Abstract

RNA interference (RNAi) pathways regulate gene expression in almost all eukaryotes in a sequence-specific manner that depends on complementarity between a small RNA and an mRNA target. In order to effect target silencing, small RNAs are loaded onto Argonautes that can silence their targets post-transcriptionally in the cytoplasm, or transcriptionally in the nucleus in a process called nuclear RNAi. Nuclear RNAi pathways play important roles in organismal development, defense against viruses and transposable elements, and chromosomal organization. Nuclear RNAi links small RNA pathways with chromatin modifications that can, in some cases, be passed on to the next generation. In *C. elegans* for example, nuclear RNAi triggers heterochromatic histone methylation that can be inherited for many generations. While many of the major genes involved in the pathway are known, it remains unclear how Argonautes and the nuclear RNAi machinery are regulated and how they recruit chromatin-modifying proteins. Through a combination of genetic and biochemical assays, the research presented in this thesis outlines specific mechanisms through which nuclear RNAi in *C. elegans* is regulated and demonstrates physical links between proteins that provide clarity on how silencing is achieved. We identified a novel family of nuclear Argonaute binding proteins (the ENRIs) and demonstrated the mechanism by which they control the strength and target specificity of nuclear RNAi. ENRI-1/2 prevent misloading of the nuclear Argonaute NRDE-3 with aberrant small RNAs, and in doing so prevent the premature nuclear localization of NRDE-3 in early embryos. Loss of ENRI-3 impairs inheritance of RNAi phenotypes and leads to misregulation of small RNAs. In addition, we showed that post- translational modifications of ENRI-2 affect many aspects of its function. Phosphorylation of ENRI-2 impairs nuclear RNAi and alters its localization. Finally, we showed how the nuclear RNAi machinery assembles in the nucleus and demonstrated physical links between some of these proteins for the first time. We found that two of these proteins, NRDE-1 and NRDE-4, form a stable complex that form a signaling platform that is required for heritable nuclear RNAi through the recruitment of a phosphatase. Overall, this thesis delineates novel modes and layers of regulation of the nuclear RNAi pathway and provides important biochemical insight into the pathway's mechanism.

McGill University

Graduate and Postdoctoral Studies

Final Oral Examination
for the Degree of
Doctor of Philosophy
of **Alexandra LEWIS**

of the Department of Biochemistry**, on Wednesday, June 9, 2022 @ 10:00 am** Hybrid. **In-person**: **Goodman Cancer Research Centre, Karp Conference Room (501)** & Via Zoom.

COMMITTEE:

Dr. Ante L. Padjen, Psychology	(Pro Dean)
Dr. Sidong Huang	(Deputy Chair)
Dr. Thomas Duchaine	(Thesis Supervisor)
Dr. Paul Lasko	(Internal Examiner)
Dr. Christian Rocheleau	(Internal Member)
Dr. Martin Sauvageau **IRCM**	(External Member)

Dr. Josephine Nalbantoglu
Dean

Members of Faculty and Graduate
Students are invited to be present

CURRICULUM VITAE

NAME: **Alexandra Lewis**
CITIZENSHIP: Canadian

ACADEMIC BACKGROUND:

Ph.D. McGill University
2015 - Present Department of Biochemistry
Thesis Supervisor: Thomas Duchaine

Thesis title: The Biochemical Mechanisms Underlying Nuclear RNAi Interference in *C. elegans*

B.Sc. McGill University
2012-2015 B.Sc. in honors biochemistry

PUBLICATIONS:

Lewis A., Berkyurek AC., Greiner A., Sawh AN., Vashisht A., Merrett S., Flamand MN., Wohlschlegel J., Sarov M., Miska EA., Duchaine TF. (2020). A Family of Argonaute-Interacting Proteins Gates Nuclear RNAi. *Molecular Cell.*

ABSTRACTS:

Presentation at *Worm Small RNA Meeting Seminar Series*. (2021). Online (Zoom). The Functional Architecture of the Molecular Complexes Involved in Nuclear RNAi. **Lewis A.**, Jang SM., Jami Y., Leventis R., Lyu C., Wohlschlegel J., Côté J., Duchaine TF.

ABSTRACTS:

Conference presentation at *The 22nd International C. elegans Conference presented by The Genetics Society of America*. (2019). UCLA, California, USA. A Family of Argonaute-Interacting Proteins Gates Nuclear RNAi. **Lewis A.**, Berkyurek AC., Greiner A., Sawh AN., Vashisht A., Merrett S., Flamand MN., Wohlschlegel J., Sarov M., Miska EA., Duchaine TF.

Poster presentation at *Toronto RNA Enthusiast's Day – TREnD* (2019). Peter Gilgan Centre for Research and Learning, Toronto, Canada. The Functional Architecture of the Nuclear RNAi Pathway. **Lewis A.**, Wohlschlegel J., Duchaine TF.

Conference presentation at *Toronto RNA Enthusiasts' Day (TREnD)* (2018). Peter Gilgan Centre for Research and Learning, Toronto, Canada. A Family of Argonaute-Interacting Proteins Gates Nuclear RNAi. **Lewis A.**, Berkyurek AC., Greiner A., Sawh AN., Vashisht A., Merrett S., Flamand MN., Wohlschlegel J., Sarov M., Miska EA., Duchaine TF.

Poster presentation at the *9th Canadian Developmental Biology Conference.* (2018). Mont Tremblant, Canada. A Novel Family of Nuclear Argonaute-Interacting Proteins Regulates Argonaute Loading in *C. elegans*. **Lewis A.**, Sawh AN., Vashisht A., Berkyurek A., Olbert D., Flamand M., Wohlschlegel J., Miska E., Sarov M., Duchaine TF.

Poster presentation at *The Complex Life of mRNA Meeting*. (2016). EMBO/EMBL, Heidelberg, Germany. A Family of Nuclear RNAi Inhibitors. **Lewis A.**, Sawh AN., Vashisht A., Flamand M., Suffert G., Wohlschlegel J., Miska E., Sarov M., Duchaine TF.