SELEX-sequencing

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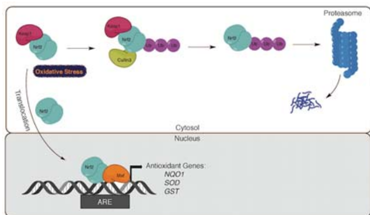
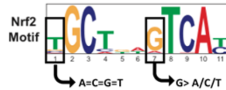
Introduction



Oxidative stress damages proteins, lipids, and DNA and thus contributes to diseases such as cancer and neurodegeneration.

The transcription factor Nrf2 is a master regulator of the response to oxidative stress. Nrf2 binds a consensus DNA sequence, the ARE (antioxidant response element).

We found that Nrf2 target genes are differentially responsive to Nrf2 activity: the differences between these responses is correlated with ARE motif quality. Some Nrf2 target genes are regulated by possessing a perfect ARE (TGCTGAGTCAT; strong binding), while most others contain various combinations of mismatches in the variable (n) ARE positions (weaker binding).



My work in the Slattery lab suggests 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 more linear manner. This data suggested that subtle changes to the ARE can have a significant impact on Nrf2 binding and the corresponding regulatory output of gene expression.

Single Nucleotide Substitution Data

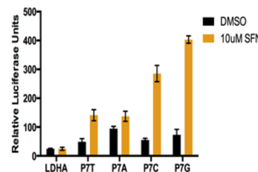
Gene transcription following Nrf2 binding was investigated as a result of positional ARE modification using a combination of *in vitro* and *in vivo* methods

Direct binding of Nrf2 to the ARE was measured *in vitro* via EMSAs

- 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
- At position 7, the differences in binding due to ARE modification were minimal

In vivo Luciferase reporter assays measured how positional changes in ARE affect Nrf2 activated 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 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

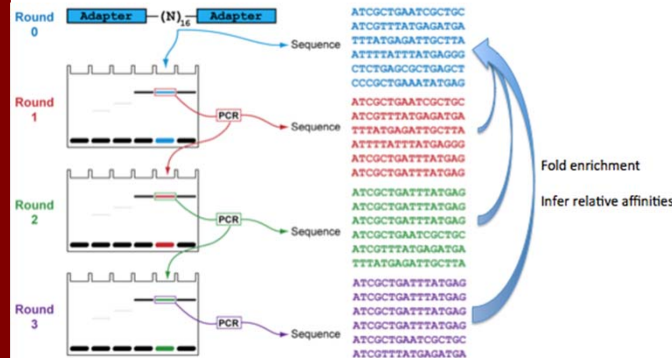


Single Nucleotide Substitution Conclusions

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 exist (e.g., G in position 7 is only preferred when position 6 is A)
- Consequently, these methods will not lead to a comprehensive understanding of how the positional variations in ARE binding motif translates to Nrf2 binding and gene expression changes

Current Work: SELEX-sequencing



Currently I am using systematic evolution of ligands by exponential enrichment coupled with DNA sequencing (SELEX-seq)

- DNA library containing a 16 base pair randomized region that is flanked by defined regions
- DNA bound by the complex is then separated from unbound DNA using EMSA
- Bound DNA is amplified by PCR, sequenced, and used for subsequent rounds of DNA binding and selection
- Comprehensive view of Nrf2:ARE binding preferences

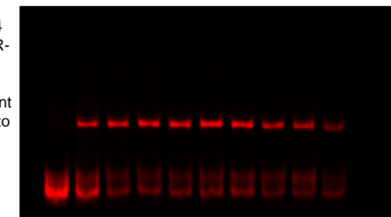
Sequencing & Analyzing Data

- Sequencing is done by the University of Minnesota Genomics Center in Minneapolis, MN
- Markov Models are used to accurately estimate the frequencies of all possible 16-base pair sequences in the initial pool
- Sequence counts from multiple rounds SELEX are compared to initial pool in order to infer relative affinities

Future Directions

- Inappropriate expression of the transcription factor DUX4 causes facioscapulohumeral muscular dystrophy (FSHMD)
- The Slattery lab has collaborated with Dr. Michael Kyba's group (University of Minnesota – Twin Cities) to identify a high affinity DUX4 binding site
- I am currently optimizing DUX4 EMSA conditions, using a probe based on the high affinity binding site, in order to run SELEX-seq on this transcription factor

Figure 1: DUX4 binding to the IR-labeled probe. Cold competitor added in different concentrations to assess binding affinities. Both WT and mutant CC used.



Lane 1: probe only
Lane 2: no CC
Lane 3: CC MUT 1x
Lane 4: CC MUT 5x
Lane 5: CC MUT 10x
Lane 6: CC MUT 50x
Lane 7: CC WT 1x
Lane 8: CC WT 5x
Lane 9: CC WT 10x
Lane 10: CC WT 50x

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