MULTIPLE DNA REPAIR PATHWAYS CONTRIBUTE TO CELL LETHALITY IN CHECKPOINT MUTANTS

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ABSTRACT

The checkpoint kinases Mec1 and Rad53 play a critical role in stabilising stalled DNA replication forks. We have conducted a genetic screen to identify mutants that render replication forks more sensitive to cell killing by the DNA damaging agent hydroxyurea (HU). We screened the yeast deletion library for mutants with heightened resistance to HU and MMS, etc. We identified several mutants in gene products involved in Base Excision Repair, Nucleotide Excision Repair, and Mismatch Repair. Mutants were retested for resistance to HU and MMS in rad53 background and all showed increased resistance to low doses of these genotoxic agents, suggesting that multiple repair pathways contribute to lethality after fork stalling. In the absence of a functional checkpoint, HU-resistance is increased when combining different repair mutations, however, none of these multiple mutants is able to rescue viability in checkpoint-deficient conditions. HU-resistance is increased when combining different repair mutants in rad53 background indicating that there are other requirements to maintain fork stability in the absence of rad53. We are currently trying to determine whether these mutants affect restart of stalled replication forks.

Possible roles of Rad53 at Replication Forks

1) Direct regulation of proteins at replication forks
2) Control of positive regulators of fork stability
3) Control of negative regulators of fork stability

A genetic screen to identify mutants able to grow in HU in checkpoint-deficient conditions

HPA - HU-resistant in high HU
HPA - HU-sensitive in high HU
HPA - HU-resistant in low HU
HPA - HU-sensitive in low HU

Conclusions:
- Different Repair Pathways contribute to lethality of rad53 cells after stalling
- BER, NER and MMR mutants
- No in HR mutants or translesion synthesis mutants
- Increased HU-resistance when combining different repair mutants in rad53 background
- Suppression in low HU, but not high HU

Model

Can replication forks restart in the absence of rad53 when repair-activities are prevented?