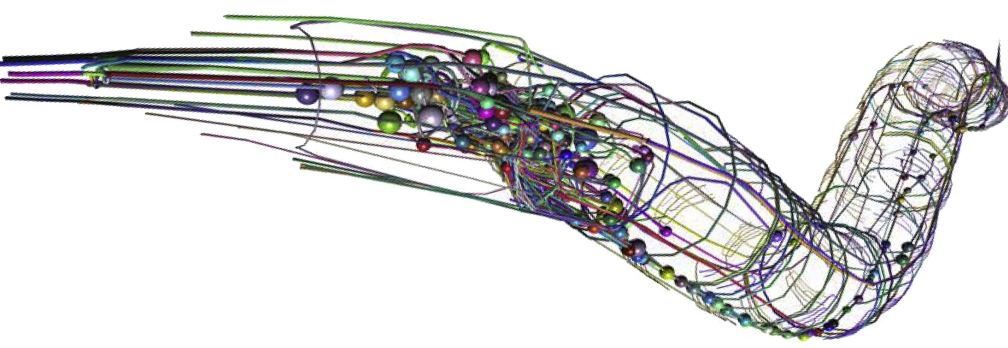
RNA-binding proteins (RBPs) bind specific sequence or structural elements in mRNAs to control their expression

*C. elegans* as a model for studying the nervous system

- C. elegans* has a uniquely well-defined nervous system:
- 302 neurons divided into 118 classes
- stereotypical neuron positions and morphologies
- completely mapped developmental cell lineage and neuronal connectome



Bright-field image of *C. elegans* overlaid with fluorescent image of neuronal nuclei expressing a fluorescent protein



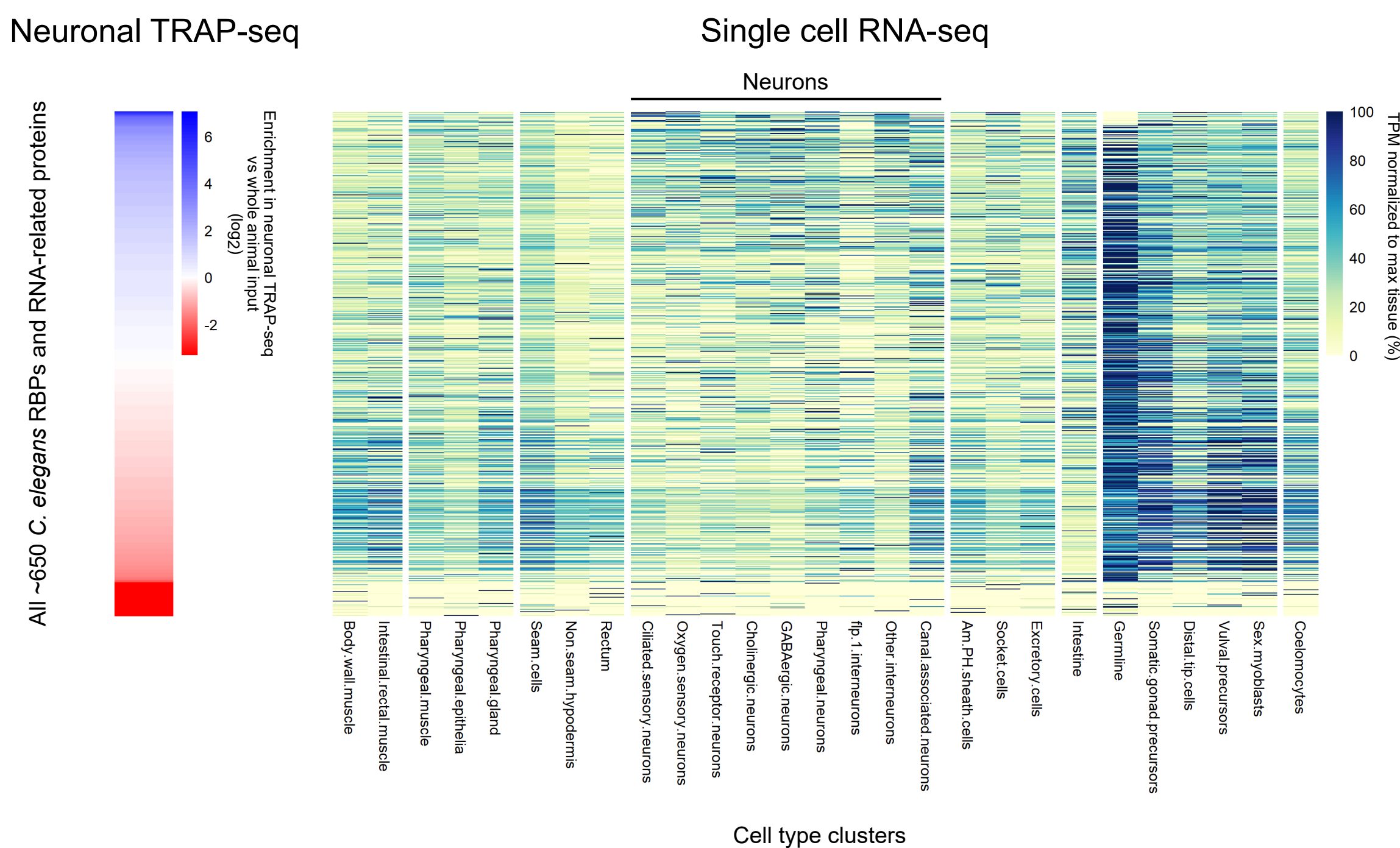
A virtual model of the *C. elegans* nervous system from the OpenWorm project  
Figure 1A, Hobert et al 2016 *Curr Biol* 26(22):R1197-R1203

