ANNOUNCEMENT: Interested members of the University Community are invited to attend the Final Oral Examination for the Degree of Doctor of Philosophy of

Maureen M. Mundia

of the Department of Molecular and Cellular Biology on Wednesday, April 27, 2016 at 1:30 p.m. in SSC 1511

Thesis Title: Analysis of the genetic requirements for Rad51-mediated 3’ polymerization during DNA repair by homologous recombination

Examination Committee: Dr. A. Bendall, Dept. of Molecular and Cellular Biology (Chair)
Dr. N. Jones, Dept. of Molecular and Cellular Biology
Dr. D. Josephy, Dept. of Molecular and Cellular Biology
Dr. K. Yankulov, Dept. of Molecular and Cellular Biology
Dr. D. Durocher, The Lunenfeld-Tanenbaum Research Institute

ABSTRACT

Maureen Mundia, B.Sc. (Hons.), M.Sc. Advisor: Dr. M. Baker

Unrepaired double-strand breaks (DSBs) may lead to genome instability, which has been linked to aging and many human diseases, including cancer. In eukaryotic cells, DSBs are repaired primarily by non-homologous end-joining or homologous recombination (HR). The central protein of HR is Rad51, which binds resected single strand DNA and, in conjunction with multiple auxiliary proteins, facilitates the initial steps of HR, which include the homology searching and strand invasion steps that prime for repair synthesis (3’ polymerization). In this study, the early steps of HR were investigated in mouse hybridoma cells by monitoring kinetics of 3’ polymerization, using a PCR-based assay that detects the repair of a plasmid-borne double-strand gap using a cognate chromosomal gene. We found that over-expression of wild-type Rad51, but not multimerization-deficient variants, stimulates the frequency of 3’ polymerization and, notably, increased wildtype Rad51 concentration and homology-length interact synergistically to promote 3’ polymerization. A significant deficiency in 3’ polymerization is observed in Rad51-depleted cell lines as well as in cells expressing the Rad51 catalytic variants that are deficient in ATP binding and hydrolysis. Notably, ATP-binding-deficient Rad51 is more toxic and uniquely reduced for the capacity to drive strand exchange through regions of heterology. Further, expression of the catalytic variants accelerates proteasome-mediated depletion of endogenous Brca2 and Rad51, and the ensuing deficit in HR selects for low levels of p53 that promote cell survival. Therefore, the proteasome plays an important role in clearing defective DNA-repair protein complexes, even in the absence of extrinsic DNA damage. The 3’ polymerization process is stimulated by moderate overexpression of Rad54 but inhibited by siRNA-mediated depletion of Rad54 or overexpression of Rad54 catalytic variants, which demonstrates the requirement for endogenous levels of Rad54 and for the Rad54 ATPase
activity in promoting HR. Moderate overexpression of human breast cancer susceptibility type 2 (hBRCA2) stimulates 3’ polymerization while depletion of mouse Brca2 or overexpression of hBRCA2 to high levels inhibits the process. Further, the 3’ polymerization defect in Brca2-knockdown cells can be augmented by over-expression of wild-type human BRCA2 but not a human BRCA2 variant bearing an in-frame deletion of the Rad51-binding BRC repeats 1-8, which highlights the role of the Brca2 BRC repeats in promoting HR in vivo.

CURRICULUM VITAE:

Maureen received her Honors Bachelor of Science (Major: Molecular Biology and Genetics, Minor: Microbiology) from the University of Guelph in 2006. She completed her M.Sc. in Molecular Biology and Genetics at the University of Guelph in the laboratory of Dr. John Dawson in 2010 and began her Doctor of Philosophy program in Molecular and Cellular Biology at the University of Guelph in 2010 with Dr. Mark D. Baker as her advisor.

AWARDS AND SCHOLARSHIPS:

University international Graduate Scholarships – 2012, 2013

SELECTED PUBLICATIONS:

Maureen M. Mundia, Alissa C. Magwood, and Mark D. Baker. 2016. Rad51 catalytic mutants differentially affect Rad51 nucleoprotein filament formation and function in vivo (manuscript under revision).

Alissa C. Magwood, Maureen M. Mundia, Dick Mosser, and Mark D. Baker. 2016. The dichotomous effects of caffeine on homologous recombination in mammalian cells (manuscript under revision).


Alissa C. Magwood, Michael Malysewich, Iulia Cealic, Maureen M. Mundia, Jennifer Knapp, and Mark D. Baker. 2013. Endogenous levels of Rad51 and Brca2 are required for homologous recombination and regulated by homeostatic re-balancing. DNA Repair. 12(12):1122-1133.


