MOL 231: Generation of transgenic zebrafish to study astrocyte to neuron transport

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Background

The brain is a complex network of neurons and glia working together to send and process signals. The glia are important helper cells which support the neurons in various ways. The most common of the glial cells, astrocytes, perform task such as metabolism of toxic compounds, injury response, and regulation of neurotransmitters in synapses (Hart & Karimi-Abdolrezaee, 2021). It has been shown in cell-cultures that astrocytes can support neurons by sending extracellular vesicles (EVs) containing neuroprotective substances (Leggio et al., 2022) and possibly also transfer healthy mitochondria. Mutations in astrocytes could harm the supportive effect they provide (de Rus Jacquet et al., 2021). The aim of this project was to generate two transgenic zebrafish lines with either fluorescent astrocytic membranes and nucleus, or mitochondria, that will enable the study of the astrocyte-neuron transport.

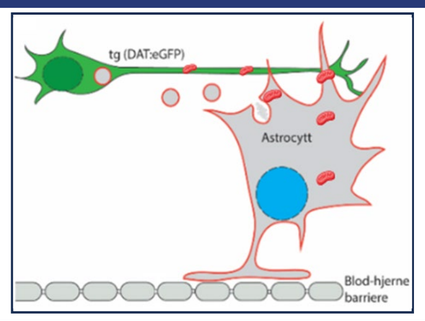
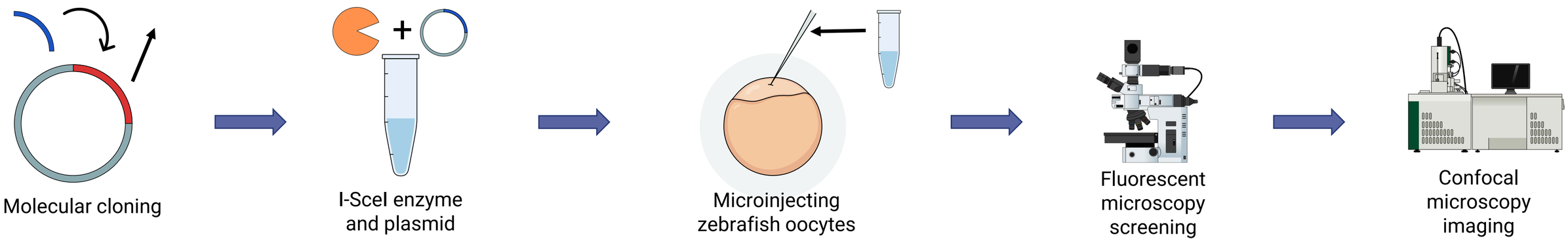


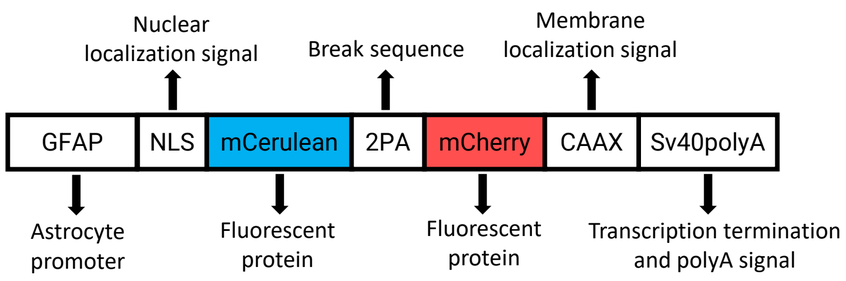
Figure 1: Proposed astrocyte-neuron transport with astrocytic red fluorescence cell membrane and extracellular vesicles, blue nucleus, and red mitochondria. The target cell expressing GFP in dopaminergic neuron expressing EGFP.

Methods

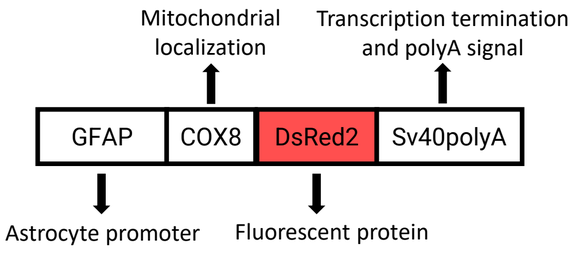
Workflow



DNA inserts



- GFAP promoter drives expression in astrocytes.
- 2PA sequence causes the resulting protein to break.
- Each set of genes are flanked by I-SceI restriction sites, allowing stable insertion into the zebrafish genome
- Target sequences are NLS (nucleus), CAAX (cell membrane) and COX8 (mitochondria)



