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Epitope tag-induced synthetic lethality between cohesin subunits and Ctf7/Eco1 acetyltransferase

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ABSTRACT

Ctf7/Eco1-dependent acetylation of Smc3 is essential for sister chromatid cohesion. Here, we use epitope tag-induced lethality in cells diminished for Ctf7/Eco1 activity to map cohesin architecture in vivo. Tagging either Smc1 or Mcd1/Scc1, but not Scc3/Irr1, appears to abolish access to Smc3 in *ctf7/eco1* mutant cells, suggesting that Smc1 and Smc3 head domains are in direct contact with each other and also with Mcd1/Scc1. Thus, cohesin complexes may be much more compact than commonly portrayed. We further demonstrate that mutation in *ELG1* or *RFC5* anti-establishment genes suppress tag-induced lethality, consistent with the notion that the replication fork regulates Ctf7/Eco1.

1. Introduction

Cohesins tether together sister chromatids from early S-phase until anaphase onset and are essential for both high fidelity chromosome transmission and transcription regulation. Cohesin complexes consist of Smc1, Smc3, Mcd1/Scc1 (herein Mcd1) and Scc3/Irr1 and accessory factors Pds5 and Rad61/WAPL [1]. While many of the components that make up the cohesin complex are known, the structure of this complex in vivo remains under intense debate. Based on early electron microscopy (EM) studies and subsequent biochemical assays, a popular model of cohesin is that Smc1 and Smc3 dimerize by hinge–hinge interactions and that their separated head domains are bridged by Mcd1. From this, a huge triangular ring is posited to result with in an inner diameter of 35 nm [2,3]. More recent Atomic Force Microscopy, Fluorescence Resonance Energy Transfer (FRET) and EM studies suggest that Smc1 and Smc3 head domains may remain in contact with one another and that their coiled-coil domains fold back to form shortened structures in which the ATPase heads are positioned closely to the hinges [4–6]. These subunit interactions provide for numerous configurations including one ring, bracelets, snaps, two-ring hand-cuffs and C-clamps [7–12].

A major obstacle in discerning the physiologically relevant cohesin architecture in vivo is that there is evidence supporting both one ring and two ring models. Both models rely heavily on analyses of epitope-tagged subunits. Intriguingly, these studies reveal that alternate tags produce cohesin complexes of different biochemical properties [3,5,13]. A critical issue thus becomes the extent to which these tagged cohesin proteins give rise to complexes that behave like wild type complexes in vivo. In either case, chromatid-bound cohesins must be converted to a pairing competent state by the establishment factor Ctf7/Eco1 (herein Ctf7). Ctf7 is an acetyltransferase that modifies Smc3 to produce sister chromatid pairing – linking establishment and cohesin architecture to sister chromatid pairing [14].

Here, we pursue a novel assay to demonstrate in vivo that epitope-tagged cohesin subunits previously used to support one-ring embrace models are unfit when challenged genetically in vivo. We uncovered cohesin tag-induced lethality in cells diminished for Ctf7 acetyltransferase activity. In turn, we exploited this in vivo cohesin architecture assay to ascertain subunit domains proximal to the site of Smc3 acetylation and thus potential regulators of Ctf7-dependent acetylation reactions. In addition, we demonstrate the molecular basis of anti-establishment factors (Rfc5 and Elg1) in regulating sister chromatid pairing reactions [15,16, Maradeo, Garg and Skibbens, submitted for publication].

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2. Materials and methods

2.1. Strain construction

All strains in this study are W303 background (Table 2). To generate Smc1-13MYC integration product PCR was preformed using primers TTGACCTATATAGATATTATTAGTTATTTGACGGGTTATAGCAGAGGTTGGTTTCATAGAGAATTCGAGCTCGTTTAAAC and AGACAGCAACAAGAAAACTCGTCGAAGATCATAACTTTGGACTTGAGCAATTACGCAGAACGGATCCCCGGGTTAATTAA with pFA6a-13MYC-KANMx6 Longtine vectors.

2.2. Genetic analysis

Diploid strains were sporulated in 0.3% potassium acetate and tetrads dissected on YPD agar media. The genotypes of the resultant spores were analyzed for wild type, single, double, triple and quadruple mutants. Isolated spores were grown at log phase in YPD followed by 10-fold serial dilutions and then maintained on YPD agar plates at a range of temperatures. For Table 1A, first *MCD1-18MYC elg1* mutant cells were crossed to *eco1-1 rfc5-1* mutant cells, sporulated, dissected and genotypes analyzed. No *MCD1-18MYC eco1-1* double mutant strains were recovered but both *MCD1-18MYC eco1-1* rfc5-1 and *MCD1-18MYC eco1-1* elg1 triple mutants were obtained. To bias the recovery of a *MCD1-18MYC eco1-1* double mutant, *MCD1-18MYC elg1* was crossed to *MCD1-18MYC eco1-1 rfc5-1*, sporulated, dissected and genotypes analyzed.

Table 1A
mcd1:myc rfc5-1 ctf7^{eco1-1} X mcd1:myc elg1.

