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PCNA promotes cohesion establishment in a context-dependent manner

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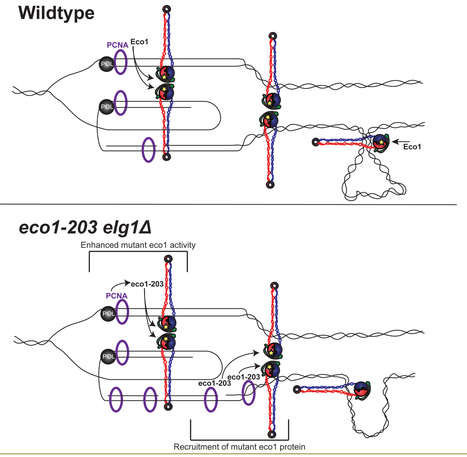
Introduction

Cell genomes undergo various structural changes throughout the cell cycle. Tethering of the same DNA molecule, *cis* tethering, ensures proper gene expression during G1, and DNA compaction during Mitosis. Tethering of two DNA molecules, *trans* tethering, maintains sister chromatid cohesion from S phase to Mitosis, and promotes DNA repair during G2/M. Cohesin is a protein complex that supports both *cis* and *trans* tethering. Once bound to DNA, cohesin must become activated through a process that requires the acetyltransferase Eco1/Ctf7. Eco1 is essential during DNA replication such that cells deficient for Eco1 activity exhibit dramatic cohesion and condensation defects [1,2]. Intriguingly, increased expression or increased retention on DNA, of the DNA replication fork processivity factor PCNA rescues *eco1* mutant cell viability and cohesion defects [1,3,4]. In combination, these studies support a model where the establishment of sister chromatid cohesion is coordinated with the process of DNA replication.

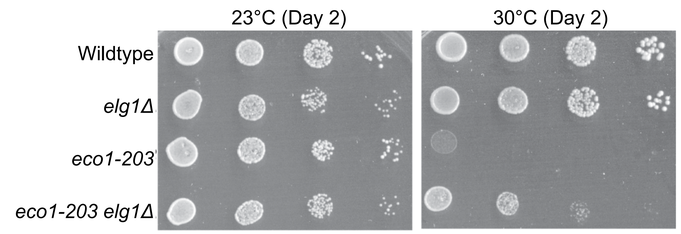
Here, we test the model that all cohesin functions are coordinated with the DNA replication fork. We confirm recent findings that elevated levels of chromatin-bound PCNA promotes Eco1-dependent Smc3 acetylation [5]. We next tested if overexpression of alternate replication factors rescue *eco1* mutant cell viability. Interestingly overexpression of the E3-ubquitin ligase component RTT101 fails to rescue *eco1* mutant cell viability, contrary to a reported study [5], as well as overexpression of Bre1. However, the overexpression of PCNA (*POL30*) rescues *eco1* mutant cell viability. We then tested if PCNA overexpression rescues the cohesion and/or the condensation defects in *eco1* mutant cells. Intriguingly, our results reveal that elevated levels of PCNA indeed rescue *eco1* mutant cell cohesion defects, but not the condensation defects, even though increased PCNA levels promote Eco1-dependent Smc3 acetylation. In combination, these findings suggest that Eco1 acetylation of Smc3, in close association with the DNA replication fork, promotes sister chromatid cohesion but that chromatin condensation occurs in a context independent of the DNA replication fork involving PCNA.



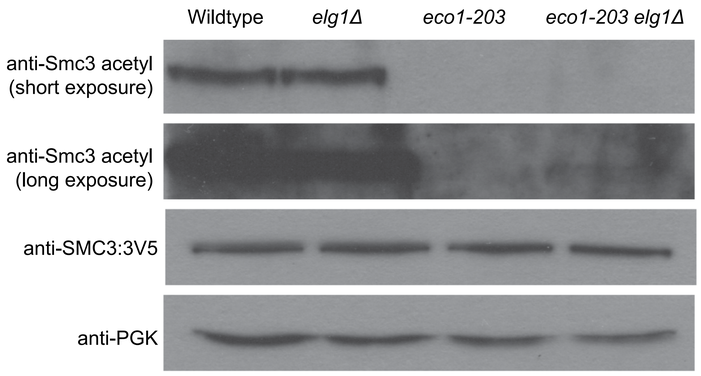
References

[1] Skibbens, et al., 1999 PMID: 9990855
[2] Guacci and Koshand, 2012 PMID: 22190734
[3] Maradeo and Skibbens, 2009 PMID: 19262753
[4] Parnas, et al., 2009 PMID: 19430531
[5] Zhang, et al., 2017 PMID: 28615292

