FALL 2020

Biochemistry and Molecular Biology Brown Bag Series

Venicia Alhawach

Ph.D. Student

"Analysis of Instabilities in ATTCT Pentanucleotide Repeats in Human Cells"

Tuesday, November 24,

2020 11:00 AM

Please contact x3249 if you would like to attend but did not receive an emailed link.

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https://science-math.wright.edu/biochemistry-and-molecular-biology

Abstract

Analysis of Instabilities at ATTCT Pentanucleotide Repeats in Human cells.

Microsatellite sequence are highly abundant unstable short repeats of DNA (1 to 6 nucleotides) whose expansion leads to many neurodegenerative diseases. When encountered by a replication fork, these repeats can impede replication progression by their ability to form non-B-DNA structures, which cause the fork to stall and eventually collapse. We show that microsatellites also cause replication-dependent DNA double strand breaks. To understand the effect of these repeats on replication and chromosomal instability, our laboratory generated a dual fluorescent system to measure replication dependent DSBs at these microsatellites quantitatively using flow cytometry. HeLa cells are used as a model system where different microsatellites such as (CTG)100, (Pu/Py)88, and (ATTCT)n have been incorporated adjacent to the human c-myc origin of replication at an ectopic site in the genome. The dual fluorescence system consists of two reporter genes: dTomato that produces a red fluorescent protein and eGFP that produces a green fluorescent protein. These reporter genes are flanked by Alu elements that are known hot spots for recombination. When breaks occur, repair can lead to different recombination products between these Alu elements that can be monitored using flow cytometry. Depending on the chosen repair pathway, cells either lose the dTomato gene and thus appear green, or lose the eGFP gene and thus appear red. Using this recently developed model, we have been able to demonstrate that the trinucleotide repeat (CTG)100 is unstable under replication stress and that the replication-dependent breaks are repaired differently than breaks induced by I-SceI endonuclease. In this work, we also study instabilities at the (ATTCT) pentanucleotide whose expansions have been correlated with the neurodegenerative disease spinocerebellar ataxia 10 (SCA 10). Previous work from our laboratory has demonstrated that (ATTCT) repeats can act as DNA unwinding element and that, when at sufficient length, are able to initiate replication origin activity. To assess how different non-Watson-Crick DNA structures affect replication fork stability and repair it is important to determine whether breaks at the pentanucleotide repeat exhibit a different pattern of repair than replication-induced breaks at the (CTG)100 and (Pu/Py)88 microsatellites.