CELL CYCLE
https://doi.org/10.1080/15384101.2020.1804221

Taylor & Francis
Taylor & Francis Group

RESEARCH PAPER

PCNA promotes context-specific sister chromatid cohesion establishment separate from that of chromatin condensation

Caitlin M. Zuilkoski and Robert V. Skibbens

Department of Biological Sciences, Lehigh University, 18015, Bethlehem, Pennsylvania, USA

ABSTRACT

Cellular genomes undergo various structural changes that include *cis* tethering (the tethering together of two loci within a single DNA molecule), which promotes chromosome condensation and transcriptional activation, and *trans* tethering (the tethering together of two DNA molecules), which promotes sister chromatid cohesion and DNA repair. The protein complex termed cohesin promotes both *cis* and *trans* forms of DNA tethering, but the extent to which these cohesin functions occur in temporally or spatially defined contexts remains largely unknown. Prior studies indicate that DNA polymerase sliding clamp PCNA recruits cohesin acetyltransferase Eco1, suggesting that sister chromatid cohesion is established in the context of the DNA replication fork. In support of this model, elevated levels of PCNA rescue the temperature growth and cohesion defects exhibited by *eco1* mutant cells. Here, we test whether Eco1-dependent chromatin condensation is also promoted in the context of this DNA replication fork component. Our results reveal that overexpressed PCNA does not promote DNA condensation in *eco1* mutant cells, even though Smc3 acetylation levels are increased. We further provide evidence that replication fork-associated E3 ligase impacts on Eco1 are more complex that previously described. In combination, the data suggests that Eco1 acetylates Smc3 and thus promotes sister chromatid cohesion in context of the DNA replication fork, whereas a distinct cohesin population participates in chromatin condensation outside the context of the DNA replication fork.

ARTICLE HISTORY
Received 5 June 2020
Revised 8 July 2020
Accepted 24 July 2020

KEYWORDS
Cohesin; cohesion; condensation; dna replication fork; pcna; elg1; eco1/Ctf7/Esco2

Introduction

Replicated sister chromatids are tethered together, termed cohesion, from S phase until chromosome segregation during anaphase. Loss of sister chromatid cohesion before anaphase is highly detrimental to the cell, leading to mis-segregation of the sister chromatids, aneuploidy, and cell death [1–8]. In addition to the *trans* tethering that occurs between sister chromatids, DNA segments must also be tethered in *cis* – intramolecular tetherings that are critical for proper gene expression during G1 and chromosome compaction during mitosis [1,9,10,11,12,13,14]. Loss of *cis* tethering further results in architectural chromatin defects that globally impact genome organization and transcription regulation [15–19].

A highly conserved protein complex, termed cohesin, ensures both *trans* and *cis* tethering. A minimal cohesin complex is comprised of three subunits: Smc1, Smc3, and Mcd1/Scc1/Rad21, but auxiliary factors (Scc3/Irr1/SA1,2 and Pds5) bind and help regulate cohesin dynamics [1,2,4,20–22]. Cohesin complexes are loaded onto chromatin throughout most of the cell cycle by the Scc2,4 deposition complex, but the cohesin population loaded during S phase is essential for proper cohesion establishment [5,23–27]. An additional factor, Eco1/Ctf7 (herein referred as Eco1) is required for, and controls, cohesion establishment [3,4]. Eco1 (ESCO1/EFO1 and ESCO2/EFO2 in higher eukaryotes) is an acetyltransferase that targets lysines 112 and 113 (K105,106 in humans) of Smc3 [3,4,28–29,30,31,32]. Eco1-dependent Smc3 acetylation is tightly coordinated with DNA replication, a model supported by three lines of evidence. First, elevated levels of the DNA polymerase processivity factor PCNA rescue *eco1* mutant cell inviability [3]. Second, deletion of the RFC subunit ELG1, whose primary function is to remove PCNA from chromatin following Okazaki

CONTACT Robert V. Skibbens, rvs3@lehigh.edu
The supplemental data for this article can be accessed here.

fragment maturation, results in retained chromatin-bound PCNA that similarly rescues *eco1* mutant cell conditional growth [33,34]. Third, Eco1 binds PCNA directly, providing a mechanism through which Eco1 is recruited to the replication fork [35,36]. More recent studies suggest that Eco1 recruitment may be supported by additional DNA replication fork-associated components [37,38,39,40]. Once acetylated, cohesin appears largely refractory to destabilizing factors such as Rad61/WAPL [41–43].

Mutations in *ECO1*, and mutations in all cohesin genes tested thus far, typically exhibit both cohesion and condensation defects [1,2,3,4,44–46]. Surprisingly, there is evidence that these cohesin functions may be separable. For instance, deletion of the anti-establishment factor *RAD61* rescues the condensation defects, but not the cohesion defects, found in *eco1* mutant cells [47]. *RAD61* deletion similarly rescues the condensation, but not cohesion, defects that arise in *pds5* mutant cells [48]. The extent to which DNA replication factors such as PCNA, which is an essential regulator of Eco1 function, rescue *eco1* mutant cell condensation defects remains untested – revealing a major deficit in our understanding regarding how fundamental aspects of chromosome biology are coordinated. Here, we test this model and provide exciting evidence that cohesin modifications that promote condensation likely occur in a temporal and spatial context that is distinct from that which promotes sister chromatid cohesion. These findings require revision of current models of Eco1 function and mechanisms through which DNA metabolism proceeds.

Materials and methods

Yeast strains

All yeast strains used in this study were performed in a W303 background strain except for yeast strains noted with an asterisk (*) – which are a S288C background Table 1. Table 2 lists bacterial plasmids used in this study. NET1 was genetically modified as previously described [49]. Primers used to C-terminal tag NET1 with GFP are (forward primer) 5′-TTTAGGTAAGAAGAAGAAGCCAAGTGGTGGATTTGCATCATTAATAAAAGATTTCAAGAAAAAACGGATCCCCGGGTTAATTAA-3′ and (reverse primer) 5′-TGCTTGATTATTTTTTTTTACTAGCTTTCTGTGACGTGTATTCTACTGAGACTTTCTGGTATCAGAATTCGAGCTCGTTTAAAC-3′ into yeast strains YBS255 and YBS514. The resulting yeast strains were transformed with either CEN vector or *2μ POL30* plasmid to obtain the resulting yeast strains (YCZ008, YCZ010, YCZ014, and YCZ016). To construct kanamycin tagged Eco1 and *eco1-203* single mutant yeast strains, PCR fragments were generated by using primers (forward primer) 5′-CAGGAGGTCCATTCCAGGGAAT-3′ and (reverse primer) 5′-TGTCCCTTCTCGTGTCTTT-3′ genetic template of YBS1991 and YBS1994. The resulting fragments were transformed into a wildtype W303 background strain (YCZ238) and integration confirmed using primers (forward primer) 5′-CAGGAGGTCCATTCCAGGGAAT-3′ and (reverse primer) 5′-CCAACCTTCTACGGCGAATA-3′. To obtain *KAN:ECO1 elg1::TRP* and *KAN:eco1-203 elg1::TRP* double mutant cells, *elg1::TRP* template was generated as previously described [49]. Primers for *ELG1* knockout are (forward primer) 5′-AGAGAAGGTTTTCCAATGAAAAGGCACGTGTCTTTATCTGATATATTGACAGGAAATAAGCGGATCCCCGGGTTAATTAA-3′ and (reverse primer) 5′-ATTTCCCCGCACTACCATGCTATATTTATTATACATACGTGTTCCTGTAACGATGCACGCGAATTCGAGCTCGTTTAAAC-3′. The resulting fragment was transformed into *KAN:ECO1* cells (YCZ237) or *KAN:eco1-203* cells (YCZ240) and integration confirmed using primers 5′-TCTTCACTGACCACCTTCGCT-3′ (upstream 5′ *ELG1*) and 5;-AACTGCATGGAGATGAGTGGT-3′ (*TRP1*). V5 tagged Smc3 strains were created using EU3430-9A, generously given by Dr. Douglas Koshland. Primers used to amplify the SMC3:5:HIS region, primers are (forward primer) 5′-TTAACGCGGTTGATTTCTACTTTCCAAAAGGTTTCTGAAAA-3′and (reverse primer) 5′-TAGCTCTGATTCTGACTCTAACTCCAGTTCGGACTCCGTATCGGATTCCAGTTCAGATTC-3′. The resulting fragment was transformed into

Table 1. Yeast strains used in this study.