I. Elevated levels of PCNA promotes Eco1-dependent Smc3 acetylation

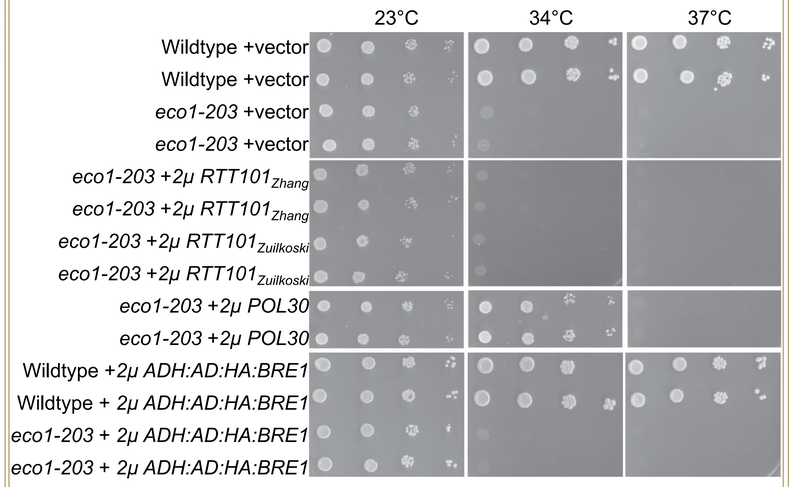


Elevated levels of PCNA (via *elg1Δ*) rescue *eco1-203* mutant temperature sensitivity.



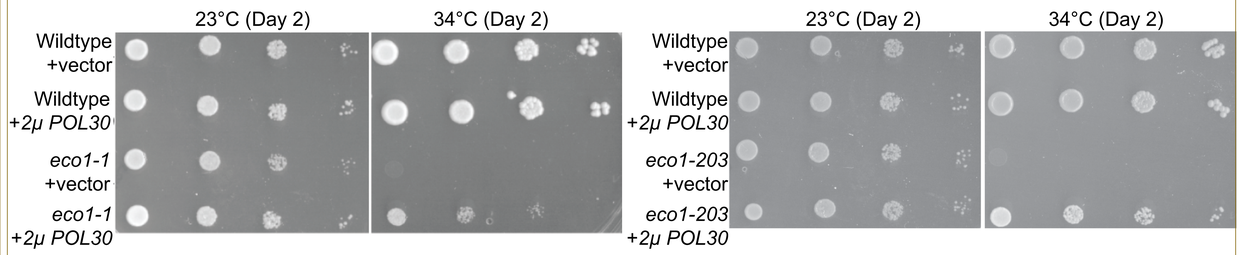
Elevated levels of PCNA (via *elg1Δ*) promotes Smc3 acetylation.

