Analysis of the subcellular localization of neurodevelopmental disease-associated proteins using Breasi CRISPR

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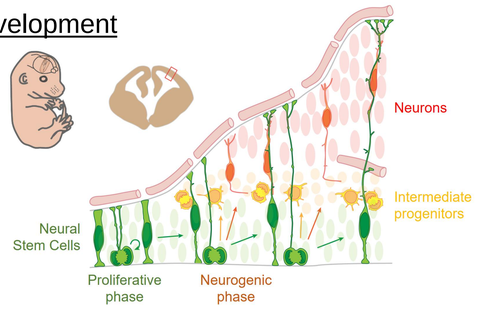
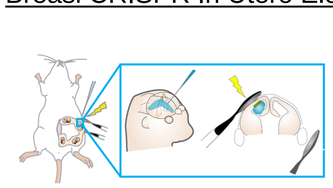
Introduction

Many rare neurodevelopmental diseases are caused by loss of expression of a single protein. Knowing the subcellular localization of these proteins in neurons during cortical development is important in helping our understanding of their roles in normal physiology and disease states. We used Breasi-CRISPR, a technology developed in the lab, which combines in utero electroporation (IUE) and CRISPR/Cas9 genome editing, to insert epitope tags in genes of interest in the developing cortex. This enables visualization of endogenous proteins in a subset of developing neurons, facilitating analysis of subcellular localization in situ.

Cortical development



Breasi CRISPR In Utero Electroporation



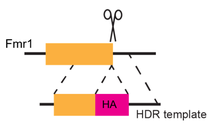
FMRP and MAP1B are important in neurodevelopment

Normal physiology
FMR1 → FMRP: RNA binding protein with many roles in RNA biology
MAP1B → MAP1B: Microtubule-associated protein

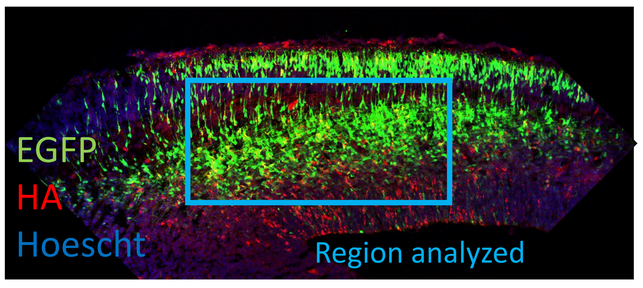
Disease state
FMR1 trinucleotide repeat expansion and gene silencing → ~~FMRP~~: Fragile X syndrome
MAP1B inactivating mutations → ~~MAP1B~~: Periventricular nodular heterotopia

