Investigating the role of protein sequestration as a response to DNA damage

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Background

• Genome maintenance is critical for cell survival. Exposure to DNA damaging stressors, both external and internal, elicit a DNA damage response which is activated, mediated, and executed by proteins. A healthy proteome is therefore essential for maintaining genome integrity.

• This is achieved by an intricate system of Protein Quality Control (PQC) pathways which aim to preserve protein function and localization. Upon replicative stress, specific proteins relocalize and form aggregates within the nucleus called INQ sites. However, due to the complexity of functions performed by the proteins relocalizing to INQ, this PQC pathway remains poorly characterized.

• Here, we establish Rpd3, a histone deacetylase, as an INQ marker and study its sequestration with respect to DNA damage response (DDR) mutants. We aim to elucidate the role of the Rpd3 sequestration in DDR and answer why INQ formation occurs upon DNA damage.

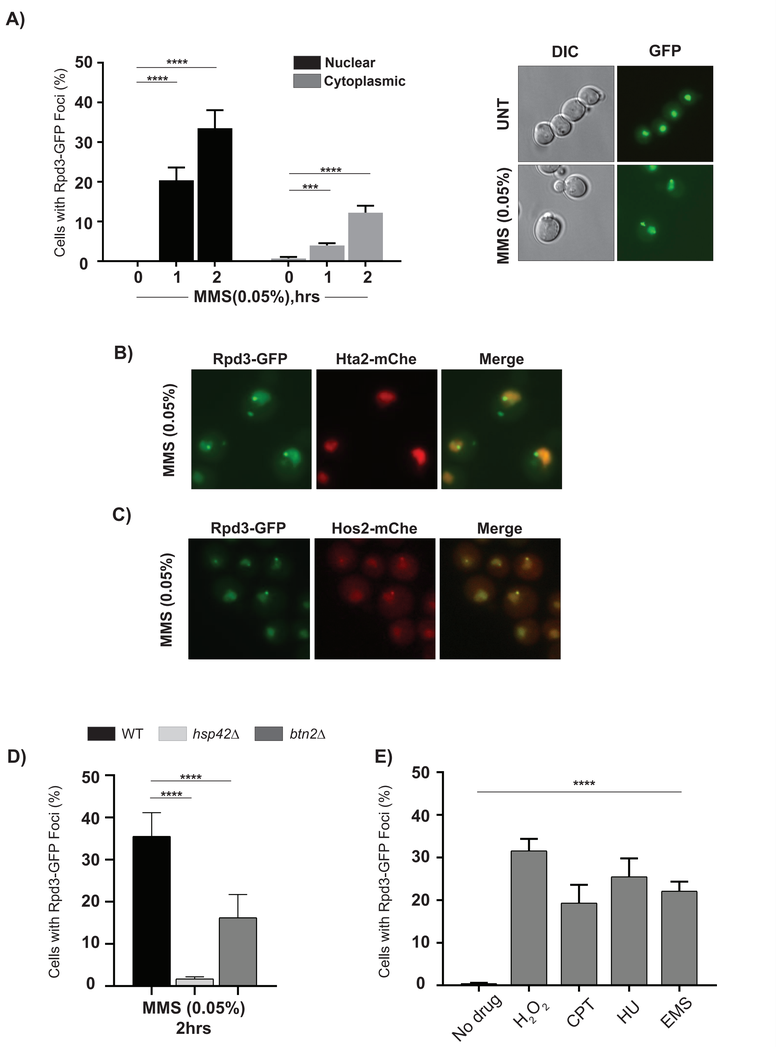

Figure 1. Characterization of Rpd3 as an INQ marker.
(A) Rpd3 aggregates form upon exposure to 0.05% Methyl methanesulfonate (MMS) which induces replicative stress. Quantification of foci is shown on the left. Representative images are shown (right). (B) and (C) Colocalization of Rpd3-GFP with Hta2-mChe and Hos2mChe respectively, under MMS treatment. (D) Deletions of compartment specific aggregases Hsp42 and Btn2 results in a significant reduction of Rpd3 INQ foci in MMS treated cells. (E) Rpd3 forms INQ with other DNA damaging agents too - H_2O_2 (2mM), Camptothecin (CPT; 25uM), Hydroxyurea (HU; 200mM) and Ethyl methanesulfonate (EMS; 0.5%) All error bars represent means ± SEM, n=3, >100 cells each. ****, p < 0.0001, ***, p<0.0002, Fisher's test.