II. PCNA overexpression rescues *eco1* mutant temperature sensitivity

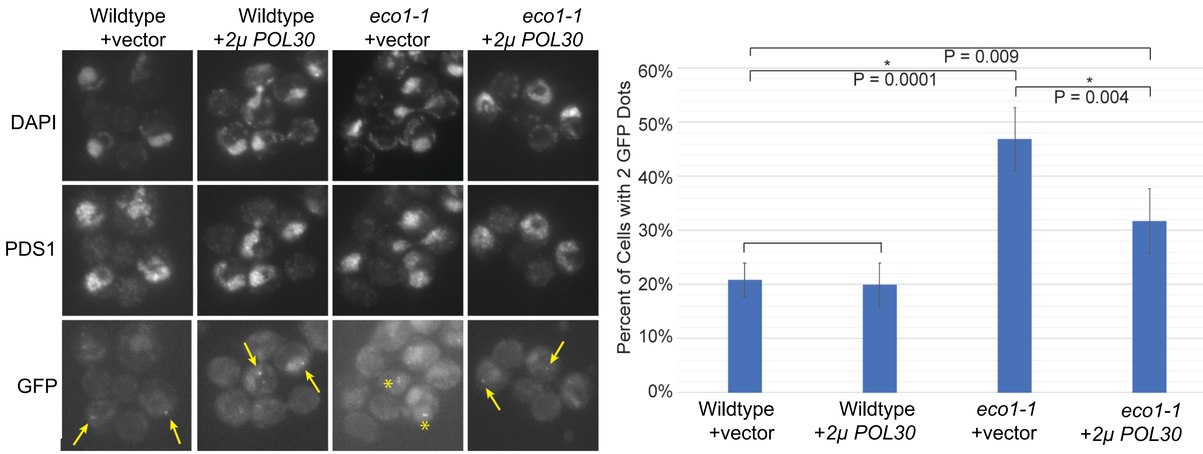


Overexpressed PCNA, and not overexpressed Bre1 or Rtt101, rescue *eco1* mutant cell temperature sensitivity. *2μ RTT101* plasmids constructed in-house (Zuilkoski) or generously provided by the Lou lab (Zhang) are indicated.

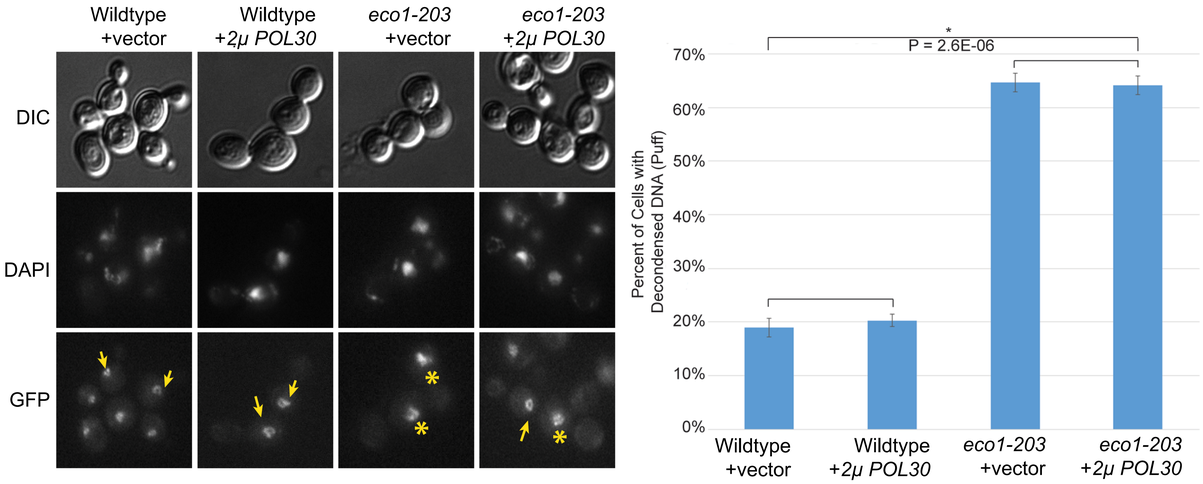
III. Overexpressed PCNA promotes sister chromatid cohesion, but not DNA condensation, in *eco1* mutant cells



PCNA overexpression (via *2μ* POL30) rescue *eco1* mutant temperature sensitivity.



Micrographs of sister chromatids (GFP, left). DNA is stained with DAPI and Pds1 is an indicator of pre-anaphase arrest. Quantification of cohesion defects in *eco1-1* cells with and without *2μ POL30* (right).



Micrographs of DNA condensation (GFP, left). DNA is stained with DAPI. Quantification of condensation defects in *eco1-203* cells with and without *2μ POL30* (right).