UNIVERSITY of GUELPH

COLLEGE of BIOLOGICAL SCIENCE

DEPARTMENT OF MOLECULAR AND CELLULAR BIOLOGY

Announcement: All interested members of the university community are invited to attend the Final Oral Examination for the degree of **Doctor of Philosophy** of

WARREN VAN LOGGERENBERG, on Fri., Nov. 24, 2017 at 9 a.m. in SSC 2315
(Co-Advisors: Dr. Ross Nazar and Dr. Stephen Seah)

Thesis Title: **Functional Significance of Structural Features and Intermediate Cleavages in the 5' ETS of the *Schizosaccharomyces pombe* pre-rRNA**

Examination Committee:

Dr. A. Bendall, Dept. of Molecular and Cellular Biology (Chair)
Dr. R. Nazar, Dept. of Molecular and Cellular Biology
Dr. J. Robb, Dept. of Molecular and Cellular Biology
Dr. D. Mosser, Dept. of Molecular and Cellular Biology
Dr. S. Abou Elela, Université of Sherbrooke

Abstract:

The pre-rRNA 5′ external transcribed spacer (5′ ETS), similar in size to that of the 18S rRNA, contains structural elements critical for rRNA maturation and is removed via a complex series of processing steps. This study further examined the functional significance of structural features and the intermediate cleavages (A2 and A1) in the 5' ETS of *Schizosaccharomyces pombe* (*S. pombe)*. Initial examination of processing intermediates in the 5' ETS based on RT-PCR indicated that the steps occurred in no specific order, suggesting alternate pathways for 5' ETS processing and degradation. Mutational analyses at the A2 intermediate cleavage site indicated that this cleavage was not critical to rRNA maturation. Systematic mutations demonstrate that structural features in at least two of seven regions are important to protect the nascent pre-rRNA from degradation. RT- PCR mapping of the 5' ETS region in cells with temperature sensitive nucleases refuted formation of the mature 5' end of 18S rRNA by RNase III-like endonucleolytic cleavage across an extended hairpin. However, the results suggested a role in spacer degradation in that the endonucleolytic cleavages served as entry points for the nuclear 5' exonuclease Dhp1p. Further mutational analyses around the 5' ETS/18S rRNA junction indicated cleavage was not dependent on sequence and/or structure, but these sequences can dramatically affect rRNA yield. The results also suggested that the mature 5' end of 18S

rRNA is usually buried upon RNA processing, since changes that extended the position of A0 cleavage affected the 18S:25S rRNA ratios. Studies related to U3 snoRNA binding based on the expression of individual U3 snoRNA domains or mutations in a site complementary to the 5' 18S rRNA pseudoknot were found also to significantly affect the 25S:18S rRNA ratio. Taken together, the present analyses raise the possibility that the 5' end of the mature 18S rRNA actually may be the result of ribozyme cleavage while U3 snoRNA acts as a chaperone to stabilize the nascent rRNA structure. Although the intermediate steps were not critical to rRNA processing, the results indicated that failure to form a stable pre-ribosomal structure, as part of a 'cellular checklist,' results in the pre-rRNA being susceptible to degradation, and provides further evidence of quality control in ribosome biogenesis.

Curriculum Vitae: After obtaining his B.Sc. (Hons) degree from the University of Guelph, Warren completed his graduate studies in the laboratory of Dr. R.N. Nazar; starting his M.Sc. in May 2012, then later transferring to the Ph.D. program in January 2014.