University of Massachusetts Medical School

GRADUATE SCHOOL OF BIOMEDICAL SCIENCES

BIOCHEMISTRY AND MOLECULAR PHARMACOLOGY

Ph.D. THESIS DEFENSE

WILLIAM SALOMON

MENTOR: Phillip Zamore, PhD

MONDAY, 12/7/2015 10:30 a.m. AS4-2072

"SINGLE-MOLECULE IMAGING REVEALS THAT ARGONAUTE RE-SHAPES THE PROPERTIES OF ITS NUCLEIC ACID GUIDES"

Small RNA silencing pathways regulate development, viral defense, and genomic integrity in all kingdoms of life. An Argonaute (Ago) protein, guided by a tightly bound, small RNA or DNA, lies at the core of these pathways. Argonaute uses its small RNA or DNA to find its target sequences, which it either cleaves or stably binds, acting as a binding scaffold for other proteins. We used Co-localization Single-Molecule Spectroscopy (CoSMoS) to analyze target binding and cleavage by Ago and its guide. We find that both eukaryotic and prokaryotic Argonaute proteins re-shape the fundamental properties of RNA:RNA, RNA:DNA, and DNA:DNA hybridization: a small RNA or DNA bound to Argonaute as a guide no longer follows the well-established rules by which oligonucleotides find, bind, and dissociate from complementary nucleic acid sequences. Counter to the rules of nucleic acid hybridization, we find that mouse AGO2 and its guide bind to microRNA targets 17,000 times tighter than the guide without Argonaute. Moreover, AGO2 can distinguish between microRNA-like targets that make seven base pairs with the guide and the products of cleavage, which bind via nine base pairs: AGO2 leaves the cleavage products faster, even though they pair more extensively. By re-writing the rules for nucleic acid hybridization, Argonautes allow oligonucleotides to serve as specificity determinants with thermodynamic and kinetic properties more typical of RNA-binding proteins than that of RNA or DNA.

Mentor(s)

Phillip Zamore, PhD

Dissertation Exam Committee

Thoru Pederson, PhD (Chair)
Anthony Carruthers, PhD
Craig Mello, PhD
Sean Ryder, PhD
Ian MacRae, PhD