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Of Yeast And Men: Unraveling The Molecular Mechanisms Of Friedreich's Ataxia

ScienceDaily (July 9, 2009) — Researchers in human genetics have long known that expansions of GAA repeats – resulting in this nucleotide triplet repeating hundreds or thousands of times – cause the most common hereditary neurological disorder known as Friedreich's ataxia. There is no cure for this condition, which damages the nervous system and can result in heart disease.

The disease's origins have been proven through analysis of genetic records of affected individuals and their families. But scientists have not been able to study the molecular processes that cause the GAA sequence to expand so profoundly because they have lacked a model that could produce the large-scale expansions for experimental purposes.

In a paper to be published in the July issue of *Molecular Cell*, a research team lead by Sergei Mirkin, White Family Professor of Biology at Tufts' School of Arts and Sciences, has created an experimental model that does indeed produce large-scale expansion of GAA repeats during DNA replication.

In doing so, Mirkin and his team were able to analyze GAA repeat expansions and then identify cellular proteins that thwarted normal replication and promoted the elongated sequence.

"In essence we believe that the replication machinery occasionally gets tangled within a repetitive run, adding extra repeats while trying to escape," says Mirkin. "And the longer the repeat - the more likely the entanglement is. That is as if a car which entered a roundabout misses the right exit due the heavy traffic and has to make the whole extra circle before finally escaping."

The researchers started with common baker's yeast because it allowed them to monitor the progress and genetic control of repeat expansions, which is not feasible in humans.

When they inserted GAA repeats of varying lengths (50-to-150 triplet repeats) into an intron of the specifically modified reporter gene they found that widespread expansions of these repeats indeed occurred. These expansions blocked RNA splicing and, as a result, deactivated the gene.

This allowed the team to measure the precise rate of repeat expansions at various experimental settings by growing yeast on the special selective medium followed by determining the repeat lengths via polymerase chain reaction.

Using this approach, the researchers observed massive expansion of GAA repeats that ranged between 200 and 450 repeats. Remarkably, they found that the likelihood of a repeat expansion increased as it

grew longer, which closely mimicked the situation observed in the genetic record of humans with Friedreich's ataxia.

Mirkin and his team then carried out a genetic screen to identify yeast proteins affecting repeat expansions. They found that the proteins within the cell that are known to facilitate the smooth replication fork progression decreased repeat expansions. Meanwhile the proteins responsible for the fork deviations, such as template switching and reversal, increased repeat expansions.

Mirkin's research is funded by the National Institutes of Health. Mirkin's team included Tufts postdoctoral fellow Alexander A. Shishkin; graduate student Irina Voineagu and Brook T. Chernot; undergraduates Robert Matera, Nicole Cherng and former laboratory member Maria M. Krasilnikova, who is currently on the faculty of Penn State. Collaborators also included Georgia Tech Professor Kirill Lobachev and postdoctoral fellow Vydhia Narayanam.