

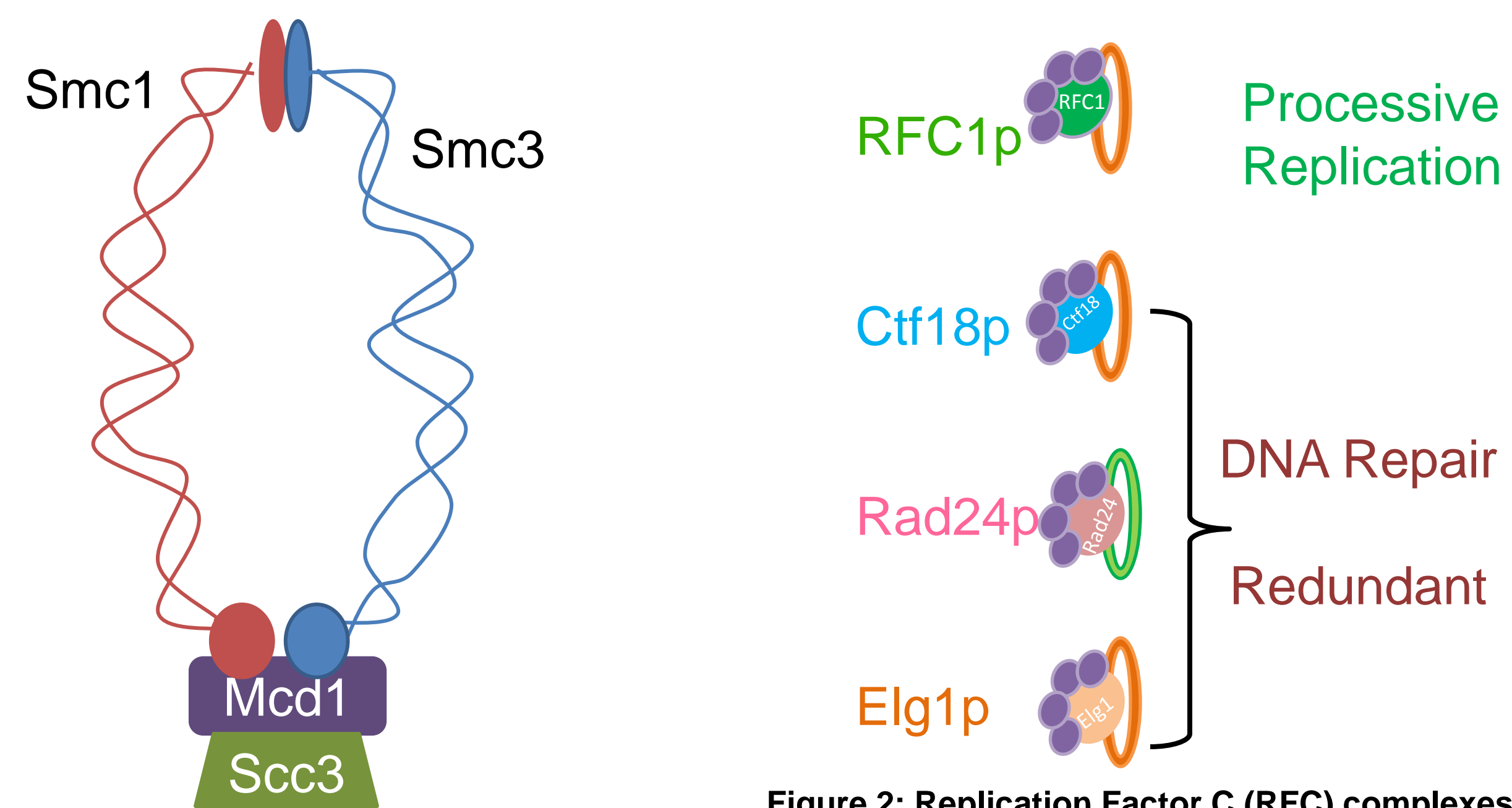
# The DNA replication fork plays a role in proper chromosome segregation

