Monoamine Signalling in a Unicellular Eukaryote: Clues to Endocrine Evolution

Debashrita Mishra

Endocrine signalling is one of the most fundamental regulatory systems in higher eukaryotes. Biogenic monoamines, such as serotonin and dopamine, function as key neuroendocrine regulators and modulate various physiological processes. Despite their well-established roles in multicellular organisms, the evolutionary origins of monoamine-based endocrine signalling remain poorly understood.

In the ciliate *Tetrahymena thermophila*, biogenic amines are known to influence physiological functions such as phagocytosis, cell growth, cilia regeneration, chemotaxis, glucose metabolism, and cell-cell communication. However, most of these studies are outdated and focus on the production and uptake of hormones and their effects on cell physiology, while direct evaluation of the pathway using genetic tools such as in situ tagging or gene knockout is still lacking.

Our study aims to understand the evolutionary origins of biogenic monoamine synthesis using *Tetrahymena* as an early-diverging eukaryotic model. Through *in-silico* tools, we have identified putative enzymes of the serotonin and dopamine biosynthesis pathways, such as aromatic amino acid hydroxylase and aromatic L-amino acid decarboxylase. We quantified cellular and secreted monoamines and their intermediates in vegetative and starvation stages using high-sensitivity HPLC-MS. Moreover, monoamines are known to exert their effects through G protein-coupled receptors (GPCRs). We have identified putative GPCR orthologs in *Tetrahymena*. Their functional characterization will be performed using heterologous expression in HEK293-G5A cells and luminescence-based reporter assays. Taken together, this study will address how monoamine signalling mechanisms function in *Tetrahymena*, further shedding light on the evolution of hormone signalling pathways across eukaryotes.