Group Name: Development and assembly of bilateral neural circuits
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Title of the MRP:

miRNA control of neuronal subtype specification in the developing spinal cord

Summary of the MRP:

The spinal cord is a premier model for understanding how neuronal diversity arises during development. Opposing morphogen gradients pattern the neuroepithelium into discrete progenitor domains, each producing distinct neuronal subtypes. A fundamental division in this process is the segregation of excitatory (glutamatergic) and inhibitory (GABAergic/glycinergic) neurons, whose balance is essential for coordinated movement, sensory processing, and the prevention of pathological states such as spasticity and chronic pain.

The transcription factor Ptf1a is a selector gene for inhibitory neuronal identity in the dorsal spinal cord. Loss of *Ptf1a* causes a fate transformation: cells that would become inhibitory interneurons instead adopt an excitatory identity. *Ptf1a* achieves this by activating inhibitory fate markers (*Pax2*, *Lhx1/5*) while repressing excitatory determinants. However, whether *Ptf1a* coordinates this fate switch through post-transcriptional mechanisms — specifically via microRNAs (miRNAs) — remains an open question.

miRNAs are small (~22 nt) non-coding RNAs that silence target mRNAs post-transcriptionally. A single miRNA can regulate hundreds of targets simultaneously, making them powerful stabilizers of gene regulatory networks. In the nervous system, miRNAs sharpen transcription factor boundaries and reinforce binary cell fate decisions — yet their specific roles regulating *Ptf1a* in spinal cord neuronal specification remain unexplored.

This project main aim is **to determine how miRNAs acting upstream of *Ptf1a* control inhibitory neuron fate in the developing spinal cord**, using the chick embryo (*Gallus gallus*) as a model system.

The specific aims of this Master Project are:
- Validate candidate miRNAs expression pattern in the chick spinal cord
- Evaluate the function of candidate miRNAs in controlling Ptf1a expression

Methods and technology involved in the MRP:

The key experimental tool is **in ovo electroporation**: DNA constructs are injected into the neural tube and driven into neuroepithelial progenitors by brief electrical pulses, allowing precise spatial and temporal gain- or loss-of-function manipulations.

Gain-of-function: Overexpression of candidate miRNAs to assess their effect on inhibitory vs. excitatory neuron ratios and *Ptf1a* target gene expression.

Loss-of-function: Delivery of miRNA sponge constructs or inhibitors to abolish endogenous miRNA activity and observe the resulting phenotype.

Lineage tracing: Co-electroporation of fluorescent reporters to track the progeny of electroporated progenitors and assess fate switches at single-cell resolution.

Readouts: Immunofluorescence for neuronal subtype markers (Pax2, Lmx1b, Tlx3), confocal microscopy, and quantitative image analysis.

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