Results

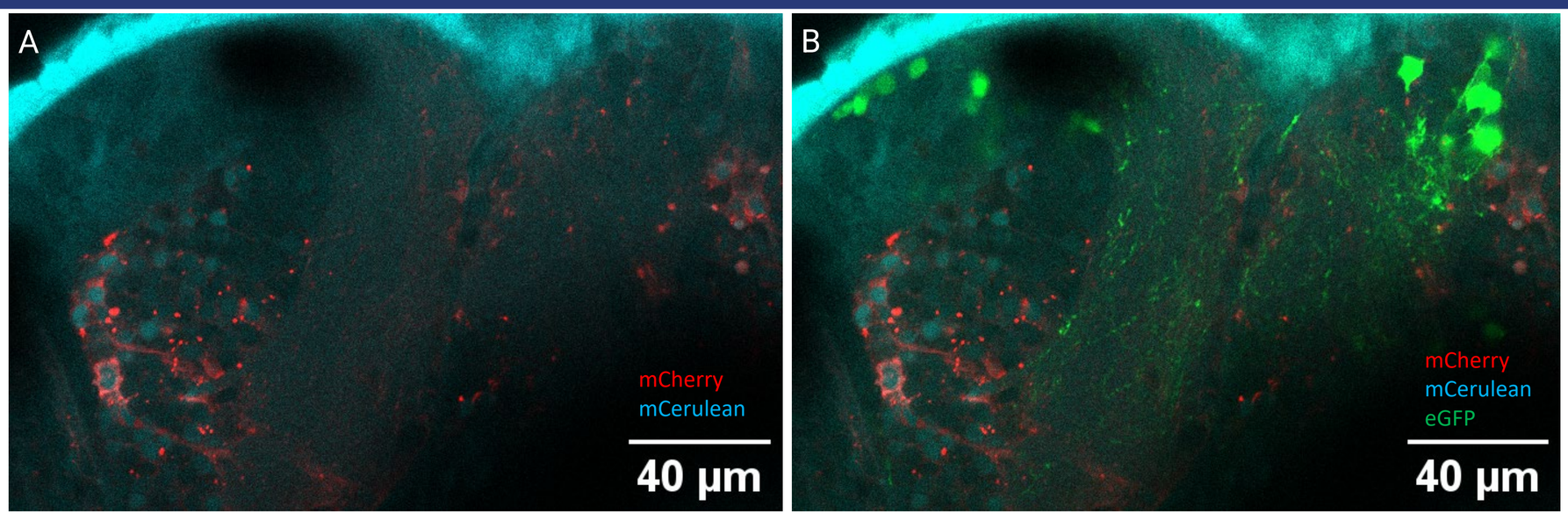


Figure 2: Confocal images of transgenic (Tg) zebrafish Tg(gfap:NLS-mCerulean-mCherry-CAAX) x Tg(DAT:eGFP). Plasmid expressing gfap driven tagged-mCherry and –mCerulean injected into eggs from fish expression GFP in dopaminergic neurons. **A)** Image shows astrocytes with cell membrane-tagged mCherry and nuclei with mCerulean. Small dots can be seen which might be either labelled extracellular vesicles or neurites. **B)** Image shows zebrafish brain expressing both astrocytic mCherry and mCerulean together with eGFP in dopaminergic cells.

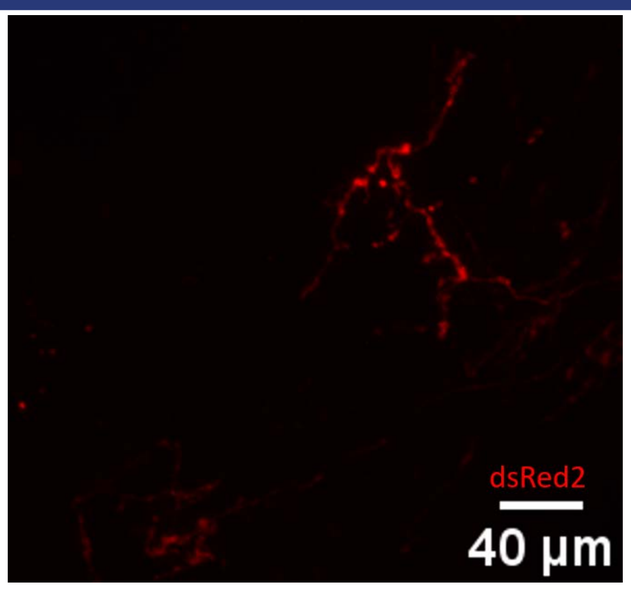
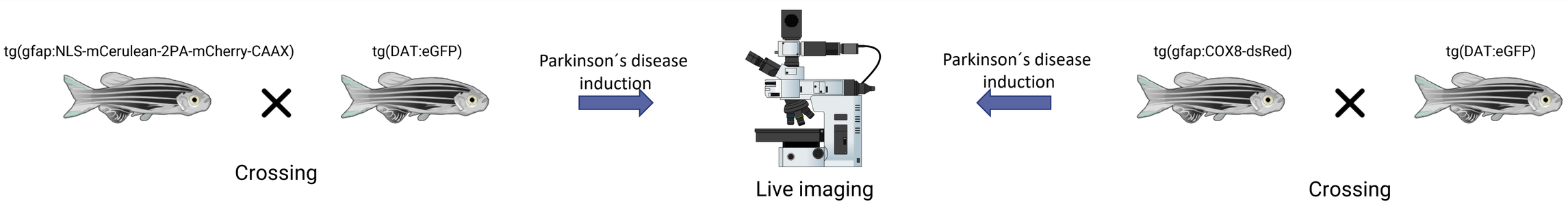


Figure 3: Confocal microscopy image of transgenic zebrafish Tg(gfap:COX8-dsRed2). Image shows red fluorescence mitochondria in astrocytes. Labelled mitochondria are observed both in the astrocyte cell body and neurites.

Conclusion

We have successfully established two F0 zebrafish lines, one with fluorescent tagged astrocyte nuclei, cell membrane and one with fluorescent astrocyte mitochondria. The lines can in combination with a line expressing fluorescent tagged dopaminergic cells be used in Parkinson´s Disease induced models to study astrocyte to neuronal transport.

Future work



References

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