Investigating roles of nonhomologous end-joining and recombination genes in repair of site-specific DNA double-strand breaks in *Saccharomyces cerevisiae*

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Abstract

The DNA in eukaryotic cells such as human and yeast cells are constantly subjected to endogenous and exogenous sources of damage through exposure to radiation and mutagenic chemicals. Among these lesions, double-strand breaks (DSBs) are one of the most lethal damages which have two DNA repair pathways dedicated to them known as the nonhomologous end-joining (NHEJ) and homologous recombination (HDR) pathways. Unlike the highly accurate HR system, the error prone NHEJ pathway in yeast cells does not require a template strand for repair and uses three major protein machineries. These are the Yku complex which binds and protects the DSB ends and recruits Mrx that tethers the DSB ends together. Mrx then recruits the Dnl4 complex, which ligates the ends together. The most commonly used assay for NHEJ repair involves transfer of circular plasmids containing a single site-specific DSB into yeast cells. NHEJ mutants (*yku*$^-$, *mrx*$^-$, or *dnl4*$^-$ yeast strains) show reduced efficiency of repair. The goals of this research project were to: (1) Investigate the role of the Mrx protein complex in the steps of the NHEJ pathway and (2) develop new assays to measure repair of DSBs by both HR and NHEJ simultaneously.

Introduction

Cellular DNA is under constant and spontaneous attack from both endogenous and exogenous sources, which can result in different types of DNA lesions. Such damage impacts cellular processes such as DNA replication and transcription. These lead to accumulation of mutations and genomic instability (1). The lesions incurred by the genomic DNA can take different forms such as alkylation of bases, bulky adduct formation, single- and double-strand breaks, and insertions or deletions (2). Two major pathways are dedicated to repairing DNA double-strand breaks, which are the most deleterious lesions exhibited by eukaryotic cells. They are known as the homologous recombination (HR or HDR) and nonhomologous end-joining (NHEJ) pathways (3). Homologous recombination is highly accurate since it utilizes an intact DNA template to repair the break which reestablishes the original sequence of the strand, whereas NHEJ simply ligates the two ends together with no regard to homology. Budding yeast makes a great model to study the functionalities of the NHEJ complexes using genetics. The NHEJ pathway utilizes three highly conserved main complexes to initiate repair in DSBs which include: Yku, Mrx and Dnl4. The most common assay for NHEJ and HR involves the transfer of circular plasmids that contain a single site-specific DSB into yeast cells. These DSBs are created by restriction enzymes cutting at specific known sites in a designated plasmid of choice (4). The Lewis lab has developed several plasmid-based systems for measuring repair by each pathway separately. More recent work has created systems for assaying both pathways simultaneously.

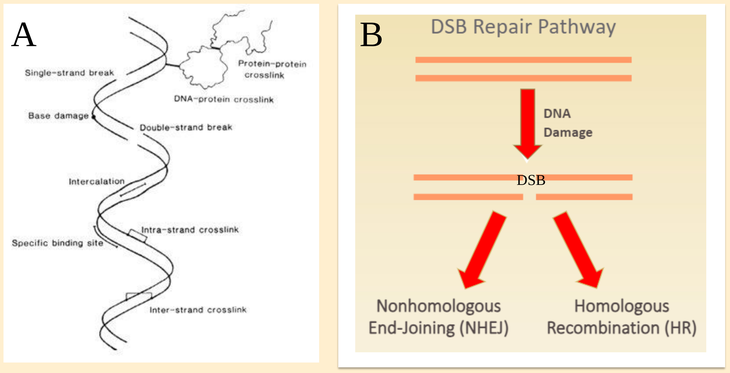

Figure 1. (A) Many types of lesions form in DNA regularly. (B) Double-strand breaks have two separate repair pathways dedicated to them.

Goals of Project

- The primary goals of this project were to (1) test a new NHEJ repair assay system and (2) develop and test a new method for measuring repair of DSBs by both HR and NHEJ simultaneously.

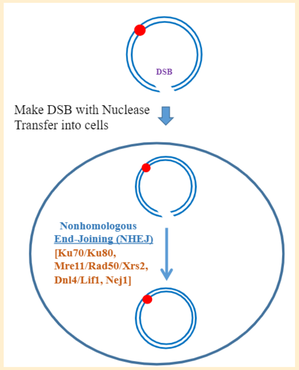

Figure 2. Schematic of Nonhomologous End-joining repair assay by inducing site specific DSB with restriction endonuclease and transferring into cells for repair

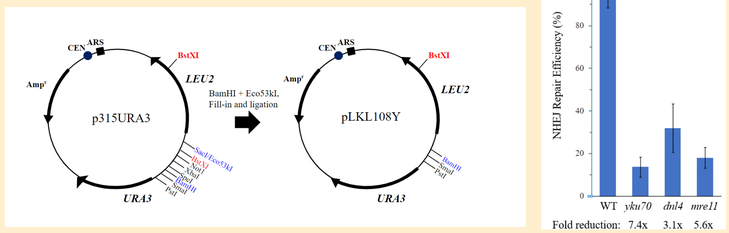

Figure 3. (left side) Construction of pLKL108Y by deleting a BstXI site from the plasmid p315URA3 that had two sites; (right side) NHEJ repair efficiency was strongly reduced in *yku70*$^-$, *dnl4*$^-$ *and mre11*$^-$ mutants using the BstXI-cut plasmid.

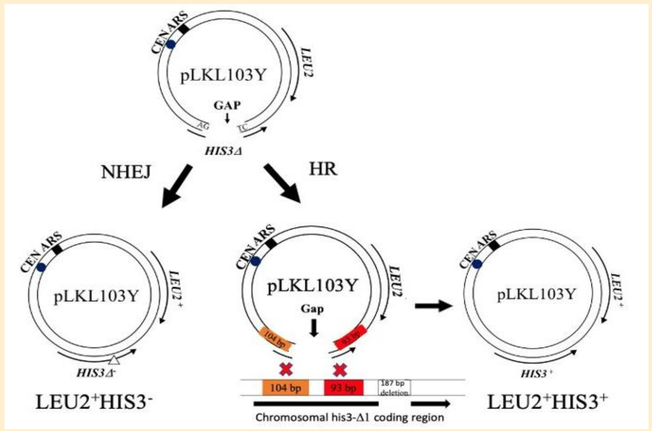

Figure 4. pLKL103Y plasmid design and assay for measuring DSB repair by both NHEJ and HR/HDR simultaneously.

The cell cycle:

G_1 → S → G_2 → M

NHEJ is High

HDR is High

New assay in log phase cells:

Strain	HDR	NHEJ
WT	68%	32%
dnl4(Nhej$^-$)	88%	12%

Figure 5. Log phase cells (mostly S+G_2) show higher DSB repair by HDR, as predicted. Nhej-defective *dnl4* mutants showed higher HDR, consistent with their reduced NHEJ repair.

Summary

- Creation of a new NHEJ assay system using a plasmid cut with BstXI
  - A new plasmid (pLKL108Y) was created that only had one site for BstXI, an enzyme that produces a DSB with 3' ssDNA overhangs
  - All three NHEJ complexes were required for efficient repair of the BstXI-induced DSB
- A new plasmid assay system that allows assessment of NHEJ and HR simultaneously was characterized
  - Repair by both pathways could be detected in Wildtype cells
  - NHEJ was reduced in NHEJ pathway-deficient *yku70* mutants
  - In future work the new system will be used to assess repair in synchronized G_1 and G_2 cells and selected DNA repair mutants

References

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