Strain	Genotype	Reference
EU3430-9A	*MATa SMC3-3V5-HIS3MX leu2-3, 112 his3-11, 15 lys2-801 trp1-1 bar1 GAL+*	[33]
YBS255*	*MATa ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52 ctf7Δ1::HIS3 CTF7:LEU2*	[6]
YBS514*	*MATa ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52 ctf7Δ1::HIS3 ctf7-203:LEU2*	[6]
YBS1147*	*MATa his3-1 leu2-0 lys2-0 ura3-0*	[50],and [51]
YBS1991	*MATa ECO1: KAN can1::STE2pr-SP-his5, -lyp1, his3D1, -leu2, -ura3, -met15, LYS2+*	This study
YBS1994	*MATa eco1 203: KAN can1::STE2pr-SP-his5, -lyp1, his3D1, -leu2, -ura3, -met15, LYS2+*	This study
YKT100	*MATa ECO1:ADE; URA:tetO; LEU:tetR-GFP;TRP:Pds51-MYC 2μ vector:HIS*	This study
YKT101	*MATa ECO1:ADE; URA:tetO; LEU:tetR-GFP;TRP:Pds51-MYC 2μ POL30:HIS*	This study
YKT106	*MATa eco1-1:ADE; URA:tetO; LEU:tetR-GFP;TRP:Pds51-MYC 2μ vector:HIS*	This study
YKT107	*MATa eco1-1:ADE; URA:tetO; LEU:tetR-GFP;TRP:Pds51-MYC 2μ POL30:HIS*	This study
YCZ008*	*MATa ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52 ctf7Δ1::HIS3 ctf7-203:LEU2 Net1:GFP:TRP 2μ POL30:URA3*	This study
YCZ010*	*MATa ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52 ctf7Δ1::HIS3 ctf7-203:LEU2 Net1:GFP:TRP, CEN vector:URA3*	This study
YCZ014*	*MATa ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52 ctf7Δ1::HIS3 CTF7:LEU2 Net1:GFP:TRP 2μ POL30:URA3*	This study
YCZ016*	*MATa ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ63 ura3-52 ctf7Δ1::HIS3 CTF7:LEU2 Net1:GFP:TRP, CEN vector:URA3*	This study
YCZ044	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1*	This study
YCZ046	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 eco1-1:ADE*	This study
YCZ052	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1*	This study
YCZ071	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 ECO1:ADE*	This study
YCZ238	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:ECO1*	This study
YCZ240	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:eco1-203*	This study
YCZ249	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:ECO1 elg1Δ::TRP*	This study
YCZ254	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:eco1-203 elg1Δ::TRP*	This study
YCZ262	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 SMC3:3V5:HIS*	This study
YCZ299	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:eco1-203 SMC3:3V5:HIS*	This study
YCZ328	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:ECO1 SMC3:3V5:HIS*	This study
YCZ334	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:ECO1 elg1Δ::TRP SMC3:3V5:HIS*	This study
YCZ342	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:eco1-203 elg1Δ::TRP SMC3:3V5:His*	This study
YCZ480	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 RTT101:3 HA:TRP*	This study
YCZ581	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:eco1-203 SMC3:3V5:HIS pCZ056*	This study
YCZ582	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:eco1-203 SMC3:3V5:HIS pCZ056*	This study
YCZ587	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:ECO1 SMC3:3V5:HIS pGADT7*	This study
YCZ589	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:eco1-203 SMC3:3V5:HIS pGADT7*	This study
YCZ591	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:ECO1 SMC3:3V5:HIS pCZ057*	This study
YCZ592	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:ECO1 SMC3:3V5:HIS pCZ057*	This study
YCZ593	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:eco1-203 SMC3:3V5:HIS pCZ057*	This study
YCZ594	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:eco1-203 SMC3:3V5:HIS pCZ057*	This study
YCZ613	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:eco1-203 SMC3:3V5:HIS pCZ050*	This study
YCZ614	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:eco1-203 SMC3:3V5:HIS pCZ050*	This study
CYZ635	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:ECO1 SMC3:3V5:HIS pCZ065*	This study
YCZ636	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:ECO1 SMC3:3V5:HIS pCZ065*	This study
YCZ637	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:eco1-203 SMC3:3V5:HIS pCZ065*	This study
YCZ638	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 KAN:eco1-203 SMC3:3V5:HIS pCZ065*	This study
YCZ651	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 ECO1:ADE pGADT7*	This study
YCZ652	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 ECO1:ADE pGADT7*	This study
YCZ653	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 ECO1:ADE pCZ065*	This study
YCZ654	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 ECO1:ADE pCZ065*	This study
YCZ655	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 eco1-1:ADE pGADT7*	This study
YCZ656	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 eco1-1:ADE pGADT7*	This study
YCZ657	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 eco1-1:ADE pCZ065*	This study
YCZ658	*MATa ade 2–1 his 3–11,15 leu 2–3,112 trp 1–1 ura3-1 eco1-1:ADE pCZ065*	This study
YCZ659*	*MATa his3-1 leu2-0 lys2-0 ura3-0 rtt101::KAN*	2,and 75
YCZ690*	*MATa his3-1 leu2-0 lys2-0 ura3-0 pGADT7*	This study
YCZ691*	*MATa his3-1 leu2-0 lys2-0 ura3-0 pGADT7*	This study
YCZ692*	*MATa his3-1 leu2-0 lys2-0 ura3-0 2 u ADH RTT101 (pCZ050]*	This study
YCZ693*	*MATa his3-1 leu2-0 lys2-0 ura3-0 2 u ADH RTT101 (pCZ050)*	This study
YCZ694*	*MATa his3-1 leu2-0 lys2-0 ura3-0 2 u ADH RTT101 (pCZ056)*	This study
YCZ695*	*MATa his3-1 leu2-0 lys2-0 ura3-0 2 u ADH RTT101 (pCZ056)*	This study
YCZ696*	*MATa his3-1 leu2-0 lys2-0 ura3-0 rtt101::KAN pGADT7*	This study
YCZ697*	*MATa his3-1 leu2-0 lys2-0 ura3-0 rtt101::KAN pGADT7*	This study
YCZ698*	*MATa his3-1 leu2-0 lys2-0 ura3-0 rtt101::KAN 2 u ADH RTT101 (pCZ050)*	This study

(*Continued*)

Table 1. (Continued).

Strain	Genotype	Reference
YCZ699*	*MATa his3-1 leu2-0 lys2-0 ura3-0 rtt101::KAN 2 u ADH RTT101 (pCZ050)*	This study
YCZ700*	*MATa his3-1 leu2-0 lys2-0 ura3-0 rtt101::KAN 2 u ADH RTT101 (pCZ056)*	This study
YCZ701*	*MATa his3-1 leu2-0 lys2-0 ura3-0 rtt101::KAN 2 u ADH RTT101 (pCZ056)*	This study

All strains are in the W303 background [containing RAD5) except where strains labeled with * are S288C background

Table 2.: Plasmids used in this study.