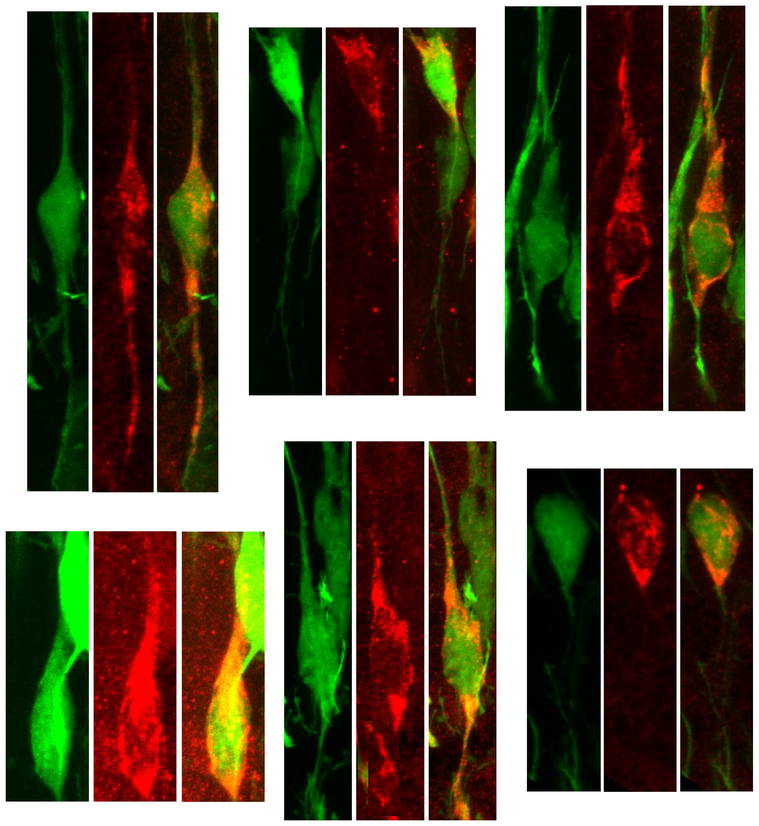
Results

Tagging of endogenous FMRP



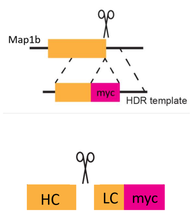
E13 → E15

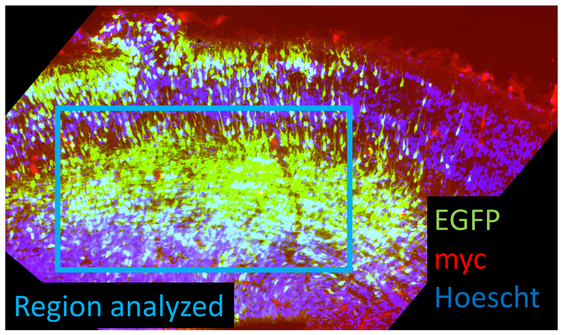


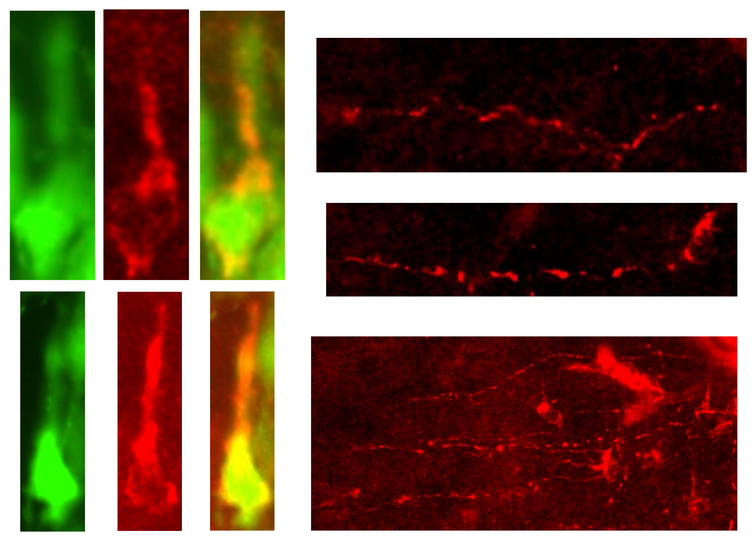


Tagging of endogenous MAP1B

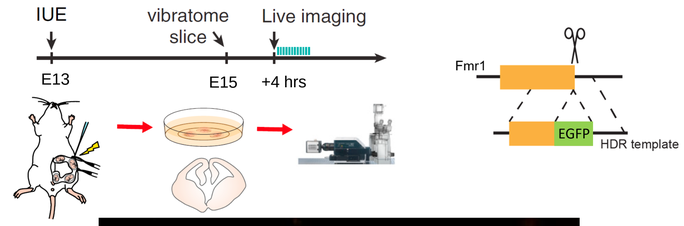
E13 → E15

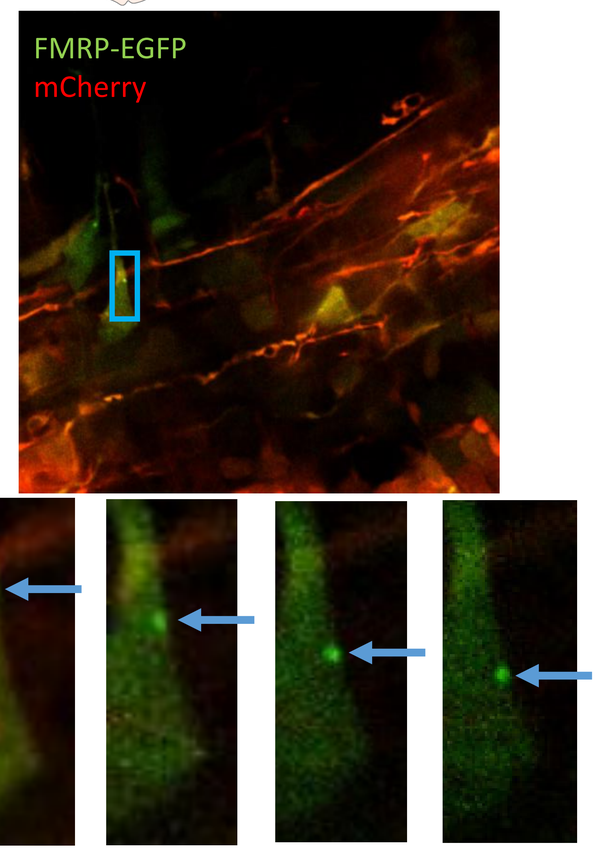






Tagging of endogenous FMRP with EGFP





Conclusion

Preliminary data indicate that FMRP is expressed in the soma and neurites of migrating neurons and the MAP1B light chain is preferentially expressed in axons in developing neurons, but is also expressed in neurites of migrating neurons. We plan to expand upon these findings by using Breasi-CRISPR to tag proteins such as FMRP and MAP1B with fluorophores, enabling us to examine their localization via live imaging of brain slices.

References

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