## Results

Half of the ~650 *C. elegans* RNA-binding proteins are enriched in neurons

Tissue-specific gene expression in *C. elegans* has been measured by:

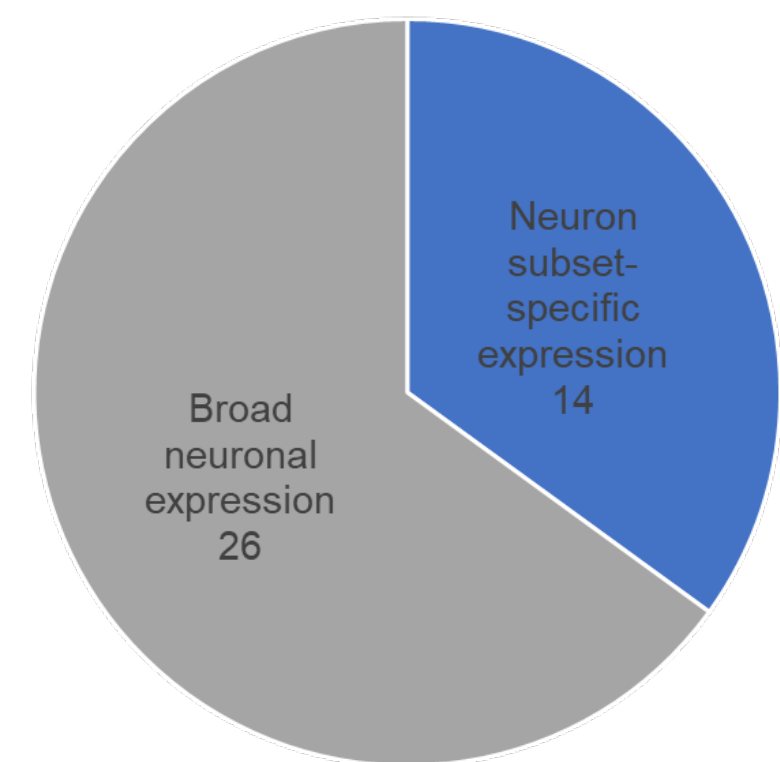
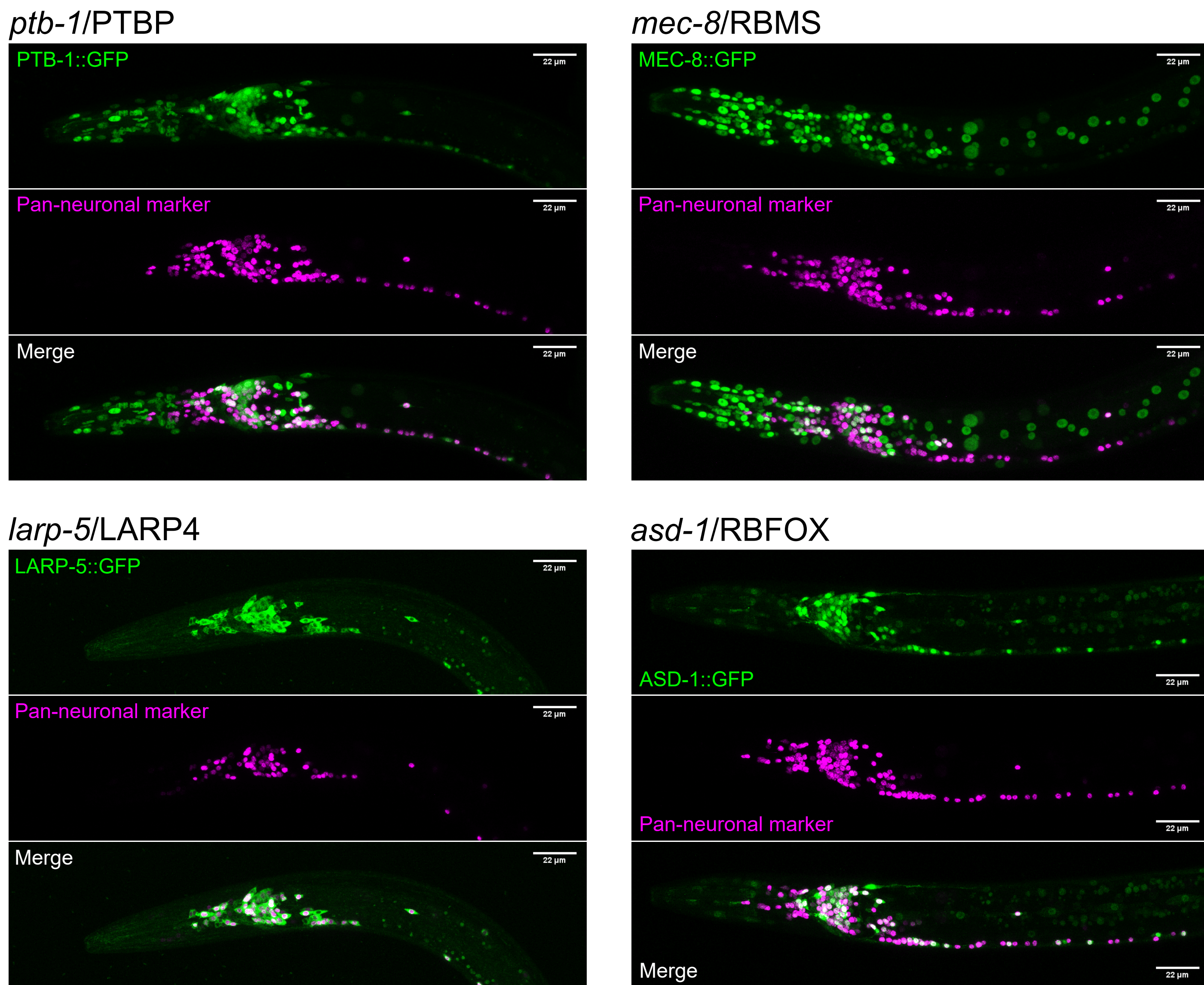
1. Translating ribosome affinity purification coupled with RNA-seq (TRAP-seq) (Gracida et al., 2017, *Cell Rep* 21:3089-101)
2. Whole animal single cell transcriptional profiling (Cao et al., 2017, *Science* 357:661-7)



