

# Role of DNA damage response in R-Loops recognition and signalling P. Pizzul<sup>1</sup>, D. Bonetti<sup>1</sup>

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### Accumulating R-Loops cells require Tel1 in presence of DNA damaging drugs



Exponentially growing cells were serially diluted (1:10) and each dilution was spotted out onto YEPD plates with or without HU or CPT.

#### Tel1 kinase activity is necessary to prevent drug sensitivity in cells with high levels of R-Loops



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DNA transcription and replication compete for the same DNA substrate. For this reason, the machineries could collide in a head-on or in a codirectional manner both causing R-Loops accumulation.

#### Saccharomyces cerevisiae strains that accumulate R-Loops



**RNA-DNA Helicases** sen1-1 R-Loop

#### The lack of Tel1 in R-Loop accumulating cells does not cause a further increase of RNA-DNA hybrids

DNA-RNA ImmunoPrecipitation (DRIP) analysis. Exponentially growing YEPD cell cultures additioned with the indicated genotoxic drug. Relative RNA-DNA hybrids enrichment in the indicated yeast strain was determined after ChIP with the S9.6 antibody and qPCR analysis at the indicated genomic loci.







#### MRX mutants show comparable phenotype to Tel1-lacking cells in presence of high levels of R-Loops

sen1-1 rad50-A78T mre11-S499P sen1-1 mre11-S499P mre11-H125N mre11-H125N sen1-1 📗



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DNA-RNA **ImmunoPrecipitation** (DRIP) analysis. Exponentially growing YEPD cell

RNA-DNA cultures. Relative hybrids enrichment the in strain indicated yeast was determined after ChIP with the S9.6 antibody and qPCR analysis at PDC1 locus.





#### **Future plans**

Genome wide investigation of R-Loops variation and Tel1 DNA-binding sites in presence or absence of CPT (camptothecin).



#### Anti-DNA-RNA Hybrid Antibody S9.6



