Sequence Determinants of Nrf2 Regulatory Output

Courtney Ryan
Swenson College of Science and Engineering
University of Minnesota Duluth

Matthew G. Slattery
UROP Faculty Adviser
Medical School, Duluth Campus
University of Minnesota

Oxidative stress damages proteins, lipids, and DNA and thus contributes to diseases such as cancer and neurodegenerative disease. The transcription factor Nrf2 is a master regulator of the response to oxidative stress. In the presence of stress, Nrf2 translocates into the nucleus and binds a consensus DNA sequence (the antioxidant response element, or ARE) that leads to up regulation of detoxification genes.

The Slattery lab has recently published that Nrf2 target genes are differentially responsive to Nrf2 activity; the differences between these responses is correlated with ARE motif quality (Lacher et al. 2015). Some Nrf2 target genes are regulated by a perfect ARE (TGCTGAGTCAT; strong binding), while most others contain various combinations of mismatches in the ARE (weaker binding). My recent work in the Slattery lab supports the hypothesis that not all AREs are equivalent. Perfect AREs respond strongly to small increases in Nrf2 and are switch-like in responding to stress; imperfect AREs respond to Nrf2 activity in a linear manner. This data suggested that subtle changes to the ARE sequence can have a significant impact on Nrf2 binding and the corresponding regulatory output of gene expression.

The nucleotides in the ARE that I have underlined (TGCTGAGTCAT, positions 2, 3, 8, 9, and 10) are known as core ARE elements and are required for Nrf2 binding and cannot tolerate substitution. The other positions have been shown to contain nucleotides that differ from the perfect ARE in many human genes that are actively controlled by Nrf2, yet we do not understand how variation in these regions can affect Nrf2 binding and the degree of regulatory output.

I used electro-mobility shift assays (EMSA) and luciferase reporter assays to test the effects of variation in two positions (1 and 7) of the ARE using both *in vitro* and *in vivo* model systems. Position 1 and 7 of the ARE were independently varied to obtain more information on how binding motif quality influences regulatory output. For example, a perfect ARE contains a "T" in position 1, and we substituted this T to an A, C, or G, while keeping the rest of the ARE the same. We repeated this process for position 7 (perfect ARE is a G, substituted for an A, C, or T). These two positions were chosen as the first to examine as positions 1 and 7 are highly variable in human ARE containing genes while keeping the core ARE nucleotides in tact.

Direct binding of Nrf2 to the ARE was measured *in vitro* via EMSA. Changing position 1 from a perfect T to a G had the most obvious decrease in Nrf2:ARE binding while changing T to a C had no change. Position 7 variation showed minimal differences in levels of binding. *In vivo* reporter assays measured how positional changes in ARE affect Nrf2-mediated luciferase gene transcription. Changing position 1 from a T (perfect) to a C resulted in no changes in luciferase expression, where as an A or G resulted in a drastic decrease of luciferase expression. Changing position 7 of the ARE from a G (perfect) to an A, C, or T did significantly decrease luciferase expression in all cases.

The differences and similarities when comparing the results from both model systems highlight why both *in vivo* and *in vitro* methods are necessary trying to understand TF biology. Furthermore, although I was able to show that variation in position 1 and 7 does lead to altered binding and luciferase expression, for specific ARE substitutions, the rules governing Nrf2:ARE interactions remain unclear and require further evaluation. Altering one base at a time and using EMSA and luciferase assays has proven to be very informative, but will not lead to a comprehensive understanding of how the positional variations in ARE binding motif translates to Nrf2 binding and gene expression changes. My research project completed in the Fall of 2015 did clearly demonstrate that the degree to which Nrf2 mediates gene expression is regulated by the strength of the ARE motif; however the rules governing Nrf2:ARE interactions are not well defined. Interdependencies between positions may exist (e.g., G in position 7 is only preferred when position 6 is A). Currently I am using technique known as SELEX-seq to gain more knowledge on the Nrf2:ARE binding preferences.

he objective of my research was to detect the impact on Nrf2 binding and corresponding regulatory output of gene expression by subtle changes to the ARE sequence. Using the approach described above and varying one position of the ARE at a time has proven to be very informative and has inspired many further questions that I plan to continue to answer while working in the Slattery laboratory and with the help of the support provided from UROP. Moreover, the support I have received from UROP has helped me realize my love for science, while simultaneously giving me the opportunity to obtain laboratory experience. I would like to continue pursuing research throughout medical school and my career. The entire UROP faculty has been extremely helpful along the way with the application process and the ending requirements and I am sincerely grateful for this opportunity.

Lacher, S.E., Lee, J.S., Wang, X., Campbell, M.R., Bell, D.A. and Slattery, M. 2015. Beyond antioxidant genes in the ancient Nrf2 regulatory network. Free radical biology & medicine 88, 452-465.