A preliminary microscopy-based survey of 40 neuronally-enriched RNA-binding proteins reveals diverse patterns of neuronal expression and subcellular localization

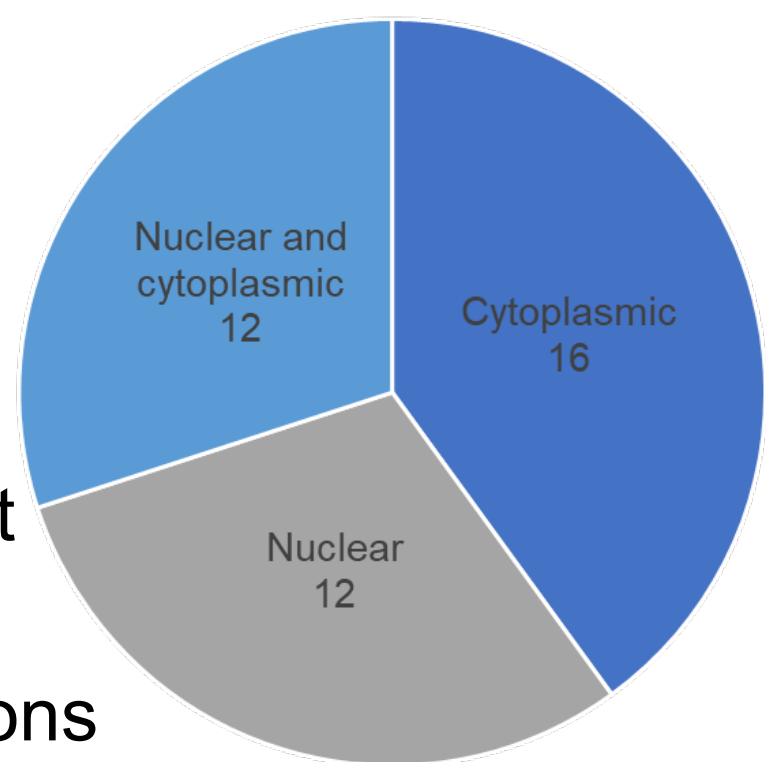
Expression of 40 RNA-binding proteins was assessed in transgenic animals co-expressing GFP-tagged RNA-binding proteins, driven by endogenous regulatory elements, and a pan-neuronal nuclear-localized fluorescent protein marker