Genotype	Observed	Expected
mcd1:myc	11	15
mcd1:myc elg1	10	15
mcd1:myc rfc5-1	11	15
mcd1:myc ctf7^{eco1-1}	0	15
mcd1:myc elg1 rfc5-1	17	15
mcd1:myc elg1 ctf7^{eco1-1}	13	15
mcd1:myc rfc5-1 ctf7^{eco1-1}	9	15
mcd1:myc elg1 rfc5-1 ctf7^{eco1-1}	13	15
Dead	36	0
Total	120	

3. Results and discussion

Sister chromatid cohesion, via conversion of cohesins to a pairing competent state, is established during S phase by Ctf7-dependent Smc3 acetylation [17,18]. Numerous studies employ cohesin-tagged subunits to characterize cohesin structure and acetylation. However, studies that challenge the physiological relevance of these constructs in vivo are severely limited. In the process of generating new strains to further characterize cohesion and acetylation regulation, we discovered that C terminally MYC-tagged Mcd1 is lethal when combined with *ctf7* alleles. Strains containing *MCD1-MYC* were crossed to cells containing *ctf7^{eco1-1}*, sporulated, dissected and the resulting tetrads analyzed. Of an expected 24 *MCD1-MYC ctf7^{eco1-1}* spores, no viable isolates were recovered (Table 1A, data not shown). In contrast, *MCD1-MYC* expression is not lethal in combination with other cohesion mutant strains (*scc2*, *scc4* or *pds5*), obviating arguments that the lethality reported here is simply based on compounded cohesion defects [19,20]. Nor is MCD1-HA expression lethal in *ctf7* mutant strains, augmenting the specificity of the synthetic lethality reported here [21]. These results reveal that *mcd1-myc* is a severe but cryptic mutant allele of *MCD1* and that the MYC-based C-terminal extension specifically diminishes the ability of Ctf7/Eco1 to acetylate Smc3 – the only known essential substrate for Ctf7 acetylation.

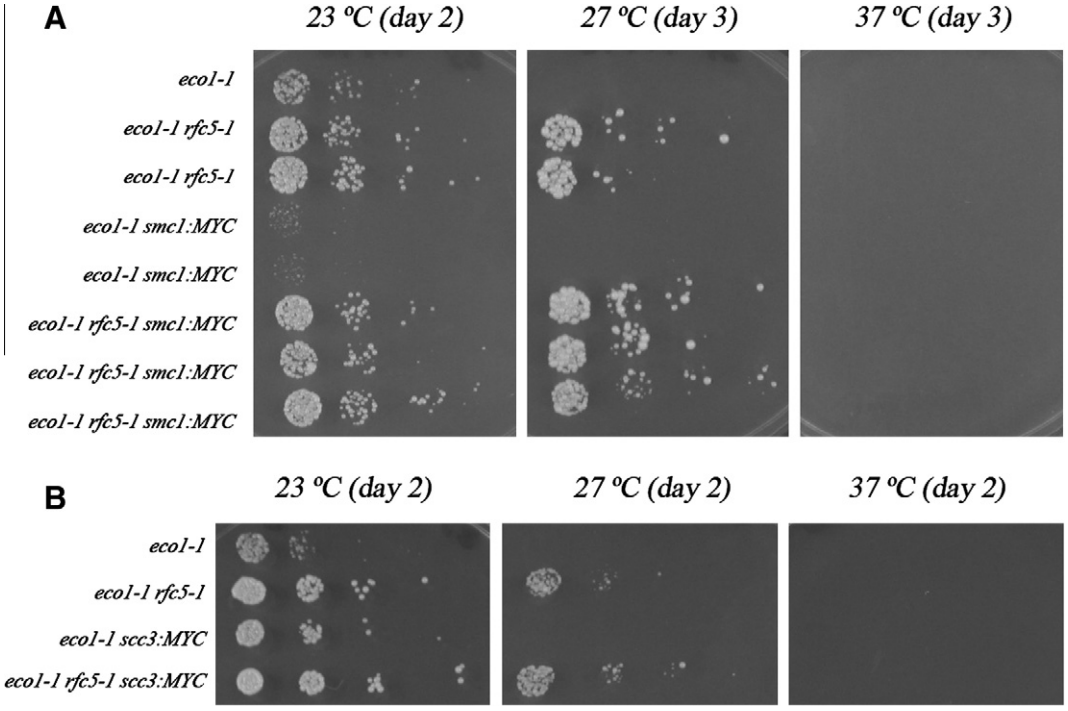

Fig. 1. (A) *SMC1-13MYC* exhibits conditional growth defects in combination with *ctf7^{eco1-1}* mutant cells that can be rescued with mutation in *rfc5*. 10-fold serial dilutions of single, double and triple mutant cells. Colony growth shown for cells on rich medium plates grown at 23, 27, and 37 °C for number of days indicated. (B) *SCC3-18MYC ctf7^{eco1-1}* mutants exhibit no conditional growth phenotypes. 10-fold serial dilutions of single, double and triple mutant cells. Colony growth shown for cells on rich medium plates grown at 23, 27, and 37 °C for number of days indicated.