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SUPPLEMENTARY INFORMATION

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Subcellular transcriptomes and proteomes of developing axon projections in the cerebral cortex

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Supplementary Discussion for Poulopoulos et al. "Subcellular transcriptomes and proteomes of developing axon projections in the cerebral cortex"

Supplementary Discussion

One major advance enabled by this new GC sorting and "subcellular RNA-proteome mapping" approach is the identification of a subcellular RNA-proteome map from this large (several mm-long) and highly polarized neuron subtype directly from the brain. Mapping Λ_R to Λ_P along the macroscopic subcellular GC-soma axis of the trans-hemispheric projection reveals how components of distinct subcellular transcriptomes distribute in somata and GCs compared to their respective distinct subcellular proteomes. This paired readout provides quantitative insight into several interesting features of projection neuron biology.

Our data show that, for any given gene, mRNA mapping is not predictive of corresponding protein mapping, since the correlation coefficient of Λ_R to Λ_P across the dataset is near zero (0.014). However, much more specific patterns emerge in subsets of genes and higher-order gene group clusters (Supplementary Table 6 and 7), suggesting that mRNA localization can serve varying biological purposes beyond simply supplying the local proteome –e.g. isolating and polarizing translational control for select proteins and pathways. Clusters of gene groups emerge in 2D maps of mRNA-to-protein subcellular distributions (Extended Data Fig. 5-6). Among these are anticipated features, such as adhesion molecules mapping mRNA toward somata and protein mapping toward GCs; adhesion molecule mRNAs are translated on rough endoplasmic reticulum in somata for translocation into the secretory pathway and subsequent anterograde protein trafficking down the axon[53]. In this same "anterograde cluster" we also identify less anticipated groups, such as proteasomes and chaperonins, indicating that these multi-subunit complexes might be produced in somata, and subsequently concentrate in GCs.

The most striking outlier to emerge from the subcellular RNA-proteome map is the "TOP cluster", encoding mostly ribosomal protein subunits and translation initiation factors. This cluster is unique in displaying a pattern of mRNA concentrated in GCs, and protein distributing in the middle of the GC-soma axis (green histograms in Extended Data Fig.6). This localization of ribosomal proteins in both GCs and somata is consistent with ribosomal proteins assembling into functional ribosomes in the nucleus[54], as well as ribosomes participating in translation in both GC and soma compartments. Localization of ribosomal mRNAs in GCs, however, indicates less canonical biology, and begs interpretation.

We mined the data extensively for correlations of individual ribosomal subunit Λ_R values with known properties of those subunits, e.g. distance from the ribosomal surface, evolutionary conservation, methylation, acetylation, or hydroxylation. We find no correlations with known properties to support specialized functions for this localization, such as local replacement of select subunits, or refurbishment of existing GC ribosomes. We thus propose a developmental interpretation, in which the supply of ribosomes is

regulated to mirror the increasing size of the neuron by spatially coupling axon growth and TOP mRNA translation through mTOR at GCs. We propose that this subcellular organization might likely be a transient feature of the axon-extension phase of projection neuron development, physically positioning mTOR[54] as the central growth regulator at sites of the most intense cellular growth. In addition to coordinating the supply of ribosomes in a growing neuron, it is intriguing to speculate that mTOR pathway foci in GCs might enable sensing of target-derived growth signals locally to optimally tune and coordinate the responsiveness of GCs as they traverse distinct microenvironments, as well as to coordinate transitions of the overall neuron's developmental program in a target-derived, stage-specific manner.

This model has interesting implications possibly toward regeneration in the adult central nervous system. Previous studies have identified mTOR and the PI3 kinase pathway as critical targets to stimulate regrowth of damaged axons in the adult CNS[55,56]. Since mTOR previously has been thought to reside largely in cell bodies of mature neurons, unidentified long-range signals have been postulated to link mTOR manipulations and the induction of regenerative growth in GCs[30]. Our results indicate that, during development, mTOR and axon growth become directly coupled both functionally and spatially at GCs. It is thus possible that reverting mTOR to this developmental GC-specific state and localization might be important in optimally enabling regenerative growth in the adult CNS.

Supplementary References

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