MULTIPLE DNA REPAIR PATHWAYS CONTRIBUTE TO CELL LETHALITY IN CHECKPOINT MUTANTS

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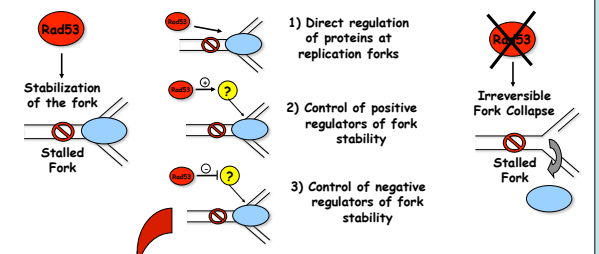
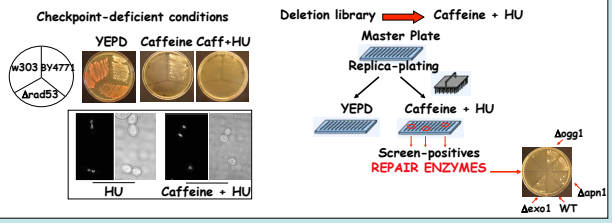
ABSTRACT

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 checkpoint-defective yeast cells more resistant to fork-stalling agents (e.g. hydroxyurea, MMS, etc). We screened the yeast deletion library for mutants with heightened resistance to hydroxyurea when the checkpoint has been compromised. From this screen 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 rad53Δ background and they 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 completely, 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.

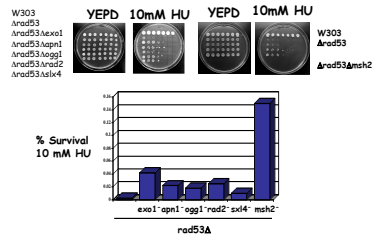
Possible roles of Rad53 at Replication Forks



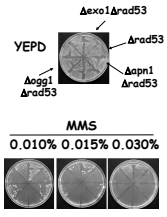
A genetic screen to identify mutants able to grow in HU in checkpoint-deficient conditions



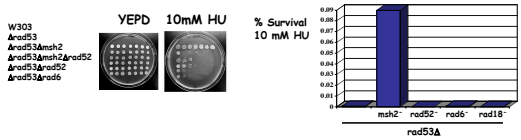
HU-resistance of BER, NER amd MMR mutants in Δrad53



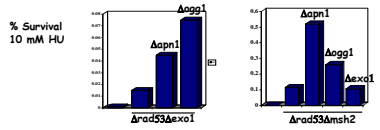
Check for MMS-resistance in repair mutants in Δrad53



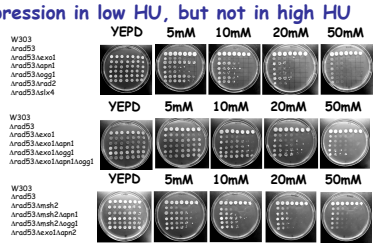
NO HU-resistance in HR and translesion synthesis mutants



Increased HU-resistance when combining different repair mutants in Δrad53 background



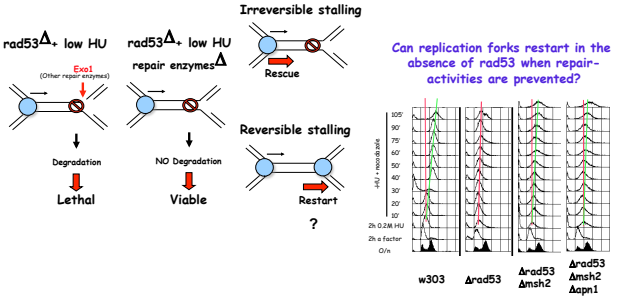
Suppression in low HU, but not in high HU



Conclusions:

- -Different Repair Pathways contribute to lethality of Δrad53 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 Δrad53 background
- -Suppression in low HU, but not high HU

Model



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