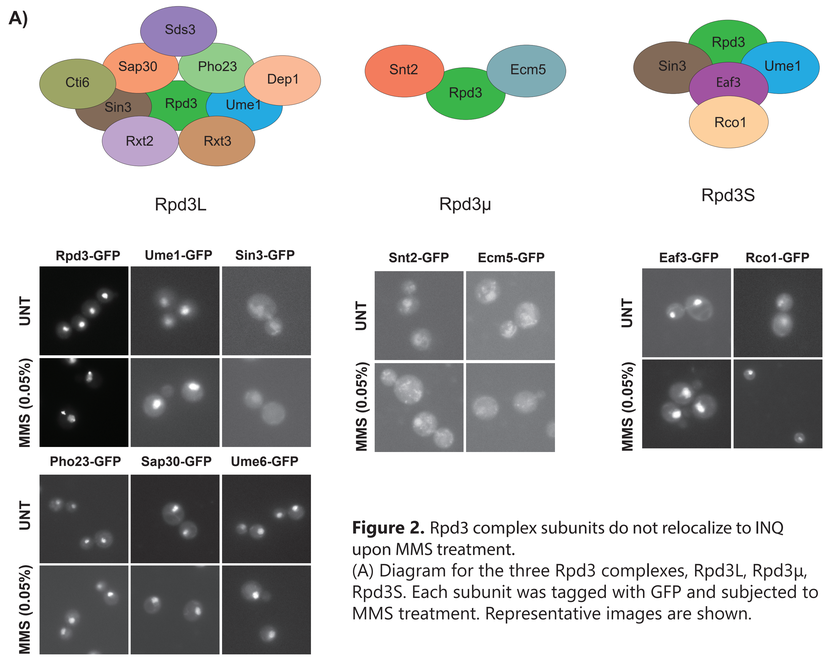

Figure 2. Rpd3 complex subunits do not relocalize to INQ upon MMS treatment.
(A) Diagram for the three Rpd3 complexes, Rpd3L, Rpd3μ, Rpd3S. Each subunit was tagged with GFP and subjected to MMS treatment. Representative images are shown.

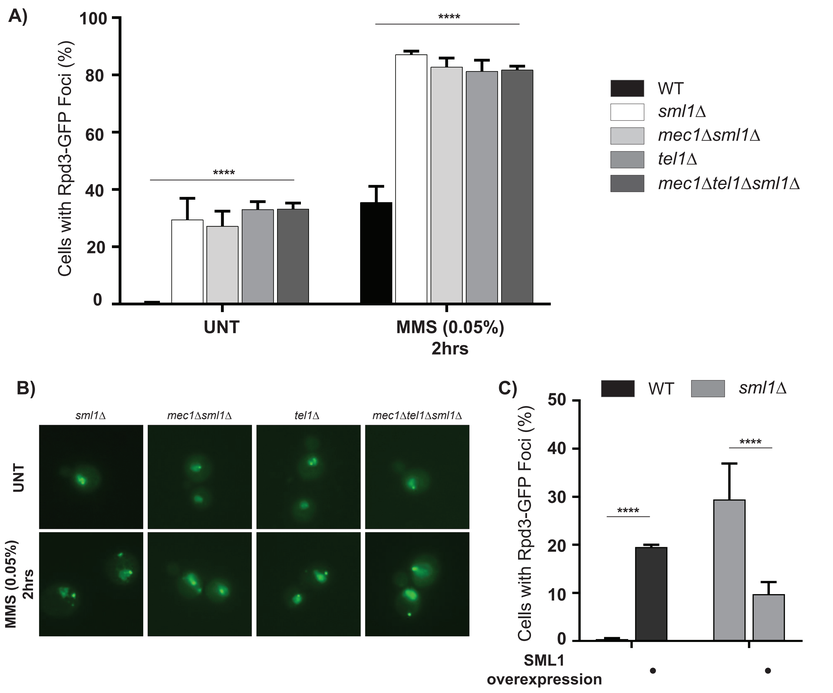

Figure 3. The DNA damage response pathway affects sequestration of Rpd3 to INQ.
(A) Deletion of kinases Mec1 and Tel1 in significantly higher Rpd3 foci in both stressed and unstressed cells.(B) Representative images are shown. (C) Overexpression of Sml1 rescues Rpd3 INQ foci in *sml1*Δ cells. Interestingly overexpression also induces foci in WT cells indicating disruption of WT level ribonucleotide levels aids in INQ formation. All error bars represent means ± SEM, n=3, >100 cells each. ****, p < 0.0001.

