

Investigating the Role of RNA Binding Proteins in Gene Expression

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Introduction: Friedreich's ataxia (FRDA) is an autosomal recessive disorder affecting 1 in 50,000 people that causes progressive nervous system damage and impaired movement. Molecularly, this disorder is characterized by abnormalities in the frataxin gene (*FXN*), which encodes for an iron chaperone protein important for iron-sulfur cluster biogenesis in the mitochondria. In unaffected people, the first intron of *FXN* contains 7-35 repeats of the nucleotide sequence GAA. In people with FRDA the first intron of *FXN* contains several hundred GAA repeats (Cook & Giunti, 2017). This repeat expansion results in a decreased *FXN* expression and localization of the locus to the nuclear periphery. It is important to note that generally, genes located at the nuclear periphery are contained in heterochromatin and have decreased expression.

Interestingly, it has been previously demonstrated that Serine and arginine-rich (SR) proteins, RNA binding proteins which play a critical role in the assembly of the spliceosome during mRNA processing, have a GAA-rich binding sequence. To assess the relationship between SR protein binding and expression of *FXN* in FRDA cell lines, we propose a model in which excess GAA repeats in the *FXN* transcript act as a sink for SR proteins. Excess SR protein binding decreases the stability of the mRNA and lowers *FXN* expression. This project aims to validate this mechanism and investigate more generally the relationship between SR proteins, gene expression, and chromatin organization.

Materials and Methods: To verify this model, we knocked down SR protein expression in FRDA and wild-type cell lines and then examined the expression of *SRSF1* (a gene encoding for a candidate SR protein) and *FXN*. Knockdown of *SRSF1* was achieved through exogenous expression of shRNA, which binds to *SRSF1* mRNA and targets it for degradation. We utilized viral infection of target cells to enable the expression of shRNA. Once infected, lentivirus integrated targeted shRNA with the cell genome, triggering shRNA expression. After infection was achieved, *FXN* and *SRSF1* mRNA expression levels were analyzed using qPCR to verify knockdown efficiency and examine *FXN* expression dynamics.

Results and Discussion: After qPCR analysis of wild-type and FRDA cell lines with and without *SRSF1* knockdown, it was found that there was no significant change in *FXN* expression in the wild-type strain or the FRDA model cell line with the *SRSF1* knockdown, as seen in figure 1. This suggests that the binding of *SRSF1* to repeat expanded *FXN* mRNA transcripts has no effect on *FXN* transcription levels or that excess *SRSF1* binding does not occur.

Conclusion: While current mechanisms for the repression of *FXN* expression in those with Friedreich's ataxia have already been described, this project aimed to elucidate a potentially novel mechanism for transcriptional repression. Ultimately, however, our data is consistent with the conclusion that SR proteins play little to no role in regulating *FXN* expression and this model for repression is most likely not relevant in FRDA cell lines. To further develop this relationship, future experiments will utilize cross-linking immunoprecipitation (CLIP) assays to determine if *SRSF1* binding is occurring at the *FXN* transcript. This will help determine if excess *SRSF1* binding is not having any effect on transcription or if this excess binding is not occurring in the first place.

References

- (1) Cook A, Giunti P. Br Med Bull. 2017;124(1):19-30

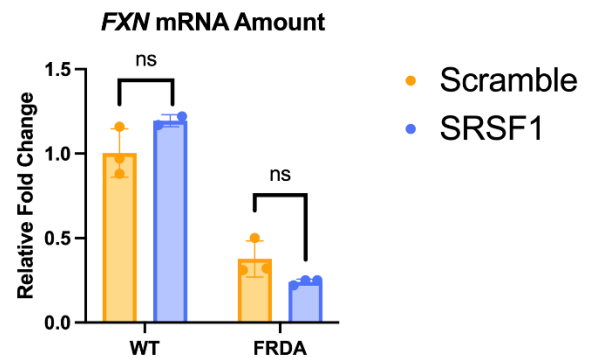


Figure 1. Quantification of *FXN* mRNA levels in the wild type (WT) and Friedrich's ataxia (FRDA) cell lines infected with a scramble control virus and an *SRSF1* knockdown virus.