Strain	Genotype	Reference
pRS202	*2μ vector:URA*	[52]
pRS316	*CEN* vector:*URA*	[52]
pBS99	*2μ POL30:URA*	[6]
pGADT7	*TakaraBio #630,442*	
pCZ050	*2μ ADH RTT101:LEU2 [Zhang]*	[40]
pCZ056	*2μ ADH RTT101:LEU2 (Zuilkoski]*	This study
pCZ057	*2μ ADH BRE11:LEU2*	This study
pCZ065	*2μ ADH HA:RTT101:LEU2*	This study

a wildtype W303 background strain (YCZ044). YCZ237, YCZ240, YCZ249, or YCZ254 was mated with YCZ262 and a genetic cross was performed to obtain the resulting yeast strains (YCZ299, YCZ328, YCZ334, and YCZ342). To obtain endogenously tagged Rtt101 yeast strains, RTT101:3 HA:TRP template was generated as previously described [49]. Primers for RTT101 tag are (forward primer) 5′-GCCAAGTTGTTACGAGACAAATTCATAACTA-GGGACGAATCAACAGCAACTTACAAGTACC-GGATCCCCGGGTTAATTAA-3′ and (reverse primer) 5′-AGGAAATAAAATGCTGTCGGA AAAAAAGTTAGGGCTGGTACGGATTATAAA-CTATCTCAGGAATTCGAGCTCGTTTAAAC-3′. The resulting fragment was transformed into YCZ052 to obtain the yeast strain YCZ480. pGADT7, pCZ050, and pCZ056 were transformed into YCZ328 and YCZ299 to obtain the resulting yeast strains (YCZ581, YCZ582, YCZ87, YCZ589, CZ591-YCZ594, YCZ613, YCZ614). pGADT7 and pCZ065 were transformed into YCZ046 and YCZ071 to obtain the resulting yeast strains YCZ651-YCZ658. pGADT7, pCZ050, and pCZ056 were transformed into YBS1147 and YCZ659, generously given by Dr. Gregory Lang, to obtain the resulting yeast strains [YCZ690 through YCZ701).

Cohesion assay

Log phase yeast strains YKT100 (*ECO1*, vector), YKT101 (*ECO1, PCNA*^{OE}), YKT106 (*eco1*, vector) and YKT107 (*eco1, PCNA*^{OE}), each harboring TetO repeats (integrated ~40 kb from centromereV) and TetR-GFP [2,48], were grown to 0.2 OD600 and synchronized in G1 by exposing cells to fresh media supplemented with alpha factor at the permissive temperature 23°C for 2.5--3 hours. The resulting G1 synchronized cells were washed and maintained for 3 hours at 34°C in fresh YPD media supplemented with nocodazole. The resulting preanaphase cells were fixed with 4% paraformaldehyde (10 minutes at 30°C). Fixed cells were spheroplasted by Zymolyase digestion and then adhered to a glass slide (coated with poly-L-Lysine) prior to microscopic analyses. MYC-tagged Pds1 was detected using MYC-directed A-14 (Santa Cruz Biotechnology) followed by goat anti-rabbit Alexa 568 (Molecular Probes, Inc., Eugene, OR). DNA detected using DAPI. Large budded cells, in which GFP spots (TetO-TetR-GFP) and Pds1 staining co-incided with DAPI, were analyzed for cohesion. Quantifications represent at least 400 cells (a minimum of 100 cells each from four iterations total) from which we determined during microscopy analysis as cohesed or prematurely separated sister chromatids. Cells were analyzed using an E800 light microscope (Nikon) equipped with a cooled CD camera (Coolsnapfx, Photometrics) and imaging software (IPLab, Scanalytics, Inc).

Condensation assay

Log phase yeast strains YCZ008 (*eco1, PCNA*^{OE}), YCZ010 (*eco1*, vector), YCZ014 (*ECO1, PCNA*^{OE}), and YCZ016 (*ECO1*, vector), each expressing Net1-GFP to enable analysis of rDNA structure [11,13,53], were grown to 0.2 OD600 and synchronized in G1 by exposing cells to fresh media supplemented with alpha factor at the permissive temperature 23°C for 3 hours. The resulting G1 synchronized cells were washed and maintained for 3 hours at 34°C in fresh YPD media supplemented with

nocodazole. The resulting preanaphase cells were fixed with 4% paraformaldehyde (10 minutes at 30°C). Quantifications of rDNA structures (loops/lines versus puffs) obtained from at least 300 cells (100 cells each from three iterations total) from which we determined during microscopy analysis as condensed or decondensed rDNA structures. Cells were analyzed using an E800 light microscope (Nikon) equipped with a cooled CD camera (Coolsnapfx, Photometrics) and imaging software (IPLab, Scanalytics, Inc).

Smc3 acetylation assay

Log phase yeast strains YCZ299 (*eco1, SMC3-V5*), YCZ328 (*ECO1, SMC3-V5*), YCZ334 (*ECO1, elg1, SMC3-3V5*), and YCZ342 (*eco1, elg1, SMC3-3V5*) were grown to 0.6 OD600 and synchronized in G1 in fresh media supplemented with alpha factor at the permissive temperature of 23°C for 3 hours. The resulting G1 arrested cells were washed and then released in fresh medium supplemented with nocodazole for 3 hours at 30°C to arrest cells in preanaphase. Cell cultures were normalized to an OD600 between 0.3–0.6, washed and resuspended in sterile water. Once frozen, 40 µl of IPH50 buffer (50 mM TRIS pH 8, 150 mM NaCl, 5 mM EDTA, 0.5% IGEPAL 630 (Sigma), 10 mM Sodium Butyrate, 1 mM DTT) and glass beads were added and cells lysed by bead beating (BioSpec). Cell lysates were supplemented with IPH50 buffer and protease inhibitor cocktail (AEBSF, 1,10-Phenanthroline, Pepstatin A, E-64 (Sigma)) before centrifugation at 15,000rpm for 20 minutes. Cells lysates were washed with sterile water before centrifugation at 15,000rpm for 10 minutes. Cell lysates were supplemented with SBIIA (0.5 M Tris pH 9.4, 6% Sodium Dodecyl Sulfate) buffer and 1 M DTT before a 10-minute incubation at 50°C. SBII (50% glycerol supplemented with bromophenol blue) buffer was added to the cell lysates followed by a 5-minute incubation at 65°C. Whole cell protein samples were resolved by SDS-PAGE electrophoresis and analyzed by Western blot using anti-V5 (1:40,000) (Invitrogen) with goat anti-mouse HRP (1:40,000) (Bio-Rad), anti-PGK (1:20,000) (Promega) with goat anti-mouse HRP (1:40,000) (Bio-Rad) or by anti-Smc3 K112/K113 Acetylation (1:1,000, gift from Dr. Katsuhiko Shirahige) in combination with goat anti-mouse HRP (1:20,000) (Bio-Rad) and ECL prime (GE Healthcare) for visualization.

Rtt101 and Bre1 expression assay

Log phase yeast strains YCZ480 (*ECO1, RTT101-3 HA*), YCZ587 (*ECO1, SMC3-3V5, pGADT7*), YCZ589 (*eco1, SMC3-3V5, pGADT7*), YCZ635 (*ECO1, SMC3-3V5, pCZ065*), YCZ636 (independent isolate of *ECO1, SMC3-3V5, pCZ065*), YCZ637 (*eco1, SMC3-3V5, pCZ065*), and YCZ638 (an independent isolate of *eco1 SMC3-3V5, pCZ065*) were harvested to assess Rtt101 protein expression. Log phase yeast strains YCZ587 (*ECO1, SMC3-3V5, pGADT7*), YCZ589 (*eco1 SMC3-3V5, pGADT7*), YCZ591 (*ECO1 SMC3-3V5, pCZ057*), YCZ592 (independent isolate of *ECO1, SMC3-3V5, pCZ057*), YCZ593 (*eco1, SMC3-3V5, pCZ057*), and YCZ594 (independent isolate of *eco1, SMC3-3V5, pCZ057*) were harvested to assess Bre1 protein expression. Log phase cell cultures were normalized to an OD600 between 0.3–0.6, washed and resuspended in sterile water. Once frozen, 40 µl of 100% TCA and glass beads were added to the sample and cells were lysed using bead beating (BioSpec). Cell lysates were supplemented with 5% TCA before centrifugation at 15,000rpm for 20 minutes. Cells lysates were washed with sterile water before centrifugation at 15,000rpm for 10 minutes. Whole cell lysates were supplemented with SBIIA buffer (0.5 M Tris pH 9.4, 6% Sodium Dodecyl Sulfate) before a 10-minute incubation at 50°C. SBII buffer (50% glycerol supplemented with bromophenol blue) and 1 M DTT was added to the cell lysates followed by a 5-minute incubation at 65°C. Whole cell protein samples were resolved by SDS-PAGE electrophoresis and analyzed by Western blot using anti-HA (F-7) (1:1,000) (Santa-Cruz Biotechnology) with goat anti-mouse HRP (1:20,000) (Bio-Rad), or anti-PGK (1:20,000)

(Promega) with goat anti-mouse HRP (1:40,000) (Bio-Rad) and ECL prime (GE Healthcare) for visualization.