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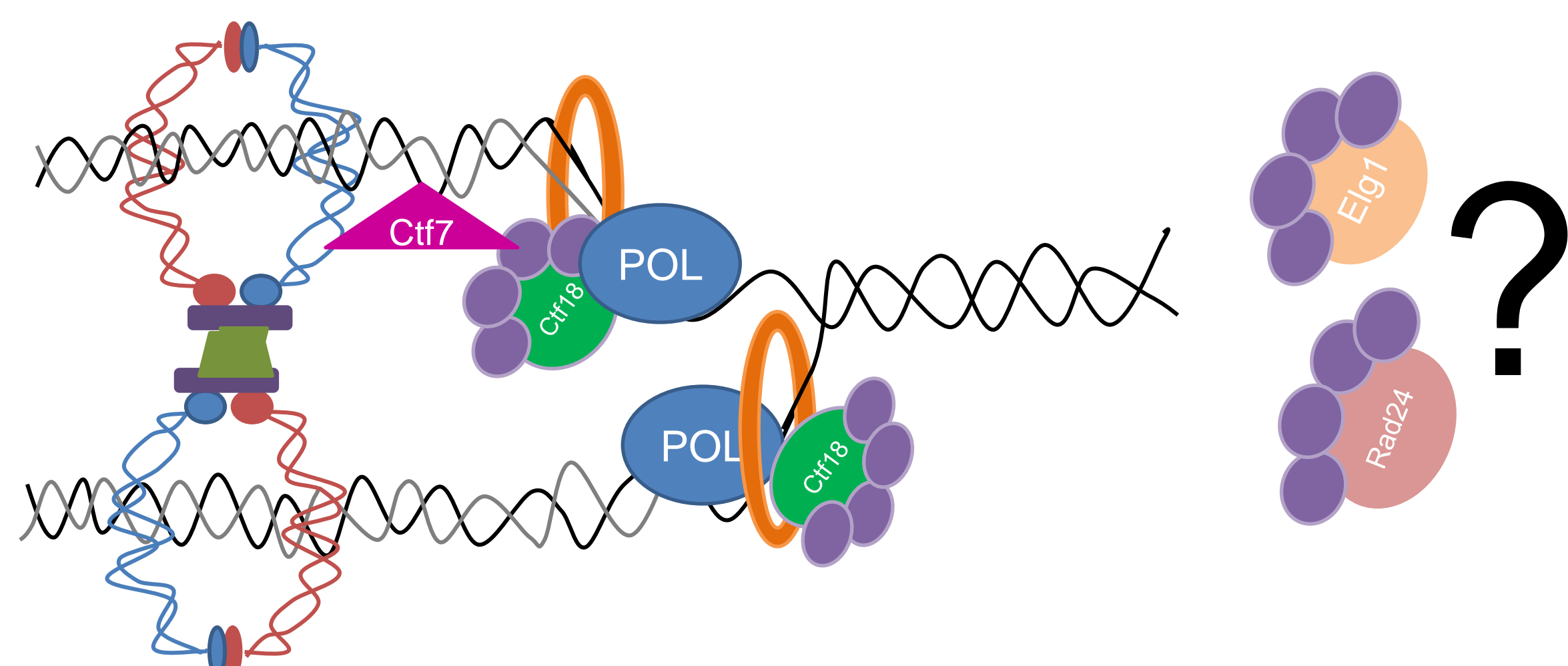
## INTRODUCTION

- Sister chromatid cohesion maintains sister identity from S phase until anaphase onset
  - Cohesins are loaded onto DNA during G1 and S phases (Figure 1).
  - Replication Factor C complexes (RFC) catalyze the loading of polymerase sliding clamps (PCNA-like molecules) onto DNA.
  - Of the four RFC complexes, Ctf18p, is the only RFC known to function in cohesion.
  - Rad24p functions in DNA damage repair while Elg1p is involved in various facets of genomic maintenance (Figure 2).
  - Cohesion is established during S phase by Ctf7p/Eco1p linking cohesion establishment to the replication fork (Figure 3).
- Here, we show a new role for RFCs in cohesion.**