Examples of RNA-binding protein expression patterns in the head of *C. elegans*:



One-third of RNA-binding proteins screened are expressed in a specific subset of neurons

RNA-binding proteins exhibit a variety of subcellular localization patterns in neurons



## Results

Annotating neuronal RNA-binding protein expression patterns using NeuroPAL: a Neuronal Polychromatic Atlas of Landmarks

NeuroPAL: a transgenic *C. elegans* strain with individual neurons labelled with 1-4 spectrally-resolvable, nuclear-localized fluorescent proteins (developed by Eviatar Yemini)

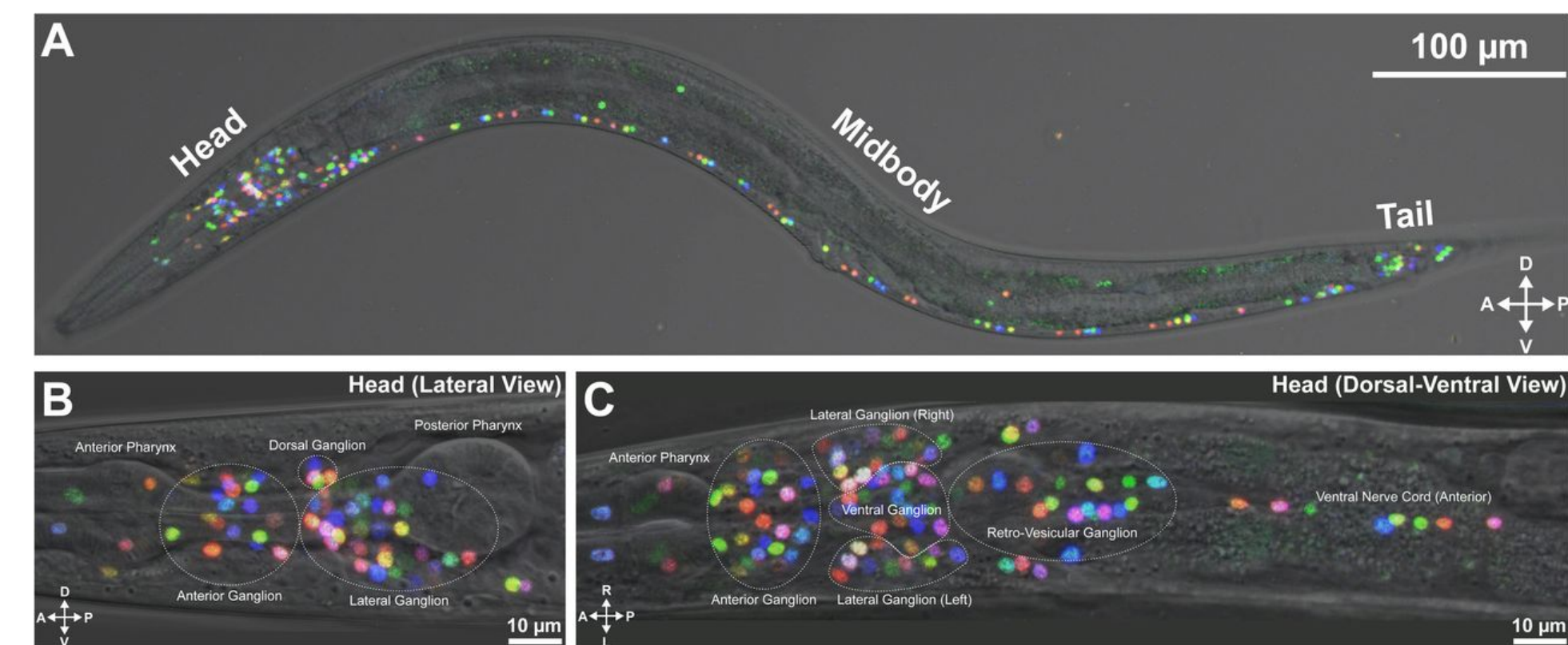
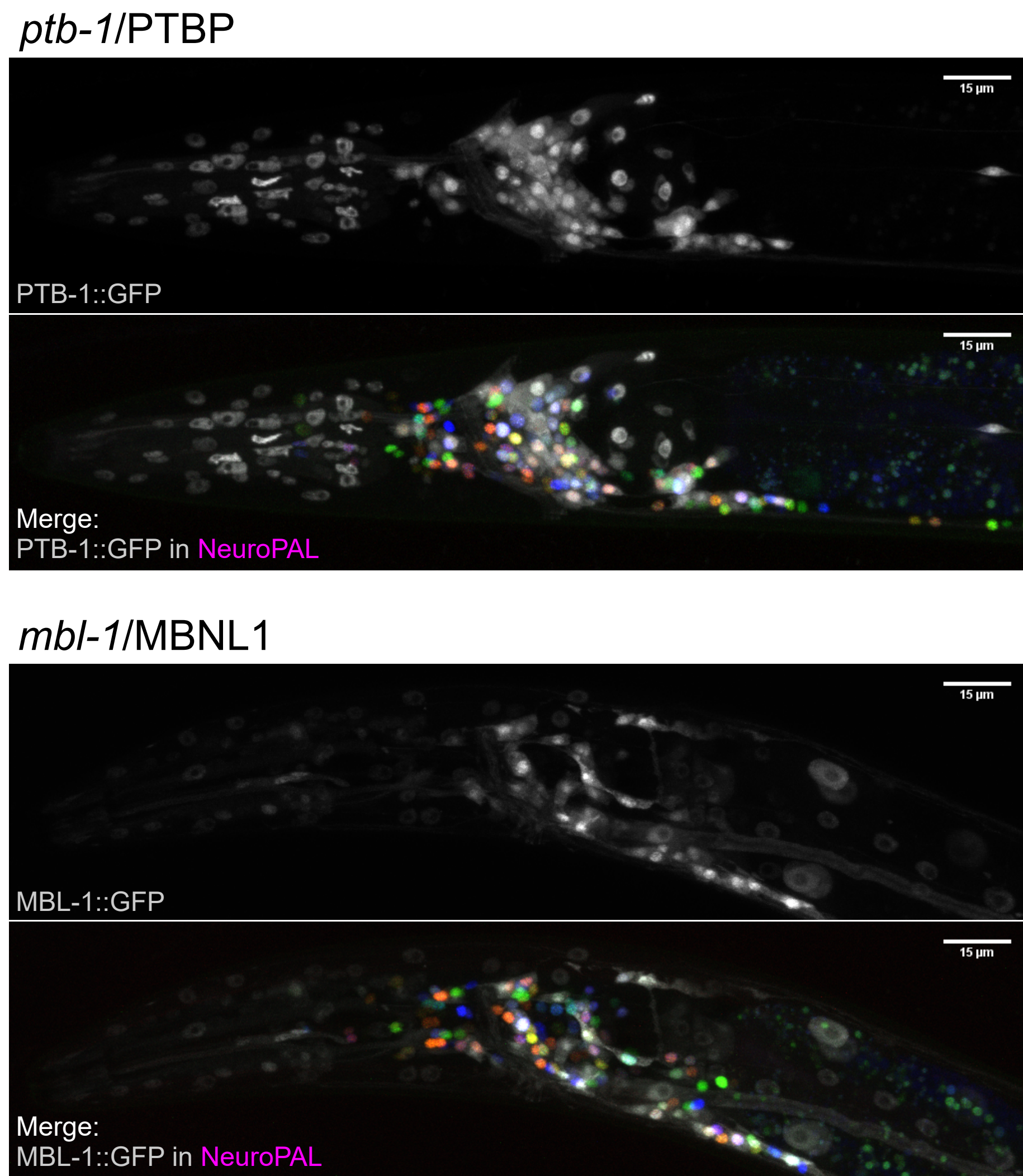


Figure 2, Yemini et al., 2019, bioRxiv 676312

Transgenic animals have been generated expressing different GFP-tagged RNA-binding proteins in the NeuroPAL strain

Examples of RNA-binding protein expression patterns in the head of NeuroPAL animals:



## Future Directions

Annotation of neuronal RNA-binding protein expression patterns at single-cell resolution using NeuroPAL

Assessment of phenotypic consequences of neuron-specific RNA-binding protein depletion

Identification of neuron-specific mRNA targets of RNA-binding proteins

## Acknowledgments

We thank Wesley Hung, Mei Zhen, Arneet Saltzman, and members of the Calarco and Saltzman labs for help and advice.

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## Objectives

1. Determine the expression and localization of RNA-binding proteins in neurons with single-cell resolution
2. Understand the roles of RNA-binding proteins in specification and function of neurons and neuronal subtypes