RESULTS

Overexpressed PCNA (*POL30*) suppresses the cohesion defect in *eco1* mutant cells.

We first mapped the temperature range through which elevated PCNA levels rescue *eco1* mutant cell temperature sensitivity. The results show that *POL30* (PCNA), expressed from a 2 μm plasmid, rescues *eco1-1* mutant cell viability at 34°C but not at 37°C, both of which are non-permissive for *eco1-1* mutant cells Figure 1a, consistent with prior studies [3,40]. We next assessed the extent to which PCNA overexpression rescues defects in sister chromatid cohesion. Wildtype and *eco1* mutant cells, both of which harbor TetO repeats (integrated ~40 kb from centromere V) and TetR-GFP to enable detection of sister chromatids, and MYC-tagged Pds1 [3,48], were transformed with either vector (2 μm) or vector that contained *POL30*. Log phase cultures of the resulting wild-type and *eco1-1* mutant strains were synchronized in G1 (medium supplemented with alpha factor) and then released to the restrictive temperature of 34°C in fresh medium supplemented with nocodazole to arrest cells in preanaphase. Cell cycle synchronizations were monitored by flow cytometry Figure 1b. Preanaphase large-budded cells that retained Pds1 (an indicator of pre-anaphase) were scored as having either tethered sister chromatids (one GFP signal) or precociously separated sister chromatids (two GFP signals) Figure 1c, D. Wildtype cells, harboring either vector alone or vector containing *POL30*, exhibited low levels (21% versus 20% two GFP signals, respectively) of separated sister chromatids Figure 1d, levels similar to those previously reported [42,48]. *eco1-1* mutant cells that harbor vector alone exhibited a high level of cohesion defects (47%). Importantly, *eco1-1* mutant cells that harbor a *2μ POL30* plasmid exhibited significantly lower levels of separated sister chromatids (32%) Figure 1d. These results confirm the fundamental role that PCNA plays in promoting *eco1* mutant cell viability and specifically in regulating Eco1-dependent sister chromatid cohesion [3,40].

Persistence of chromatin-bound PCNA (via *elg1Δ*) promotes Eco1-dependent Smc3 acetylation

Smc3 acetylation is essential for cohesion establishment [31,32]. To test if elevated PCNA levels indeed increases Smc3 acetylation, we attempted to epitope-tag (3 HA) Smc3 in wild-type and *eco1* mutant cells via transformation [49], but recovered few viable *eco1-1* mutant cells. An adverse genetic interaction was confirmed through tetrad analysis (Supplemental

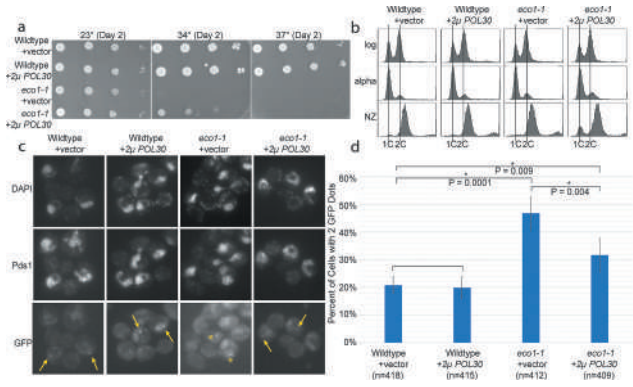

Figure 1. PCNA overexpression rescues *eco1-1* mutant cell cohesion defects (a) 10-fold serial dilution of wildtype and *eco1-1* mutant cells that harbor either 2μ vector alone or 2μ vector that contains *POL30*. Cells were plated on selective medium plates and incubated at either 23°C, 34°C, or 37°C for 2 days. (b) DNA content of wildtype and *eco1-1* mutant cells that harbor either vector alone or *2μ POL30* during log phase, G1 synchronized (alpha factor), and pre-anaphase (NZ) synchronization of cells released from G1 at 34°C. (c) Micrographs of preanaphase wildtype and *eco1-1* mutant cells as described in (B) above. GFP dots were counted in large budded cells in which the anaphase inhibitor Pds1p (Pds1) was readily detected within the DNA mass (DAPI). Tethered sister chromatids (one GFP dot) are indicated with arrows and separated sister chromatids (two GFP dots) are indicated with asterisks (*). (d) Percent of pre-anaphase cells with precocious sister chromatid separation. n = number of total cells counted. n reflects counts of at least 100 cells, per strain, obtained from at least 4 biological replicates. Student's T-Tests were used to assess statistical significance differences and error bars represent standard deviation. Statistically significant differences (*) are based on $P < 0.05$.

Figure 1A and B). We did, however, successfully V5-tag Smc3 (construct generously provided by Dr. Douglas Koshland) in *eco1-203* mutant cells by genetic crosses, from which we obtained wildtype cells, *elg1Δ* and *eco1-203* single mutant cells, and *eco1-203 elg1Δ* double mutant cells that each harbor Smc3-3V5 (Supplemental Figure 1 C, D). We confirmed that *eco1-203* mutant cell temperature sensitive growth is suppressed by the deletion of *ELG1* Figure 2a [33,54]. To assess the impact of Smc3 acetylation on cohesion, the resulting strains were synchronized in G1, washed and released at 30° (restrictive for *eco1-203* cells) into fresh media containing nocodazole Figure 2b. The resulting pre-anaphase cultures were assessed for Smc3 acetylation using an anti-Smc3 K112/K113 acetylation antibody (generously provided by Dr. Katsuhiko Shirahige). We independently verified the anti-Smc3 K112/K113 acetylation antibody obtained from Dr. Shirahige: Smc3 acetylation was detected in wildtype strains, but not in *ecolΔ rad61Δ* double mutant cells, where deletion of *RAD61* provides for viability of cells deleted for the essential gene *ECO1* [31,33,42,43]. Commercially available acetylated Smc3-directed antibodies provided variable results that included detection of Smc3 in *ecolΔ rad61Δ* double mutant cells (Supplemental Figure 1E). Interestingly, wildtype and *elg1Δ* single mutant cells exhibited identical levels of Smc3 acetylation Figure 2c, revealing that wildtype levels of PCNA is not limiting for Smc3 acetylation. Smc3 acetylation was largely undetectable in *eco1-203* single mutant cells, similar to prior reports [40,55]. Intriguingly, *eco1-203 elg1Δ* double mutant cells exhibited increased Smc3 acetylation, but at levels well below that observed in wildtype cells and *elg1Δ* single mutant cells Figure 2c, Supplemental Figure 2A and B). These results, combined with *elg1Δ*-dependent suppression of *eco1-203* mutant cell conditional growth and cohesion defects, suggest that PCNA promotes Eco1-dependent acetylation of Smc3 in the context of the DNA replication fork.

