Undergraduate Research Symposium May 17, 2013 Mary Gates Hall

Online Proceedings

POSTER SESSION 1

Commons East, Easel 57

11:00 AM to 12:30 PM

Genetic Investigations of the Sterol Biosynthesis Pathway of *Trypanosoma cruzi*

Omeed Mohammad (Omeed) Faghih, Junior, Biochemistry, Neurobiology
Mentor: Frederick Buckner, School of Medicine

Trypanosoma cruzi is a protozoan parasite prevalent in South and Central America that causes life-long infection in humans. Approximately 30% of infected individuals develop a condition called Chagas disease which usually manifests as life-threatening cardiomyopathy or pathologies in the gastrointestinal system. Over 8 million people are believed to be infected. Unfortunately, current drugs for treating Chagas disease have low antiparasitic activity, are expensive, and are known to cause harmful side effects. As a result, research needs to focus on discovering new drug targets in *Trypanosoma cruzi* to lead to improved drugs. Most eukaryotic organisms synthesize sterols for essential biological functions that when manufactured incorrectly can lead to cell death. This research is investigating the effects of blocking the synthesis of ergosterol by deactivating an enzyme called sterol 14-demethylase. This enzyme catalyzes one of the intermediates of 20 steps of ergosterol synthesis. Inhibitors of sterol 14-demethylase, known as azoles, have been shown to be extremely active on *T. cruzi* in vitro and are now the center of research regarding anti *T. cruzi* drug discovery. We are developing novel azole compounds that target the sterol 14-demethylase homolog of *T. cruzi* to deprive the cell of its necessary sterols. However, further evidence shows that blocking sterol 14-demethylase can lead to an accumulation of sterol intermediates that are converted into toxic agents in the presence of another enzyme, ERG3 enzyme, which is normally active in a later step in the biosynthesis of ergosterol. The objective of this research project is to analyze the ERG3 homologs of *T. cruzi*. The putative homologs have been cloned and sequenced. No significant mutations were found. The research now is focused on engineering genetic knockouts of the ERG3 genes to test the hypothesis that the knockout parasites may be resistant to treatment with azole drugs.

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Development of a *Trypanosoma cruzi* Methionyl-tRNA Synthetase Assay Using Bioluminescence for Compound Screening

Kristi M Kajita, Freshman, Pre-Sciences
Mentor: Frederick Buckner, School of Medicine
Mentor: Ranae Ranade, Department of Medicine

Chagas Disease is a tropical disease caused by the parasite *Trypanosoma cruzi.* An estimated 100 million people are at risk in 21 endemic countries and ˜8 million persons are currently infected. Left untreated, Chagas disease can cause fatal heart rhythm abnormalities, a dilated heart, a dilated esophagus, and/or a dilated colon. The efficacy of current treatments decreases the longer the person is infected, and 40% of treated patients experience adverse reactions, demonstrating the need for new and improved drugs. A drug target has been identified in *T.cruzi:* the Methionyl-tRNA synthetase (MetRS). Like tRNA synthetase enzymes in other species, the *T.cruzi* MetRS is crucial for protein production and survival. To exploit this target in T.cruzi we developed a robust assay (Z' factor $\geq$ 0.5) to identify hits against the MetRS enzyme using a luminescence ATP detection method (Promega's Kinase-Glo^{TM}). Compounds that inhibit the *T.cruzi* MetRS enzyme will be tested for specificity by assaying the compound with the human MetRS homolog. Specific *T.cruzi* MetRS inhibitors will be tested for potency against intracellular *T.cruzi* amastigotes and cytotoxicity against mammalian cells. Compounds that are toxic to *T. cruzi*, not cytotoxic to mammalian cells, and have good pharmacological properties will be selected for hit–to–lead drug development.