

BIOLOGY
BLUE ASH COLLEGE

SUMMER RESEARCH OPPORTUNITIES FOR UNDERGRADUATE students

APPLICATION DEADLINE: 02/25/2022

PROJECT TITLE: TOR kinase-mediated Enolase Regulatory Mechanism in Naegleria

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Project Description

Lab Location: UC Blue Ash College

Research Background: The long-term goal of our research is to identify drug targets in the TOR (target of rapamycin) kinase signaling axis and to develop pharmacological agents against *Naegleria fowleri*. The UPRISE student will engage in research that requires molecular biology, biochemistry, cell biology, and some data science.

N. fowleri is a pathogenic free-living amoeba that causes rare (151 reported cases as of 2020 in the US) but highly fatal (greater than 97%) primary meningoencephalitis in the central nervous system. The thermophilic amoeba lives in warm freshwater in the southern states (e.g., Texas and Florida) and gains access to the cerebrum when contaminated water enters the nasal cavity. The onset of symptoms such as headache and nausea start typically a week after infection, and it only takes another week for the patient to succumb to the disease. The high mortality rate comes from the fact that current medications are not fast and effective enough.

In order to identify kinase-mediated cell signaling pathways in *Naegleria*, non-pathogenic *Naegleria gruberi* was used as a model organism because the species has been studied the most, and various omics data and cDNAs are readily available. Recently, former UPRISE students identified 3884 phosphorylation sites in 2084 phosphoproteins out of 15,727 predicted proteins and 48 metabolites including acetylcholine by phosphoproteomics and metabolomics analyses. The students also showed that the proliferation of *N. gruberi* was severely suppressed by TOR kinase inhibitors including Torin-1. The phosphoproteome of starved or Torin-1 treated *Naegleria* cells identified 228 common proteins that were completely dephosphorylated. Protein-protein association analysis on the dataset identified enolase having associations

with 7 other phosphoproteins within the dataset, and three serine residues of enolase are dephosphorylated. Among them, S93 is of particular interest as the residue is essential for ligand binding and conserved from yeast to humans.

Enolase is one of ten enzymes responsible for glycolysis and has functions in fibrinolysis, myogenesis of cardiac and skeletal muscles and implicated in the development of cancer, Alzheimer's disease, and rheumatoid arthritis. Non-classical functions of enolase in part come from its subcellular localization to organelles such as the nucleus, vacuole, mitochondrial membrane, cytoskeleton, exosome, and cell surface. The regulation of subcellular localization of enolase is largely unknown, and we hypothesize that TOR kinase regulates the phosphorylation and subcellular localization of enolase in *Naegleria*.

Naegleria species have a fascinating and unique feature that they can transform into three different morphological stages depending on environmental conditions. The trophozoite (amoeboid) is the normal stage of the life cycle and is mitotically active, while the cyst form is metabolically inert. The third form is flagellate, having a pair of flagella, with which *Naegleria* swims away from the unfavorable environment. Interestingly, when treated with TOR inhibitor, Torin-1, *Naegleria* changes its morphology to a cyst-like form within 30 to 60 min of treatment. Enolase has been shown by another group to localize in the cytoplasmic vesicles that promote encystation and in the cyst wall in *N. fowleri*.

Approach: Does TOR kinase activities inhibit the localization of enolase to cytoplasmic vesicles and the formation of the cyst by phosphorylating S93 of enolase? It is well documented that inhibition of TOR kinase is required for the formation of autophagosomes during starvation in mammals and yeasts. Therefore, it is reasonable to hypothesize that starvation induces encystment via inhibition of TOR in *Naegleria*. TOR is also known to localize on the lysosomal membrane. To test the hypothesis, first, colocalization of fluorescently labeled enolase-GFP and TOR-RFP on the cytoplasmic vesicles upon starvation will be monitored by live-cell confocal microscopy in real-time. Next, by the CRISPR-Cas9 system, TOR kinase complexes 1 and 2 will be disrupted by deleting raptor and rictor genes - the gene products are necessary for the formation of complexes. The effect of the raptor and/or rictor mutations on the enolase phosphorylation will be determined by LC-MS. The ability of encystment and the localization phenotype of enolase-GFP in mutants will be evaluated by confocal microscopy.