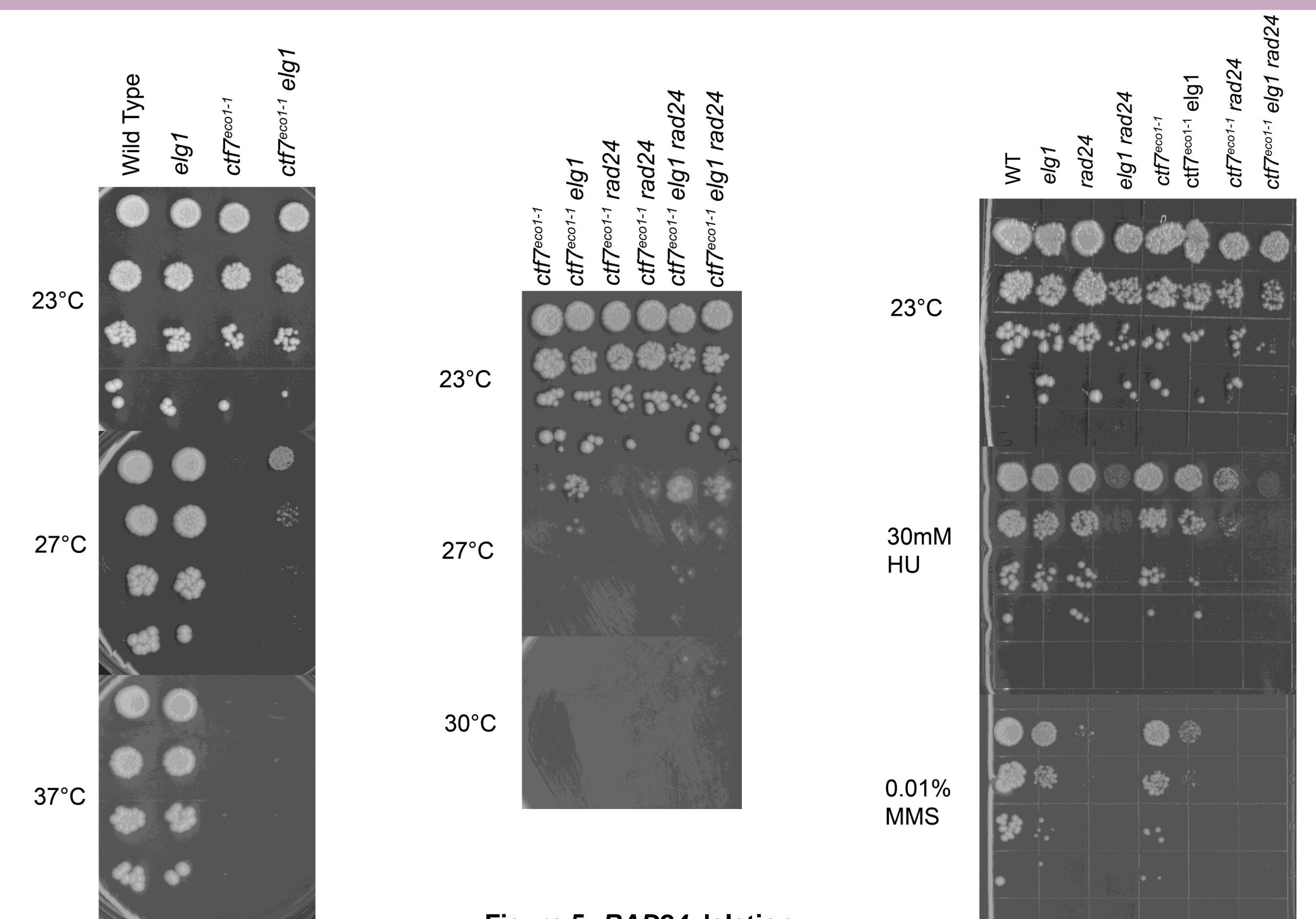
**Figure 1: Schematic of the cohesin ring.** The cohesin complex is a large proteinaceous ring made up of five subunits, Smc1, Smc3, Mcd1, Scc3 and Pds5 (not shown).

**Figure 2: Replication Factor C (RFC) complexes function in DNA replication and repair.** Rfc1 is the only essential large RFC. The RFC1 subunit of the RFC complex can be replaced with either Ctf18, Rad24 or Elg1.



**Figure 3: Model of cohesion establishment.** To date, Ctf18-RFC is the only RFC known to function in cohesion. Since Rad24 and Elg1 both have redundant functions to Ctf18, we propose that Elg1 and Rad24 also play a role in cohesion establishment.