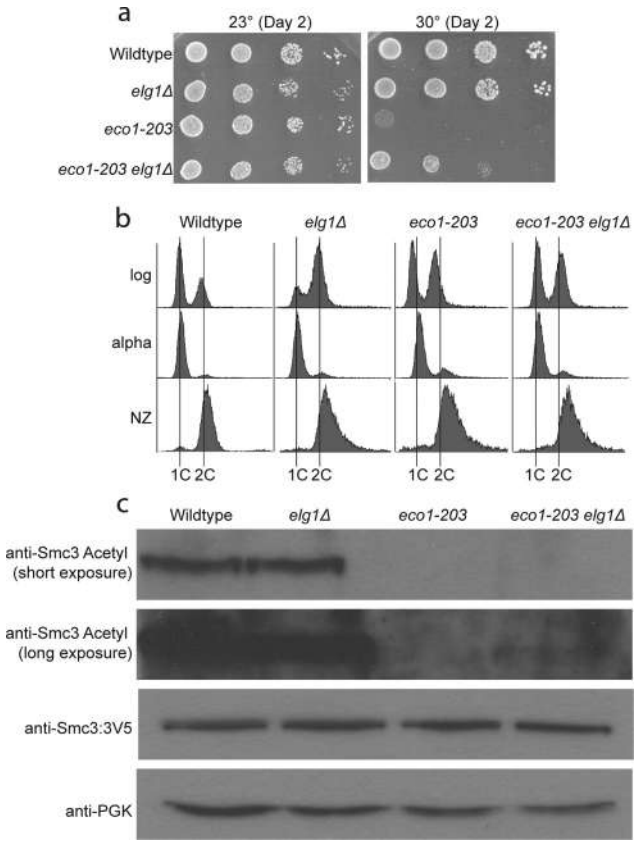

Figure 2. Excess PCNA on chromatin (via *elg1Δ*) partially rescues Smc3 acetylation levels in *eco1-203* mutant cells. (a) 10-fold serial dilution of wildtype, *elg1Δ* and *eco1-203* single mutant cells, *eco1-203 elg1Δ* double mutant cells containing Smc3-3V5 plated on medium rich plates and incubated at 23° C and 30°C for 2 days. (b) DNA content of wildtype, *elg1Δ* and *eco1-203* single mutant cells, *eco1-203 elg1Δ* double mutant cells during log phase, synchronized in G1, then shifted to 30° C and arrested in pre-anaphase. (c) Excess PCNA on chromatin partially rescues Smc3 acetylation levels in *eco1-203* mutant cells. Smc3 was detected by a V5 specific antibody, and acetylated Smc3 was detected by a K112/K113 acetylation antibody. PGK was used as a loading control.

Overexpressed Bre1 does not rescue *eco1* mutant cell viability

Elg1, in association with Rfc2-Rfc5, promotes PCNA unloading while Ctf18, in association with Rfc2-Rfc5, promotes PCNA loading onto DNA [34,56,58]. These roles in PCNA regulation are consistent with findings that *elg1Δ*, which results in increased chromatin-bound PCNA, promotes Eco1-dependent cohesion establishment and suppresses *eco1* mutant

cell growth defects while *ctf18Δ* exacerbates *eco1* mutant cell viability [3,33,54]. It thus became important to test the extent to which other DNA replication-associated factors, beyond PCNA regulators, might influence Eco1-dependent cohesion activity. Of particular interest is the DNA replication-fork associated E3 ubiquitin ligase complex that contains Bre1 [57,58]. Relevant here is that Bre1 promotes Eco1 recruitment to DNA and that *bre1Δ* cells exhibit both cohesion defects and reduced levels of acetylated Smc3 [59]. These findings predict that elevated levels of Bre1 should suppress *eco1* mutant cell conditional growth, similar that obtained by elevated levels of PCNA. To test this prediction, we overexpressed Bre1 (*2μ ADH BRE1*) in both wildtype and *eco1-203* mutant cells. The resulting wildtype cells exhibited normal cell growth in the presence of either the 2μ ADH vector alone or containing *BRE1*. As expected, *eco1-203* mutant cells that harbored vector alone exhibited growth defects at the restrictive temperature of 34°C. *eco1-203* mutant cells that harbor *2μ ADH BRE1* continued to exhibit severe cell inviability at the restrictive temperature of 34°C (Figure 3a, Supplemental Figure 4A). We confirmed that the *BRE1* coding sequence was free of mutation by DNA sequencing and also that Bre1 protein was expressed from the overexpression vector by Western blot (Figure 3b, Supplemental Figure 3A). Our results suggest that, though Bre1 may be important for cohesin acetylation and sister chromatid cohesion [59], Bre1 is unlikely to be a direct regulator of Eco1 activity.

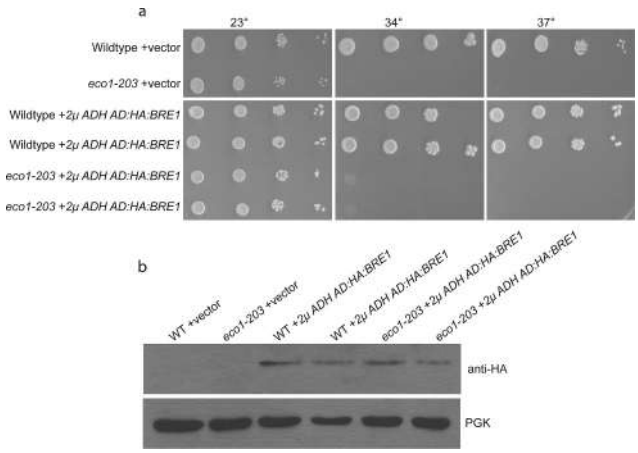

Figure 3. Overexpressed Bre1 does not rescue *eco1-203* mutant cell viability. (a) 10-fold serial dilution of wildtype and *eco1-203* mutant cells harboring either 2μ vector or *2μ ADH BRE1* plated on selective medium plates and incubated at 23°C, 34°C, and 37°C for 3 days. (b) Detection of AD-HA tagged Bre1 using HA-directed antibody (Santa Cruz Biotechnology) in log phase wildtype and *eco1-203* single mutant cells.

Overexpression of *RTT101* does not rescue either *eco1-203* or *eco1-1* mutant cell viabilities.

An additional DNA replication fork-associated complex, Rtt101-Mms22-Mms1, was also recently reported to rescue both *eco1* mutant cell viability and cohesion defects, and significantly increase Smc3 acetylation levels [40]. Rtt101-Mms22-Mms1 promotes histone deposition and stabilizes the replisome during replication stress [60,61,62,63], a specific context previously posited for Eco1 [64,65]. Elevated levels of Rtt101 produced the most robust rescue *eco1* mutant cells [40]. Thus, we first decided to confirm Rtt101 rescue of *eco1-203* mutant cells by generating an *RTT101* overexpression vector, in parallel to obtaining the *RTT101* overexpression plasmid engineered by Dr. Huiqiang Lou [40]. *eco1-203* mutant cells that harbor a PCNA overexpression plasmid were included as a positive control [3, Figure 1d]. We were surprised that neither our in-house constructed *RTT101* expressing plasmid, nor that obtained from our colleague Dr. Lou, rescued *eco1-203* mutant cell viability. In contrast, elevated levels of PCNA provided the expected rescue of *eco1-203* mutant cell temperature sensitivity (Figure 4a, Supplemental Figure 4A). We sequenced both *RTT101* constructs to confirm that neither contained mutations within the *RTT101* coding sequence. We next confirmed that *RTT101* was indeed being expressed: *rtt101Δ* cells (generously provided by Dr. Gregory Lang) are sensitive to the DNA damage agent methyl methanesulfonate (MMS). MMS sensitivity was independently rescued by both *RTT101* overexpression plasmids (Figure 4b, Supplemental Figure 4B). Note that *RTT101* overexpression in

