Genetic interaction between Mec1 and Mrc1 in response to replication stress

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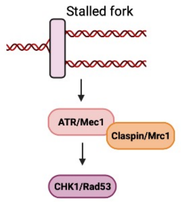
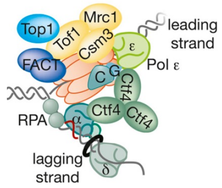
INTRODUCTION

Mec1/ATR and Mrc1/Claspin in the replication checkpoint

A.



B.

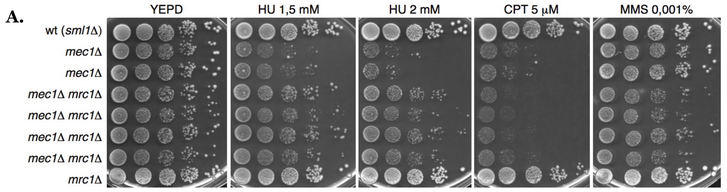


A. The replication stress, includes a set of events that interferes with the progression of the replication forks (i.e. stalled forks). A replication checkpoint whose key proteins are ATR/Mec1, Claspin/Mrc1 and CHK1/Rad53 is activated in response to replication stress.

B. Mrc1/Claspin has a role in unperturbed DNA replicatio. It interacts with components of the replisome and enhances replisome speed (Bell and Labib, 2016).

AIM OF THE PROJECT

A.



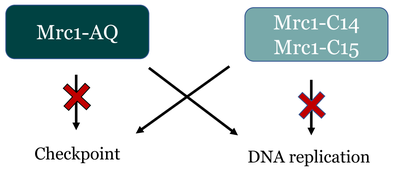
A. Exponentially growing cells with the indicated genotypes were serially diluted (1:10) and each dilution was spotted on YEPD plates with or without hydroxyurea (HU), camphothecin (CPT) or methyl methanesulfonate (MMS) at the indicated concentrations.

How does Mrc1 contribute to the HU sensitivity in *mec1Δ* cells?

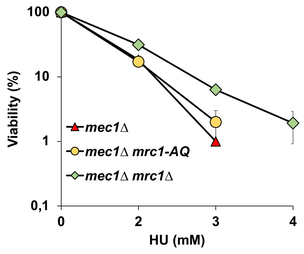
RESULTS 1

The Mrc1 replicative function contributes to HU sensitivity of *mec1Δ* cells

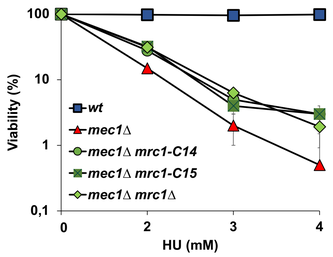
A.



B.



C.



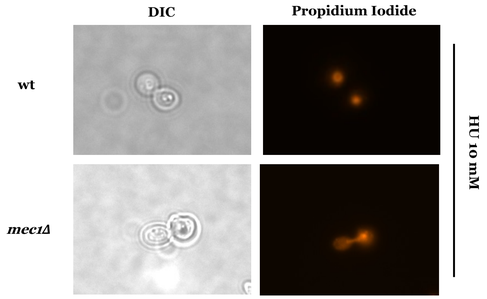
A. Schematic representation of Mrc1 variants with separated functions. The Mrc1-AQ variant is checkpoint defective, Mrc1-C14 (1-843) and Mrc1-C15 (1-903) are replication defective. Mrc1-AQ has the S/TQ residues mutated in AQ; Mrc1-C14 and Mrc1-C15 contain a deletion in the C-terminus.

B. and **C.** A defined number of cells has been plated on YEPD plates with or without HU at different concentrations. Plates were incubated 3 days at 25°C to determine the colony-forming units (CFU) .

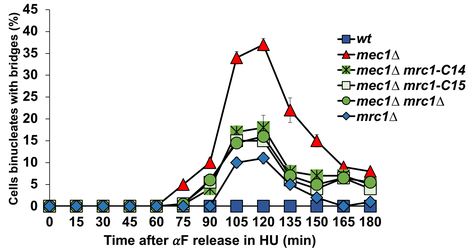
RESULTS 2

Mrc1 contributes to the formation of mitotic DNA bridges in *mec1Δ* cells

A.



B.



A. Cells were arrested in G1 with alpha factor and then released in YEPD containing HU 10 mM. Samples were treated with propidium iodide to allow nuclei visualisazion. Mitotic bridges are detectable as filamentous structures between partially divided nuclei.

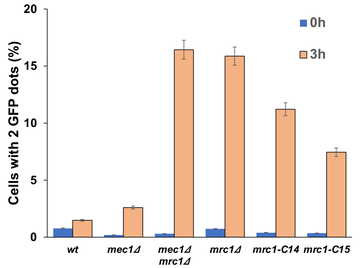
B. The percentage of binucleated cells with bridges in the presence of HU was quantified in the strains with the indicated genotypes.

RESULTS 3

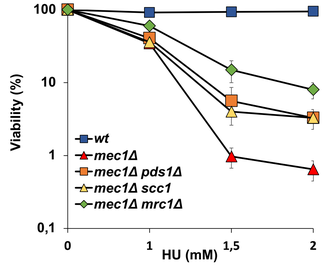
A sister chromatid cohesion defect suppresses HU sensitivity

A.

B.

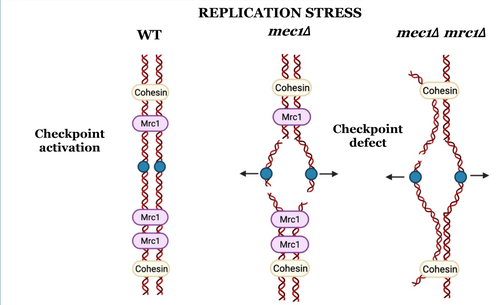


C.



A. The GFP reporter gene is inserted at chromosome VI to detect chromatid cohesion.
B. Cells were arrested in G1 with αF and then released in YEPD containing HU 10 mM and nocodazole. Sister chromatid cohesion defect was quantified counting the number of cells with 2 GFP dots after 3h.
C. A defined number of cells has been plated on YEPD plates with or without HU at different concentrations. Plates were incubated 3 days at 25°C to determine the colony-forming units (CFU) .

WORKING MODEL



References

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