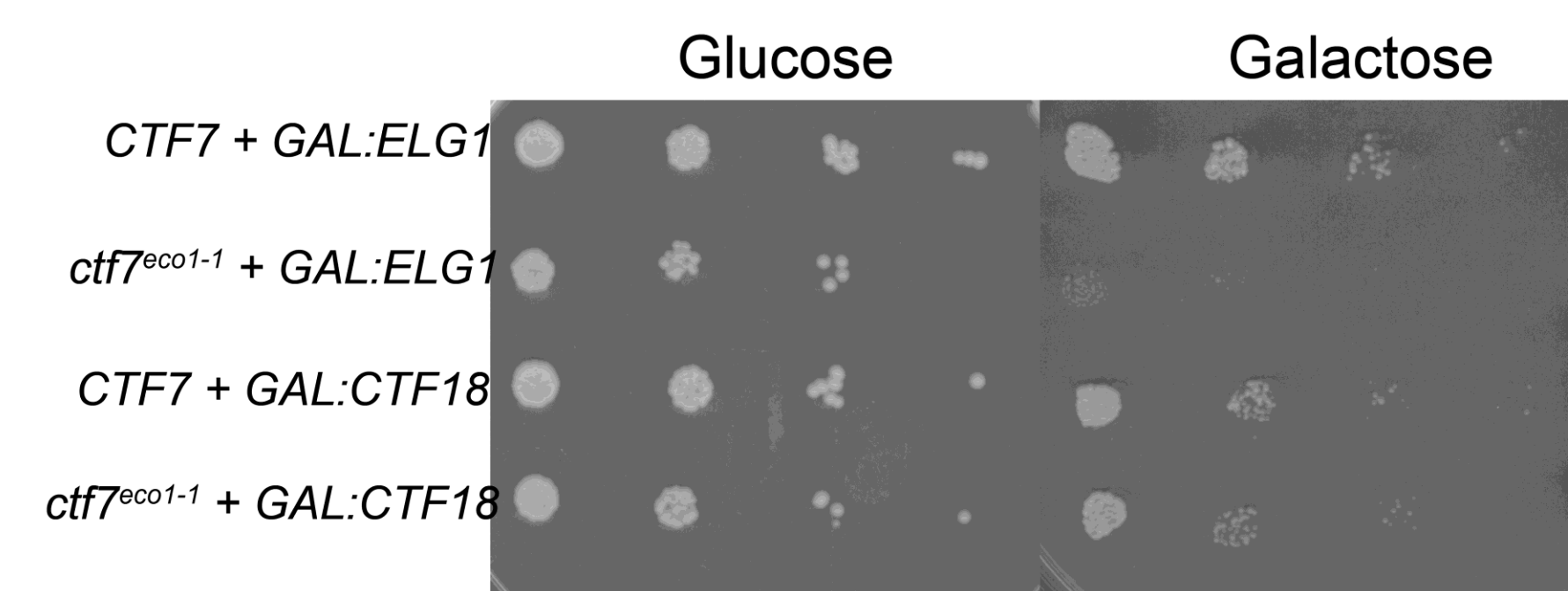
## RESULTS



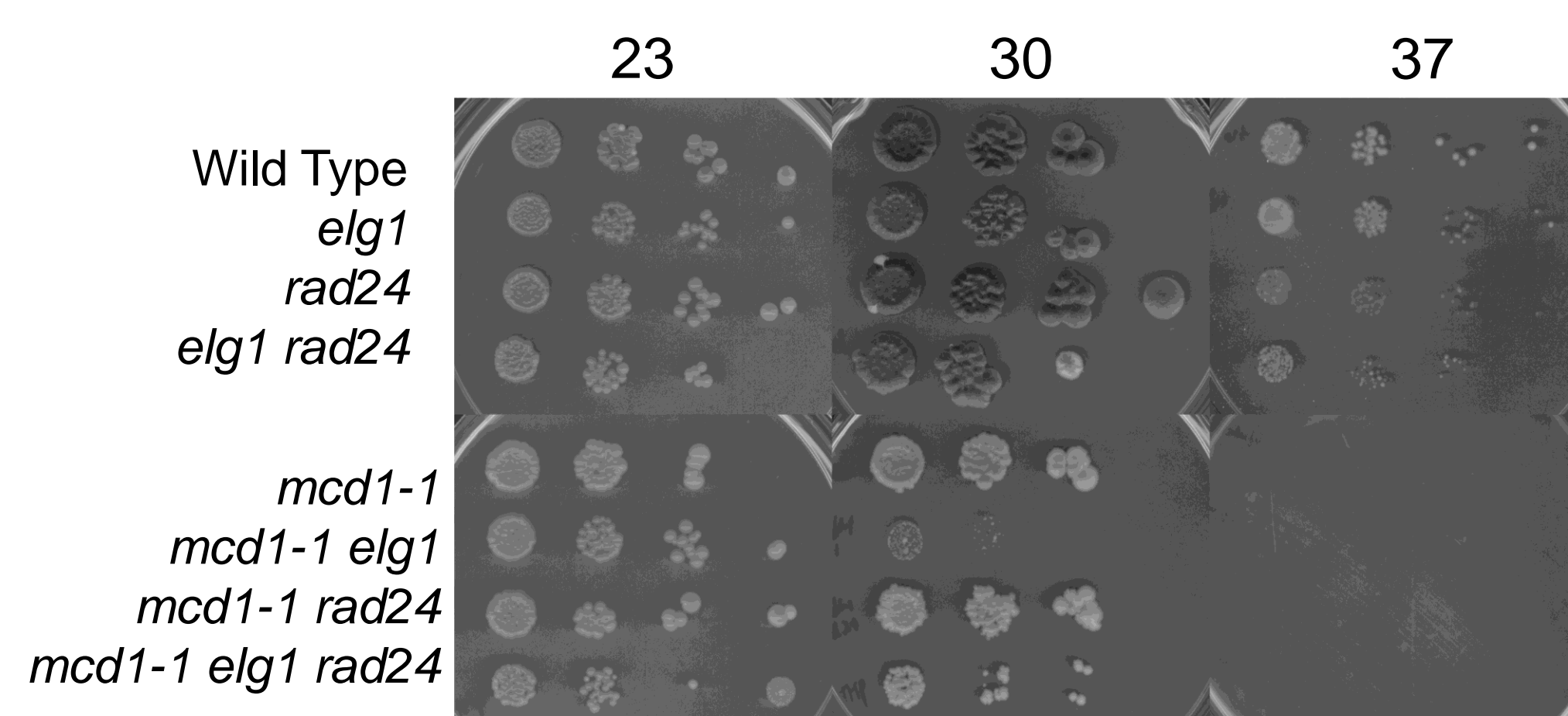
**Figure 4: ELG1 deletion suppresses *ctf7* mutant cell conditional growth.** 10-fold serial dilutions of wildtype, *ctf7* and *elg1* single mutant strains compared to *ctf7 elg1* double mutant strains. Colony growth shown for cells on rich medium plates maintained at 23, 27 and 37 for 7 days

**Figure 5: RAD24 deletion neither rescues nor exacerbates *ctf7* mutant cell conditional growth.** 10-fold serial dilutions of *ctf7* single mutant strains compared to *ctf7 elg1* and *ctf7 rad24* double mutant strains as well as to *ctf7 elg1 rad24* triple mutant cells. Colony growth shown for cells on rich medium plates maintained at 23, 27 and 30 for 7 days.