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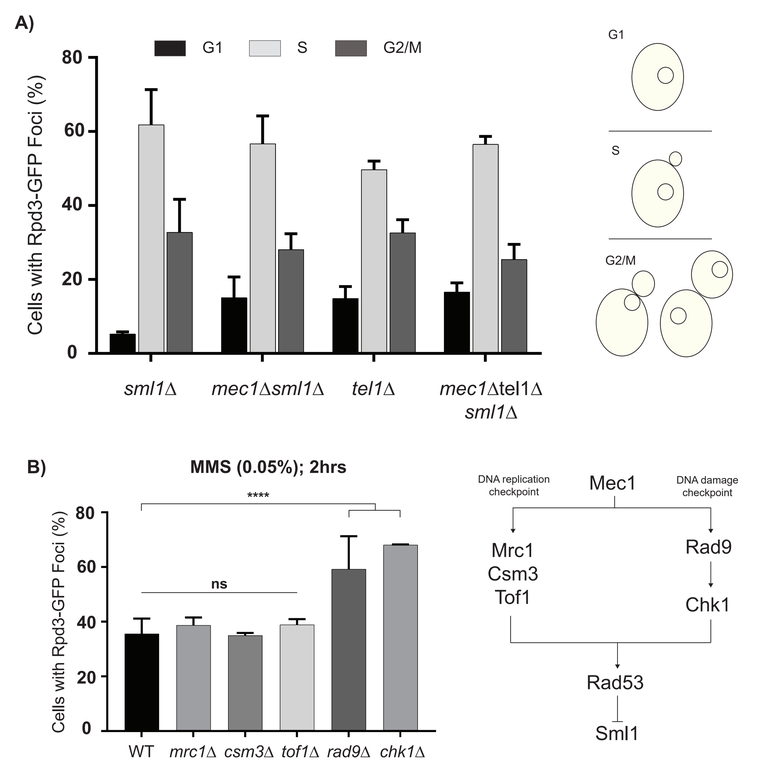

Figure 4. The DNA Damage Checkpoint prevents formation of Rpd3 INQ under stressed conditons.
(A) Budding index for Rpd3-GFP foci in different genetic backgrounds (unstressed). Spontaneous INQ formation is S-phase specific. (B) Mutations in the DNA damage checkpoint and not the DNA replication checkpoint result in the increase of Rpd3 INQ in MMS treated cells. All error bars represent means ± SEM, n=3, >100 cells each. ****, p < 0.0001, *, p < 0.03, ns, p>0.01, Fisher's test.

Conclusions:

• Rpd3 gets sequestered to INQ upon DNA damage in a chaperone-dependent manner. Live-cell imaging of the Rpd3 complexes under methylmethane sulfonate (MMS) treatment indicates that the complexes might disassemble upon DNA damage.
• Deletion of both checkpoint kinases Mec1(ATR) and Tel1(ATM) results in high levels of Rpd3 INQ in both stressed and unstressed conditions. Surprisingly, deregulation of ribonucleotide levels, both by Sml1 deletion and overexpression also increases INQ. This coupled with budding index data showing Rpd3 INQ in unstressed cells is S-phase specific suggests that Rpd3 sequestration might be dependent on high ribonucleotide levels i.e. during DNA replication.
• The DNA damage checkpoint (Rad9,Chk1) and not the DNA replication checkpoint prevents increased INQ formation under stressed conditions.

Future Directions:

• Spontaneous S-phase specific INQ in Mec1/Tel1 mutants suggests Rpd3 sequestration could be related to DNA replication. Future experiments will aim at investigating Rpd3 occupancy at early and late origins in S phase using FACS and ChIP-qPCR.
• Using different cloning strategies, we will aim to create an Rpd3 mutant which can no longer be sequestered to INQ. Cell-cycle progression and origin firing will be retested in Mec1/Tel1 mutants using an INQ-less Rpd3 mutant.

We hypothesize that Rpd3 is sequestered to INQ to initiate early firing of replicating origins in Mec1/Tel1 mutants thus explaining their fast S-phase progression phenotype.

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