



Research Fellowship: Visual dissection of GAA-mediated mechanisms of *FRDA* repression and identification of novel candidate factors involved in frataxin function

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Scientific abstract

Friedreich's Ataxia (FA) is the most common genetic recessive ataxia. Currently, there is no treatment for FA, probably due to a limited understanding of the pathological mechanism underlying the disease. It is known that an expansion of GAA repeats in intron 1 of the frataxin-coding gene *FRDA* is responsible for decreased levels of frataxin, but how this reduction in expression occurs and what the exact functional role of frataxin (FXN) is remain to be elucidated.

In this proposal we focus on the use of new tools to address these two unsolved issues. We have generated genomic DNA-reporter fusion vectors, which carry an in-frame insertion of luciferase at the end of the *FRDA* gene (*FRDA*-Luc vector) in the whole 80 kb *FRDA* genomic locus, and a 300 GAA repeat expansion in intron 1 (*FRDA*-GAA-Luc vector). We have generated human clonal cell lines carrying either *FRDA*-Luc or *FRDA*-GAA-Luc genomic vectors. We will use these cell lines for two different areas of research. First, we will study the effect of GAA repeats on frataxin transcription, by using real-time transcription imaging. By fluorescence *in-situ* hybridization we will also assess whether GAA repeats can affect the location of the *FRDA* gene in the nucleus. Secondly, we will use a transposon-mediated mutagenesis screening which will allow us to identify new candidate genes involved in FXN functional pathways.

Lay summary

Friedreich's ataxia (FA) is caused by a reduction in the levels of a protein called frataxin. What is known about the cause of FA is that the low levels of frataxin are due to a mutation in the gene coding for frataxin (*FRDA*), in particular a 3-base pair sequence (GAA) repeated up to 1700 times in the mutated *FRDA* gene. The way this mutation affects frataxin levels is not completely clear although several hypotheses have been described. Another subject that remains to be understood is the role of frataxin in the cell, since understanding its function will get us closer to a therapy. In our laboratory, we have generated cells that show the same effect of the GAA mutation on frataxin levels observed in Friedreich's ataxia. Furthermore these cells have been modified with a reporter gene, which flashes light whenever the *FRDA* gene is activated, making quantification of frataxin levels easy and rapid. Our aim is to use these cells to better understand the two main missing pieces of information, namely the pathological mechanism of the GAA mutation and most importantly the function of frataxin.



First, we will study the direct effect of the GAA mutation on RNA synthesis, the first step involved in the cellular production of the protein frataxin, and observe the way the GAA mutation affects this process. Second, we will investigate the role of frataxin by randomly mutating the genes of the cells we have generated, until we find mutations that affect the levels of frataxin. When this occurs, we will have found genes that are very likely to be implicated in frataxin cellular role, since their activity can influence frataxin levels.

This study will help us understand more about the mechanism through which the GAA mutation affects the levels of frataxin and also provide more information about the cellular role of this important protein. Both studies could help us develop novel therapeutic strategies.

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