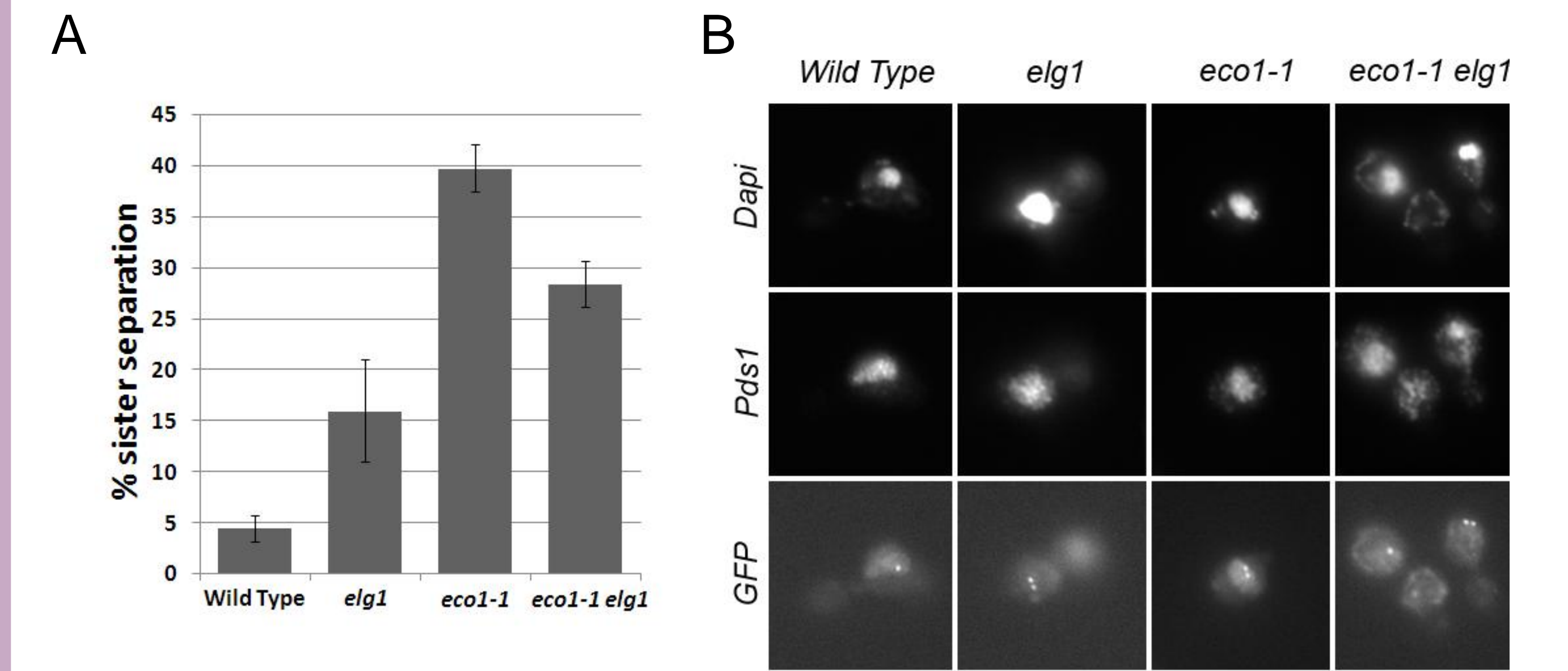
**Figure 6: Ctf7p function in DNA repair.** 10-fold serial dilutions of *ctf7*, *elg1* and *rad24* single mutant strains, *elg1 rad24*, *ctf7 elg1*, *ctf7 rad24* double mutant strains and *ctf7 elg1 rad24* triple mutant strains grown at 23 on rich medium and medium supplemented to either 30 mM hydroxyurea (HU) or 0.01% methyl methyl sulfanate (MMS).



**Figure 7: ELG1 over-expression reduces *ctf7* mutant cell growth.** 10-fold serial dilutions of wildtype and *ctf7* mutant cells harboring plasmid from which either *ELG1* or *CTF18* expression is repressed (Glucose) or elevated (Galactose).

