

Lost in Translation: How Pancreatic Beta-cells Save Themselves from Stress

The prevalence of diabetes in the Canadian population is ~5%, and rates are as high as 40% have been reported in indigenous populations. Despite considerable study, the underlying molecular defects that cause diabetes remain elusive. Diabetes is caused by insufficient production of insulin, which is produced in vast amounts by pancreatic beta-cells, of which there are limited numbers. Due to the body's extensive requirements for insulin, beta cells are under a significant amount of stress. Any disturbance in homeostasis, such as excessive amounts of circulating lipids (lipotoxicity) in obesity, can lead beta-cell malfunction and death, which in turn leads to type 2 diabetes. Specifically, the endoplasmic reticulum (ER), which is a key component of the protein production process, may experience a loss of function should it give in to the stress of the continuous and extensive production of insulin in response to extensive nutrient uptake in obesity. Evidence points to a particularly importance of ER stress as a common mechanism involved in beta-cell dysfunction and death. Under ER stress, general protein translation is turned off. However, a select pool of proteins continues to be translated in the ER and it is known this mechanism is crucial for the recovery of beta-cells from ER stress and continued production of insulin. Specific translation initiation factors are thus necessary to maintain the translation of these proteins while global translation is turned off, however none have been identified to date.

One of the Johnson group's research directions is focused on identification of novel pathways in fatty acid-induced beta-cell death in type 2 diabetes. The goal of this project is to determine which regulatory factors are involved in this process, and which proteins are continually translated in a manner dependent on these factors. Johnson lab recently showed that eukaryotic initiation factor 2A (eIF2A) is one of the key players in selective translation initiation during ER stress in pancreatic beta-cells. Wildi's involvement in this project focuses on determining which proteins are selectively translated via an eIF2A-dependent mechanism. Wildi will perform unbiased survey of eIF2A-dependent polysome-associated mRNAs during ER stress. Using a mouse pancreatic beta cell line (MIN6) that stably overexpresses eIF2A or GFP (control), Wildi will monitor protein translation during ER stress at high-resolution on a genome-wide scale for the first time. Wildi will employ ribosome profiling and RNAseq using expertise of Dr. Eric Jan and Dr. Elizabeth Rideout in the Life Sciences Institute. Wildi will isolate ribosome-protected mRNA after 1 hour of palmitate treatment (lipotoxicity model). By comparing the coverage and number of reads per mRNA in untreated cells versus stressed cells, with and without overexpression of eIF2A, Wildi will measure the translational efficiency of each mRNA and identify genes requiring eIF2A-dependent translation. While waiting for sequencing results, Wildi will test candidate eIF2A-dependent mRNAs (ATF4, CHOP) using Western blotting and luciferase assay, which have been already identified in the lab by Dr. Evgeniy Panzhinskiy.

These studies will hopefully solve the mystery of how key beta-cell genes escape global translational repression in the context of diabetes-related stresses. This work will define new pathways that can be targeted for the prevention of ER-stress in diabetes. Wildi is very excited to participate in work that will both uncover novel biochemical mechanisms and help us understand the processes that underlie type 2 diabetes.