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Vivian Tsang

Washington University in St Louis

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Recommended Citation
Tsang, Vivian, "The Role of PIWI Protein in Leishmania braziliensis RNA Interference " (2012). Washington University Undergraduate Research Digest, Volume 8, Issue 1.
http://openscholarship.wustl.edu/vol8_iss1/152

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Toward a Better Understanding of...

The Role of PIWI Protein in *Leishmania braziliensis* RNA Interference

Vivian Tsang

Mentor: Stephen Beverley

The RNA interference (RNAi) pathway uses small non-coding RNAs to target and degrade complementary messenger RNAs via an RNA-induced silencing complex (RISC), which contains an Argonaute family protein. Regulation of gene expression by RNAi is conserved across many organisms, including the trypanosomatid *Leishmania braziliensis*, a protozoan parasite that causes the disease Leishmaniasis in South America. Bioinformatics studies in *L. braziliensis* have identified two putative Argonaute family proteins: ARGONAUTE1 (AGO1) and PIWI. AGO1 has been demonstrated to participate in the 23nt siRNA-dependent RNAi pathway of *L. braziliensis*, but the precise function of PIWI and its importance in *L. braziliensis* RNAi remains unknown. Surprisingly, knockdown of PIWI in *L. braziliensis* reduced RNAi activity, which indicates that PIWI forms part of the RNAi pathway. In contrast, PIWI knockouts show little phenotype in African trypanosomes and those *Leishmania* species that lack RNAi pathways.

To fully characterize the role of PIWI in *L. braziliensis* RNAi, I am working to generate *piwi/-* null mutants, and complemented lines bearing an epitope-tagged PIWI protein. RNAi activity assays and virulence tests in a *piwi/-* null mutant will test the importance of PIWI in the RNAi pathway. Immunoprecipitation of the tagged PIWI and high-throughput sequencing of any co-precipitated small RNAs will be used to characterize the distribution of small RNAs bound to PIWI. In addition, sequencing of total small RNAs will be performed on the *piwi* mutant. Epitope-tagged PIWI will also allow the identification of interacting protein partners, as well as immunofluorescence to determine the cellular localization of the PIWI protein. These studies may reveal novel classes of small RNAs and protein factors participating in *L. braziliensis* RNAi, and provide insights on the evolution of the RNAi pathway in other *Leishmania* species which retain PIWI but not ARGONAUTE.