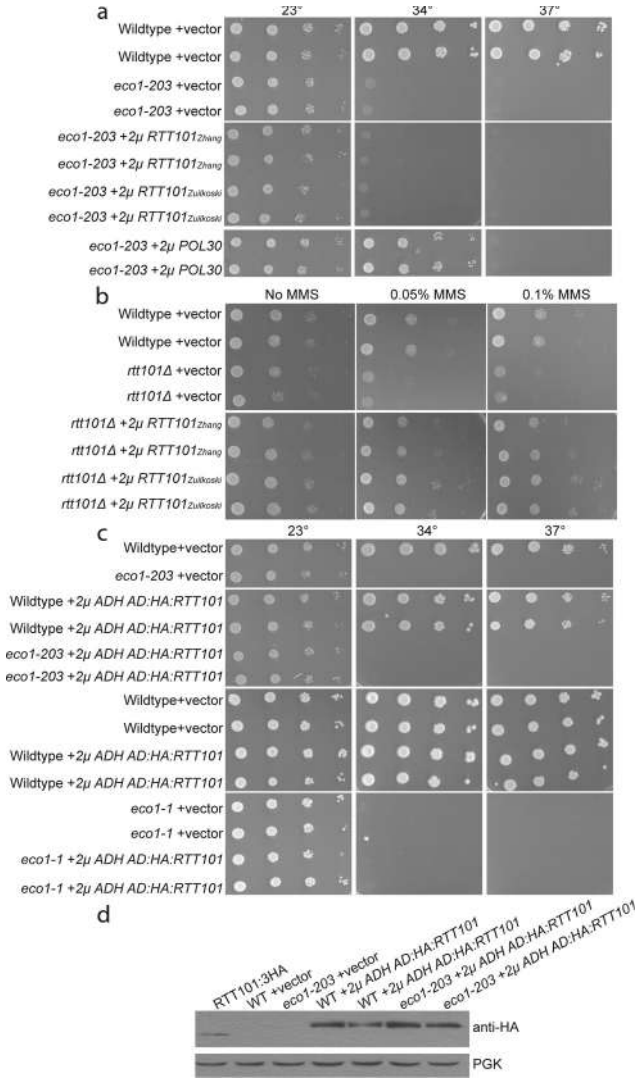

Figure 4. Overexpression of *RTT101* in *eco1-203* or *eco1-1* mutant cell viability.

fact makes *rtt101Δ* mutant cells mildly resistant to genotoxic agents, compared to wildtype levels of Rtt101.

Mms22 binds Eco1 through a short region that requires amino acids 61 L and 63D [40]. We were concerned that our *eco1-203* strains might have incurred spontaneous mutations that abolished this site – a possibility that we negated through DNA sequencing. Finally, we generated an *RTT101* overexpression plasmid in which Rtt101 protein was epitope-tagged (AD-HA) so that we could unambiguously confirm elevated expression. This construct similarly failed to rescue either *eco1-203* or *eco1-1* single mutant cell growth defects, despite driving elevated levels of Rtt101 protein (Figure 4c and d, Supplemental Figure 3B, Supplemental Figure 4A). In the study conducted by Zhang and colleagues, the strains were constructed in a BY4741 background, where our *eco1* alleles were derived from W303 and S288C backgrounds. We hypothesized that there is a strain specific rescue of *eco1* alleles by *RTT101* overexpression, which was independently confirmed (Dr. Huiqiang Lou – personal communication). Currently, the basis for these strain-specific impacts remain unknown.

(A)10-fold serial dilution of wildtype and *eco1-203* single mutant cells, harboring either *2μ* vector alone or with *RTT101* or *POL30*, plated on selective medium plates and incubated at 23°C, 34°C, and 37° C for 2 days. Plasmids constructed in-house (Zuilkoski) or generously provided by the Lou lab (Zhang) are indicated. (B) 10-fold serial dilution of wildtype and *rtt101Δ* cells, both harboring vector or *2μ RTT101*, plated on selective medium plates containing no MMS, 0.05% MMS or 0.1% MMS and incubated for 2 days. (C) 10-fold serial dilution of wildtype, *eco1-203* and *eco1-1* single mutant cells, each harboring either vector or *2μ AD-HA-RTT101*, plated on selective medium plates and incubated at 23°C, 34°C, and 37°C for 3 days. (D) HA-tagged Rtt101, either endogenously expressed (RTT101:3 HA, lane 1) or expressed from a 2μ high-copy vector (lanes 4–7) detected using an HA-directed antibody (Santa Cruz Biotechnology). PGK (Novex) was used as a loading control.

PCNA overexpression does not rescue the condensation defect in *eco1* mutant cells.

Given the inability of either *BRE1* or *RTT101* expression to suppress *eco1* mutant cell temperature sensitivity, we turned to PCNA to address the critical question of whether condensation defects are rescued in coordination with cohesion defects. The validity of this approach is augmented by findings that PCNA overexpression Figure 5a and *ELG1* deletion Figure 2a, both provide significant rescue of *eco1* mutant cell conditional growth [3,33,40]. Wildtype cells and *eco1-203* mutant cells were each modified to express Net1-GFP,

enabling detection of rDNA structure as either uncondensed puffs or condensed lines or loops [11,13,53] and transformed with either a *CEN* vector alone or *2μ POL30*. The resulting strains were arrested in G1 at the permissive temperature, and released at 34°C (restrictive temperature for *eco1-203* mutant cells) into fresh medium containing nocodazole to arrest cells in pre-anaphase Figure 5b. The synchronized yeast strains were then scored for condensation defects (elevated number of puff-like structures compared to discrete line/loop structures) Figure 5c. Wildtype cells harboring either vector alone or *2μ POL30* exhibited low level of condensation defects (20% and 21% puff-like rDNA, respectively) while *eco1-203* mutant cells (vector alone) exhibited a high level of condensation defect (60% puffs), consistent with prior results [3,,45]. Importantly, *eco1-203* mutant cells that harbored a *2μ POL30* plasmid persisted in exhibiting high levels of condensation defects (60% puffs) Figure 5d. These results reveal that PCNA promotes only a subset of cohesin functions – in this case promoting sister chromatid tethering – but not chromosome condensation.

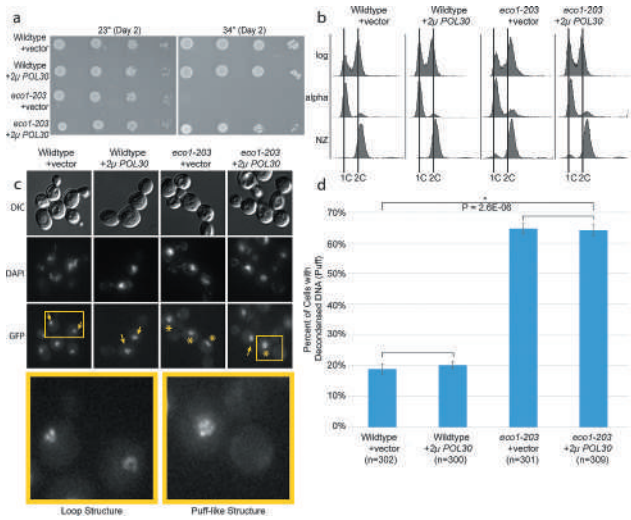

Figure 5. The overexpression of PCNA does not rescue DNA condensation defects in *eco1-203* mutant cells. (a) 10-fold serial dilution of wildtype and *eco1-203* mutant cells, harboring either a vector alone or with *2μ POL30*, plated on selective medium plates and incubated at 23°C and 34°C for 2 days. (b) DNA content of log phase wildtype and *eco1-203* single mutant cells (as described in (A)) synchronized in G1, and then shifted to 34°C and arrested in pre-anaphase. (c) Micrographs of wildtype and *eco1-203* single mutant cells (as described in (A)) in which sister chromatids (GFP) are located within the DNA mass (DAPI) of large-budded cells (DIC). Arrows indicate condensed rDNA loops and asterisks indicated decondensed rDNA puffs. (d) Percent of pre-anaphase cells with decondensed DNA. n = number of total cells counted where at least 100 cells per strain were counted over 3 biological replicates. Statistical analysis was performed using a Student's T-Test and error bars represent standard deviation. Statistically significant differences (*) are based on $P < 0.05$.