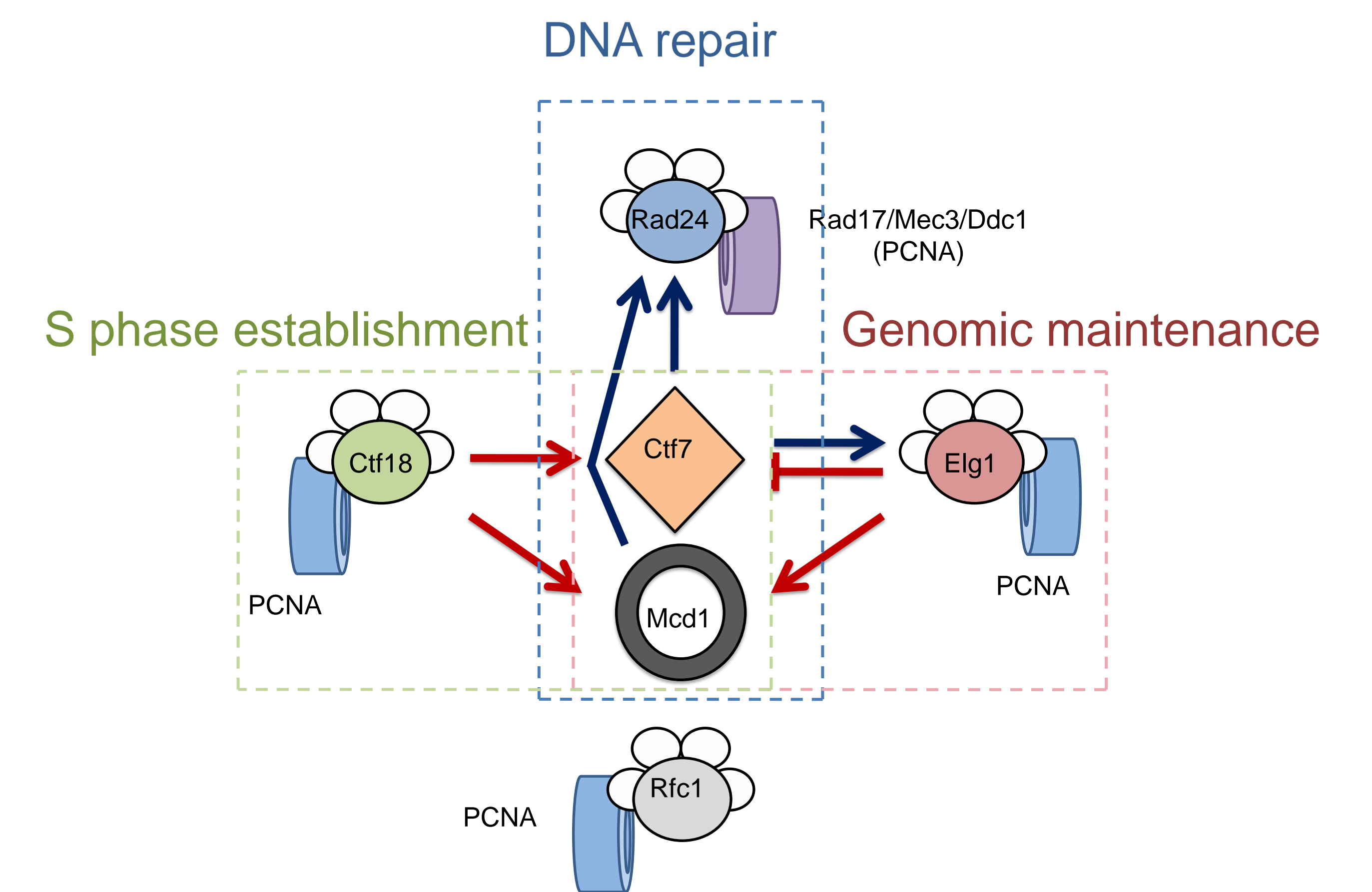


**Figure 8: ELG1 deletion, but not RAD24 deletion, exacerbates *mcd1-1* mutant cell conditional growth.** 10-fold dilutions for each single, double and triple mutant strain are shown (see text). Colony growth shown on rich medium plates maintained at 23, 30 and 37 for 7 days.



**Figure 9: Role of ELG1 deletion in cohesion.** A) Quantification of cohesion defects exhibited by wild type, *ctf7* and *elg1* single mutant strains and *ctf7 elg1* double mutant strains arrested prior to anaphase. Error bars represent standard deviation. B) Micrographs of wild type, *ctf7* and *elg1* single mutant strains and *ctf7 elg1* double mutant strains in which sister chromatid loci (GFP) and Pds1p (Pds1) are visualized within the DNA mass (DAPI).

## CONCLUSIONS



**Figure 10: Schematic highlighting RFC interactions.** Ctf18-RFC and Elg1-RFC exhibit separate and distinct activities relative to Ctf7p-dependent cohesion establishment but both promote Mcd1p-dependent cohesion maintenance. Ctf7p and Mcd1p both promote the Rad24-RFC DNA damage response pathway. Note that Ctf18-RFC, Elg1-RFC, Rfc1-RFC and Rad24-RFC all participate in PCNA clamp dynamics while Rad24-RFC functions exclusively in Rad17/Mec3/Ddc1 clamp dynamics. Cohesion-based pathways are shown in red, DNA damage repair pathways are shown in blue. Dashed boxes indicate RFC cellular roles.

## ACKNOWLEDGEMENTS

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