SPRING 2021

Biochemistry and Molecular Biology Brown Bag Series

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Professor

"What happens to the DNA ends at double strand breaks (DSBs)"?

Tuesday, February 16, 2021

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

"What happens to the DNA ends at double strand breaks (DSBs)"?

Today's brown bag talk will describe the ectopic site dual fluorescence system that we use to analyze DSB processing and characterize replication-dependent DSBs at unstable, non-B form DNA repeats. To do this, we devised dual fluorescence (DF) constructs in which a restriction enzyme cutting site or a non-B DNA structure-forming microsatellite repeat is flanked by fluorescent reporter genes (dTomato, eGFP) and Alu elements which represent preferred sites for DNA recombination. All DF constructs were integrated at the same ectopic FLP recombinase target (FRT) in the HeLa genome. We show that DSBs can be detected by the loss of the dTomato or eGFP fluorescent reporter genes, PCR analysis and next-generation DNA sequencing (NGS).

I-Sce1 cleaves at the single ectopic site in the genome of DF cells. I-Sce1 cleavage resulted largely in the loss of the eGFP reporter by single strand annealing (SSA) recombination between Alu elements, demonstrating that the primary mechanism for repair of a "clean" DSB involves 5'—>3' end resection. However, small pool analysis of flow sorted cells revealed that ca. 10% of DSBs suffered end processing followed by aberrant nonhomologous end joining (NHEJ).

In contrast to the results of cutting by I-Sce1, replication-dependent DSBs at non-B DNA forming $(CTG)_{100}$ microsatellite repeats were due to Mus81 resolvase cutting near the I-Sce1 site at one edge of the unstable microsatellites. As opposed to I-Sce1 digestion however, these DSBs produced a large fraction of $eGFP^+$ cells that had lost the dTomato marker. Similar results were obtained with a different microsatellite, $(Pu/Py)_{88}$, and under multiple conditions of replication stress.

These results suggest that replication-dependent DSBs at structured microsatellites are resistant to end processing and DNA repair. This may be due to blocking of the microsatellite ends similar to the sequestration of telomeric TTAGGG repeats by TERRA RNA and t-loop formation. That the sequence or structure of the microsatellite repeat affects end processing and recombination is supported by the finding that the $(ATTCT)_{47}$ microsatellite repeat shows a different pattern of recombination resulting in apparent duplication of the dTomato gene.

Although the great majority of replication-dependent DSB ends at $(CTG)_n$ structures are resistant to repair, a small percentage (ca. 0.001%) undergoes resection and invades the sister chromatid or nonallelic genomic sites to carry out break-induced replication (BIR). NGS analyses show that BIR initiates at the upstream side of the microsatellite DSB and is ca. 1000 x as mutagenic as normal replication. The invading 3' end of the resected DSB is prone to mismatch repair mutagenesis due to its folded structure.

Future experiments will analyze the fate of the downstream DSB end, and the contributions of different microsatellite structures and DNA damage response factors to DNA repair and BIR.