Discussion

A wealth of studies link Eco1-dependent sister chromatid cohesion establishment to the process of DNA replication, most notably through PCNA recruitment [3,33,35,36,38,39,40,54,66]. The extent to which Eco1 promotes DNA condensation in the context of DNA replication, however, remains unknown. A major finding of this study is that PCNA appears to play little, if any, role in Eco1-dependent chromosome condensation. These results reveal that Eco1 promotes condensation in a context that may be spatially and/or temporally distinct from that of the DNA replication fork, or at least in relation to PCNA Figure 6. This fundamental aspect of chromosome biology, and the manner in which Eco1 promotes chromosome condensation, thus remains an intriguing enigma.

A second key finding of the current study is the extent to which Eco1 and cohesin functions in both cohesion and condensation are separable. Several studies now document that elevated levels of PCNA suppress *eco1* mutant cell growth [3,33], a rescue that we now document occurs through rescue of cohesion defects, but not condensation defects. In contrast, previous efforts revealed that *rad61Δ* instead rescues *eco1* mutant cell condensation, but not cohesion, defects [47]. These results mirror those for the auxiliary cohesin factor Pds5: retained chromatin-bound PCNA (via *elg1Δ*) suppresses *pds5* mutant cell cohesion defects, while *rad61Δ* suppresses *pds5* mutant cell condensation defects [48,67]. Rad61 and Pds5 both associate with the core cohesin complex [21,22,42,43], and thus provide little illumination regarding the context (relative to the DNA replication fork) through which Eco1-dependent condensation may be regulated. Future efforts in biochemical analysis of

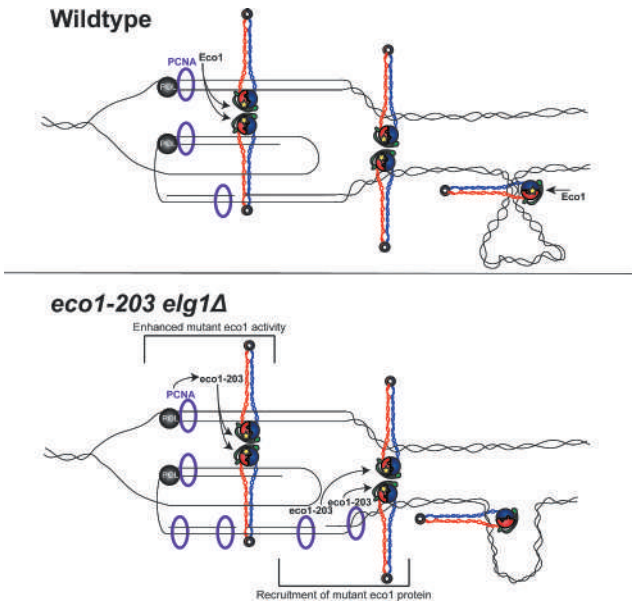

Figure 6. Contexts through which Eco1 may promote cohesion (proximal to the DNA replication fork) and condensation (distal to the replication fork). Top: In wildtype cells, Eco1 is recruited to the replication fork by PCNA to acetylate cohesin and promote sister chromatid cohesion. Cohesin acetylation outside the context of the DNA replication fork by Eco1 promotes DNA condensation. **Bottom**: In *eco1-203 elg1Δ* double mutant cells, mutant eco1 protein is recruited to the replication fork by retained chromatin-bound PCNA. Either through enhanced activity or recruitment further behind the replication fork, Eco1 acetylates cohesin in context of PCNA to establish cohesion. Even in this extended PCNA-eco1 recruitment scenario, DNA condensation defects persist.

cohesins from genetic backgrounds, capable of only condensation or cohesion, are likely to provide critical insights regarding a "cohesin code" [64] that may allow for simultaneous but distinct assemblies of *trans* and *cis* DNA locus tetherings.

Is there prioritization, in terms of cell viability, for the myriad roles of cohesins? It is worth speculating that the earliest SMC complex persisted through evolution due to a singular beneficial impact on either RNA or DNA biology. Whether this involved increased stability (reduced accessibility to damaging agents through compaction), non-mutagenic repair (proximity of template), high fidelity transmission (cohesion of sisters) or cell function (transcription) remains unknown. Early studies of current day eukaryotes document that the rescue of *eco1* mutant cell inviability by *rad61Δ* appears coordinated solely with the ability to condense chromosomes [47]. As noted by the authors, the prioritization of cohesin function in condensation is likely predicated on the idiosyncrasies of yeast that exhibit a closed mitosis and bipolar chromosome attachment during S phase. An intriguing outcome of the current study is that cell viability appears instead coordinated with cohesion: rescued *eco1* mutant cell growth defects appears to require only a partial rescue in cohesion – not condensation. One possibility is that the Net1-GFP assay employed here may not fully reveal the state of chromosome condensation or the effect that PCNA plays in condensation. Prior to these roles in cohesion and condensation, however, Rad21 (Mcd1/Scc1) was first identified as a DNA repair factor [68,69,70]. Certainly, there is sufficient evidence to conclude that all of these mechanisms are critical for cell survival. Which activity arose first, however, remains an important issue in functional evolution that warrants further research.

The role of PCNA in cohesion also impacts current views of cohesion establishment. Early notions that the DNA replication fork passes through a pre-loaded huge triangular cohesin ring (single ring entrapment of replicated sister chromatids) [26,31,71,72] appeared inconsistent with replisome size constraints and further was challenged by evidence that cohesin are loaded behind the fork [5,26,27,73]. Loading behind the fork could be explained by a two-step sister capture mechanism by individual cohesins [73], but there is compelling genetic and biochemical evidence that sister chromatid tethering involves the dimerization of cohesins deposited onto each sister chromatid [48,71,74, 75,76,77,78,79]. While cohesin oligomers proved the dominant structure by EM, current views of cohesion remain heavily influenced by EM analyses of the remaining single ring structures [80,81]. Not surprisingly, cohesin-cohesin dimerization requires Eco1 [79]. Here, our data supports the model that Eco1-dependent cohesin acetylation promotes cohesion (cohesin dimerization) in the context of PCNA [82]. Furthermore, our results reveal that only a small pool of acetylated cohesins is required to support cohesion, consistent with prior studies [40,83]. Much less is known

regarding Eco1-dependent regulation of condensation. Recent publications reveal that cohesin extrudes DNA into a loop-like structure, plausibly through cohesin monomers [84,85,86]. Further studies are required to determine the mechanism through which Eco1, independent of the DNA replication fork, establishes DNA condensation.

Acknowledgments

We thank the R.V.S. laboratory members (Michael Mfarej, Annie Sanchez, Nicole Kirven, and Shaya Ameri) for helpful comments during the progression of this study. We also thank Dr. Douglas Koshland, Dr. Gregory Lang, Dr. Huiqiang Lou, and Dr. Katsuhiko Shirahige for generously sharing yeast strains and reagents. This work was supported by an award to R.V.S. from the National Institutes of Health [R15GM110631]. Any opinions, findings, and conclusions or recommendations expressed in this study are those of the authors and does not necessarily reflect the views of the National Institutes of Health. No competing interests are declared.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Lehigh University [Nemes Fellowship Research Award]; National Institutes of Health [R15GM110631].

ORCID

Caitlin M. Zuilkoski http://orcid.org/0000-0002-7793-5512

References

[1] Guacci V, Koshland D, Strunnikov A, et al. A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of MCD1 in S. cerevisiae. Cell. 1997;91(1):47–57.

