University of Victoria | Graduate Studies

Notice of the Final Oral Examination
for the Degree of Doctor of Philosophy

of

DAVID JOHN DILWORTH

BSc (University of Waterloo, 2009)

"Functional Characterization of the Nuclear Proline Isomerase FKBP25: A Multifunctional Suppressor of Genomic Instability"

Department of Biochemistry and Microbiology

Thursday, July 27, 2017
9:00 A.M.
David Strong Building
Room C144

Supervisory Committee:
Dr. Chris Nelson, Department of Biochemistry and Microbiology, University of Victoria (Supervisor)
Dr. Caren Helbing, Department of Biochemistry and Microbiology, UVic (Member)
Dr. Julian Lum, Department of Biochemistry and Microbiology, Uvic (Member)
Dr. Jürgen Ehlting, Department of Biology, UVic (Outside Member)

External Examiner:
Dr. Michael Hendzel, Department of Oncology, University of Alberta

Chair of Oral Examination:
Dr. Peter Wan, Department of Chemistry, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies

Abstract

The amino acid proline is unique. In proteins, peptidyl proline exists in a bent cis peptide bond conformation at a relatively high frequency. This feature has ramifications on protein structure and function. It presents both a barrier for de novo protein folding and an opportunity for the post-translational regulation of protein function. Peptidyl-prolyl isomerases (PPIs), an enzyme superfamily, catalyze the interconversion between conformers. PPIs are ubiquitous - expressed from bacteria to humans, constituents of most cellular compartments in eukaryotes, present in all cell types in multicellular organisms, and engaged in diverse cell processes. The functions and substrates of most PPIs are unknown. This knowledge gap has significant implications for human health, as PPIs are the intracellular receptors of a widely used class of immunosuppressant drugs. These drugs and their analogs also show promise in the treatment of disease, including neurodegenerative disorders and cancer. Understanding the function of PPIs will be critical for the rational design of effective second generation PPI inhibitors tailored to the treatment of specific diseases.

The PPI FKBP25 is one of few nuclear resident isomerases. Currently, a functional picture of its biology is lacking. While it has been shown to bind DNA and associate with chromatin, suggesting a role in transcriptional regulation, there is little evidence as to its actual function within the cell. Here, I discover that FKBP25 is a multifunctional dsRNA binding protein required for the maintenance of genomic stability. In Chapter 2, I identify the significance of FKBP25's dsRNA binding properties, showing that this feature of the protein results in recruitment to pre-ribosomal particles in the nucleolus. In Chapter 3, I explore the involvement of FKBP25 in transcriptional regulation, prompting an unexpected discovery - FKBP25 is a microtubule-associated protein and contributes to microtubule dynamics. This function influences the stress response, cell cycle, and chromosomal stability. As part of this study, I also characterize the regulation of FKBP25's localization and nucleic acid binding activity throughout the cell cycle. Finally, in Chapter 4, I uncover a role for FKBP25 in the repair of DNA double-stranded breaks, a particularly dangerous type of genomic lesion. Importantly, this function requires FKBP25's catalytic activity, identifying for the first time a functional requirement for cis-trans prolyl isomerization by FKBP25. Collectively, this work is a significant advance in the understanding of FKBP25 and PPIs in general. This thesis contributes to the exploration of PPIs as drug targets and will aid the rational design of therapeutics that target individual PPI family members.