



PROGRAMME

The Final Oral Examination
For the Degree of

MASTER'S OF SCIENCE
(Medical Genetics)

SHELLEY MILLER

BSc University of British Columbia, 2006

Monday, August 11, 2008, 9:00 am

12th Floor Conference Room, BC Cancer Research Centre
675 West 10th Avenue, Vancouver, BC

**"Development of Embryonic Stem Cells Expressing Endogenous
Levels of a Fluorescent Protein Fused to the Telomere Binding
Protein TRF1"**

EXAMINING COMMITTEE

Chair:

Dr. Keith Humphries (Medical Genetics)

Supervisory Committee:

Dr. Peter Lansdorp, Research Supervisor (Medical Genetics)

Dr. Ann Rose (Medical Genetics)

University Examiner:

Dr. Dixie Mager (Medical Genetics)

ABSTRACT

Telomeres are the repetitive DNA sequence and associated proteins found at the ends of linear chromosomes. They have a role in biological processes including meiosis and aging as well as implications in a number of genomic instability disorders and cancers. Telomeres maintain genomic stability by protecting chromosome ends from terminal fusions and misidentification as DNA damage sites. Their wide range of functions has resulted in an increased interest in developing tools to study the dynamics of telomeres in live cells. To do this, current studies use the ubiquitously expressed protein Telomere Repeat Factor 1 (TRF1) tagged with a fluorescent protein. TRF1 is a negative regulator of telomere length that binds exclusively to telomere repeats. Over-expression of the fluorescent protein fused to TRF1 has been a useful tool to track telomere movement. The foci formed by the tagged TRF1 protein accurately represent the number of telomeres expected in the cells and the localization is maintained throughout the cell cycle. A caveat with this system is that over-expression of TRF1 leads to accelerated telomere shortening, as well as replication defects that can stall telomere replication. These caveats make it difficult to draw conclusions about telomere dynamics based solely on observations of cells over-expressing fluorescently tagged TRF1. To eliminate problems associated with protein over-expression, I have tried to develop knock-in embryonic stem (ES) cells expressing fluorescently tagged TRF1 from the endogenous *Trf1* promoter. To do this, I have used a recombineering technique using Bacterial Artificial Chromosomes (BACs). BAC recombineering allows for the direct knock-in of a fluorescent tag into the mouse *Trff* gene locus. Genetic constructs with the correct sequence inserts have been obtained and have been used for transfection of ES cells. While no correctly targeted ES cells have been identified so far, the expectation is that ES cell lines with correctly targeted fluorescently tagged TRF1 will be obtained in the near future. Such lines will be used to study telomere dynamics in ES cells, differentiated cells generated from ES cells, as well as to generate mice.

BIOGRAPHICAL NOTES

Born: February 2, 1979, Sparwood BC

Academic Studies: BSc University of British Columbia, 2006

Current Position: MSc candidate, UBC

GRADUATE STUDIES

Field of Study: Telomeres and Genetic Engineering, Medical Genetics

Courses

MEDG 520	Advances in Human Molecular Genetics	Instructors	Dr. A. Brooks-Wilson
MEDG 530	Advanced Human Genetics		Dr. L. Clarke
MEDG 521	Molecular and Cellular Biology of Cancer		Dr. S. Dunn
MEDG 545	Current Topics in Medical Genetics Research		Drs. P. Hoodless and R. Kay
MEDG 505	Genome Analysis		Dr. S. Jones
MEDG 548	Directed Studies		Dr. P. Lansdorp

PRESENTATIONS

Shelley Miller, Evert-Jan Uringa and Peter M. Lansdorp. (2007) The Role of Telomeres in Meiosis. Sixth Canadian Telomeres & Telomerase Symposium. Winnipeg, Canada.

Shelley Miller, Evert-Jan Uringa and Peter M. Lansdorp. (2007) The Role of Telomeres in Meiosis. Stem Cell Network Annual General Meeting. Toronto, Canada.

Shelley Miller, Evert-Jan Uringa and Peter M. Lansdorp. (2007) The Role of Telomeres in Meiosis. University of British Columbia Medical Genetics Research Day. Vancouver, Canada.

SUPERVISORY COMMITTEE

Dr. Peter Lansdorp, Research Supervisor (Medical Genetics)

Dr. Louis Lefebvre (Medical Genetics)

Dr. Wendy Robinson (Medical Genetics)

Dr. Ann Rose (Medical Genetics)