[2] Michaelis C, Ciosk R, Nasmyth K. Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. Cell. 1997;91(1):35–45.

[3] Skibbens RV, Corson LB, Koshland D, et al. Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery. Genes Dev. 1999;13(3):307–319.

[4] Tóth A, Ciosk R, Uhlmann F, et al. Yeast cohesin complex requires a conserved protein, Eco1p(Ctf7), to establish cohesion between sister chromatids during DNA replication. Genes Dev. 1999;13(3):320–333.

[5] Ciosk R, Shirayama M, Shevchenko A, et al. Cohesin's binding to chromosomes depends on a separate complex consisting of Scc2 and Scc4 proteins. Mol Cell. 2000;5(2):243–254.

[6] Mönnich M, Kuriger Z, Print CG, et al. Model of roberts syndrome reveals that Esco2 depletion interferes with development by disrupting the cell cycle. PLoS One. 2011;6(5):e20051.

[7] Whelan G, Kreidl E, Wutz G, et al. Cohesin acetyltransferase Esco2 is a cell viability factor and is required for cohesion in pericentric heterochromatin. Embo J. 2012;31(1):71–82.

[8] Cucco F, Musio A. Genome stability: what we have learned from cohesinopathies. Am J Med Genet Part C Semin Med Genet. 2016;172(2):171–178.

[9] Lavoie BD, Hogan E, Koshland D. In vivo dissection of the chromosome condensation machinery: reversibility of condensation distinguishes contributions of condensin and cohesin. J Cell Biol. 2002;156(5):805–815.

[10] Dorsett D, Eissenberg JC, Misulovin Z, et al. Effects of sister chromatid cohesion proteins on cut gene expression during wing development in Drosophila. Development. 2005;132(21):4743–4753. doi:10.1242/dev.02064

[11] Lopez-Serra L, Lengronne A, Borges V, et al. Budding yeast Wapl controls sister chromatid cohesion maintenance and chromosome condensation. Curr Biol. 2013;23(1):64–69.

[12] Lu S, Lee KK, Harris B, et al. The cohesin acetyltransferase Eco1 coordinates rDNA replication and transcription. EMBO Rep. 2014;15(5):609–617.

[13] Shen D, Skibbens RV. Temperature-dependent regulation of rDNA condensation in Saccharomyces cerevisiae. Cell Cycle. 2017;16(11):1118–1127.

[14] Bloom MS, Koshland D, Guacci V. Cohesin function in cohesion, condensation, and DNA repair is regulated by Wpl1p via a common mechanism in saccharomyces cerevisiae. 2018;208:111–124.

[15] Ball AR Jr., Chen Y, Yokomori K. Mechanisms of cohesin-mediated gene regulation and lessons learned from cohesinopathies. Biochim Biophys Acta. 2014;1839(3):191–202.

[16] Rao SSP, Huang SC, Glenn St Hilaire B, et al. Cohesin loss eliminates all loop domains. Cell. 2017;171(2):305–320.

[17] Wutz G, Várnai C, Nagasaka K, et al. Topologically associating domains and chromatin loops depend on cohesin and are regulated by CTCF, WAPL, and PDS 5 proteins. Embo J. 2017;36(24):1–27.

[18] Haarhuis JHI, van der Weide RH, Blomen VA, et al. The cohesin release factor WAPL restricts chromatin loop extension. Cell. 2017;169(4):693–707.e14.

[19] Maya-Miles M, Andújar E, Perez-Alegre M, et al. Crosstalk between chromatin structure, cohesin activity and transcription. Epigenetics Chromatin. 2019;;12 (1):47.
[20] Strunnikov AV, Larionov VL, Koshland D. SMCI: an essential yeast gene encoding a putative head-rod-tail protein is required for nuclear division and defines a new ubiquitous protein family. J Cell Biol. 1993;123 (6):1635–1648.
[21] Hartman T, Stead K, Koshland D, et al. Pds5p Is an essential chromosomal protein required for both sister chromatid cohesion and condensation in saccharomyces cerevisiae. J Cell Biol. 2000;151 (3):613–626.
[22] Panizza S, Tanaka T, Hochwagen A, et al. Pds5 cooperates with cohesin in maintaining sister chromatid cohesion. Curr Biol. 2000;10:1557–1564.
[23] Uhlmann F, Nasmyth K. Cohesion between sister chromatids must be established during DNA replication. Curr Biol. 1998;8(20):1095–1102.
[24] Tanaka T, Cosma MP, Wirth K, et al. Identification of cohesin association sites at centromeres and along chromosome arms. Cell. 1999;98(6):847–858.
[25] Rollins RA, Korom M, Aulner N, et al. Drosophila nipped-B protein supports sister chromatid cohesion and opposes the stromalin/Scc3 cohesion factor to facilitate long-range activation of the cut gene. Mol Cell Biol. 2004;24(8):3100–3111.
[26] Lengronne A, Mcintyre J, Katou Y, et al. Establishment of Sister Chromatid Cohesion at the S. cerevisiae Replication Fork. Mol Cell. 2006;23(6):787–799.
[27] Watrin E, Schleiffer A, Tanaka K, et al. Human Scc4 Is required for cohesin binding to chromatin, sister-chromatid cohesion, and mitotic progression. Curr Biol. 2006;16(9):863–874.
[28] Ivanov D, Schleiffer A, Eisenhaber F, et al. Eco1 is a novel acetyltransferase that can acetylate proteins involved in cohesion. Curr Biol. 2002;12(4):323–328.
[29] Bellows AM, Kenna MA, Cassimeris L, et al. Human EFO1p exhibits acetyltransferase activity and is a unique combination of linker histone and Ctf7p/Eco1p chromatid cohesion establishment domains. Nucleic Acids Res. 2003;31(21):6334–6343.
[30] Hou F, Zou H. Two human orthologues of Eco1/Ctf7 acetyltransferases are both required for proper sister-chromatid cohesion. Mol Biol Cell. 2005;16 (8):3908–3918.
[31] Rolef Ben-shahar T, Heeger S, Lehane C, et al. Eco1-dependent cohesin acetylation during establishment of sister chromatid cohesion. Science. 2008;321 (5888):563–566.
[32] Unal E, Heidinger-Pauli JM, Kim W, et al. A molecular determinant for the establishment of sister chromatid cohesion. Science. 2008;321(5888):566.
[33] Maradeo ME, Skibbens RV. The Elg1-RFC clamp-loading complex performs a role in sister chromatid cohesion. PLoS One. 2009;4(3):18–21.
[34] Kubota T, Nishimura K, Kanemaki MT, et al. The Elg1 replication factor C-like complex functions in PCNA unloading during DNA replication. Mol Cell. 2013;50 (2):273–280
[35] Moldovan GL, Pfander B, Jentsch S. PCNA controls establishment of sister chromatid cohesion during S phase. Mol Cell. 2006;23(5):723–732.
[36] Bender D, Da Silva EML, Chen J, et al. Multivalent interaction of ESCO2 with the replication machinery is required for sister chromatid cohesion in vertebrates. Proc Natl Acad Sci USA. 2020;117(2):1081–1089.
[37] Skibbens RV. Chl1p, a DNA Helicase-Like Protein in Budding Yeast, Functions in Sister-Chromatid Cohesion. Genetics. 2004;166(1):33–42. doi:10.1534/genetics.166.1.33
[38] Borges V, Smith DJ, Whitehouse I, et al. An Eco1-independent sister chromatid cohesion establishment pathway in S. cerevisiae. Chromosoma. 2013;122 (1–2):121–134.
[39] Samora CP, Saksouk J, Goswami P, et al. Ctf4 Links DNA replication with sister chromatid cohesion establishment by recruiting the Chl1 helicase to the replisome. Mol Cell. 2016;63(3):371–384.
[40] Zhang J, