FALL 2021

Biochemistry and Molecular Biology Brown Bag Series

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"Instability at $(ATTCT)_n$ Pentanucleotide Repeats in Human Cells"

Tuesday, November 2, 2021

11:00 AM

135 Oelman Hall

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

Abstract
Instability at $(ATTCT)_n$ Pentanucleotide Repeats in Human Cells

Microsatellites are unstable, short repeats of DNA (1 to 6 nucleotides) that can expand, contract or break, leading to mutations that threaten the genomic integrity. When encountered by a replication fork, these repeats can impede replication progression due to their ability to form non-Watson-Crick structures which cause the fork to stall and eventually collapse. To understand the effect of these repeats on replication and chromosomal instability, our laboratory generated a dual fluorescent system to quantitate replication dependent DSBs at these microsatellites using flow cytometry. The HeLa cell line was the model system used where different microsatellites capable of forming triplex, quadruplex and hairpin structures have been incorporated adjacent to the human c-myc origin of replication with defined replication polarity. The system consists of two fluorescent reporter genes, dTomato that produces a red fluorescent protein, and eGFP that produces a green fluorescent protein, flanked by Alu elements that are targets for recombination. When DNA double strand breaks occur, repair can lead to different mutagenic patterns and recombination products that can be monitored using flow cytometry and DNA sequencing. Using this model system, we have been able to demonstrate that trinucleotide repeat $(CTG)_{100}$ hairpins and guanine-rich quadruplex forming DNAs are unstable under replication stress. In the current work, we are interested in studying instability at the (ATTCT) pentanucleotide whose expansions have been correlated with the neurodegenerative disease spinocerebellar ataxia 10 (SCA 10). Previous work from our lab has demonstrated that (ATTCT) repeats can act as DNA unwinding element and that sufficient length repeats initiate replication origin activity and undergo replication-dependent recombination. I will contrast the pattern of breaks at the pentanucleotide repeat and at the $(CTG)_{100}$ repeat and discuss whether interruptions in those ATTCT repeats can affect recombination and replication in our model system.