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Ancient complement-mediated mechanisms of neural selection and axonal pruning during nervous system development and regeneration in an invertebrate model (*Pomacea canaliculata*)

The complement system is a fundamental component of innate immunity, but growing evidence shows that it also performs non-immune functions. In vertebrates, complement components such as C1q and C3 mediate the recognition and elimination of weak or redundant synapses during development, whereas regulators of complement activity (RCAs), proteins containing complement control protein (CCP) domains, protect healthy neural structures from improper complement activation. Since complement predates adaptive immunity, these mechanisms may reflect an ancestral role in cellular recognition, selection and tissue remodeling.

This project investigates whether complement-mediated neural selection and axonal pruning represent an evolutionarily conserved mechanism in *Pomacea canaliculata*. This species combines a complex complement system with the absence of adaptive immunity, allowing complement-dependent processes to be studied in a simplified immunological context.

The hypothesis is that the pro-phagocytic component (PcC3) and the regulators of complement activity (PcRCAs) act in a coordinated manner: PcC3 tags neural structures undergoing developmental or regenerative remodelling for removal, while PcRCAs locally protect functional structures from bystander complement damage.

This is addressed first through, long-read Oxford Nanopore and Illumina short-read transcriptomics combined to characterize full-length PcRCA isoforms and reconstruct the neuro-immune transcriptional landscape of *Pomacea canaliculata*. Comparison with hemocyte and other tissue transcriptomes are then used to identify neural-specific regulators and separate tissue-specific functions from systemic immune responses. in silico interaction analyses are sequently carried out to probe the molecular relationship between PcC3 and its various regulators.

Second, the spatiotemporal distribution of PcC3 and PcRCAs is mapped during development, aging and regeneration by multiplexed RNA hybridization, immunolocalization, confocal microscopy and ultrastructural analysis. Building on preliminary data, behavioural assays will be conducted in *Pomacea canaliculata* to identify individuals showing signs of neurological impairment across different age groups. Ganglionic transcriptomic profiles will then be correlated with behavioural data and the cellular localization of *PcC3* and *PcRCA* transcripts to explore associations among neural function, behaviour, complement regulation, and age-related degeneration.

Finally, the causal role of complement is tested by targeted RNA interference against PcC3 during embryonic development. Developmental and structural phenotypes are quantified through three-dimensional imaging and neuronal markers, directly assessing how impaired complement activity affects nervous-system organization and development.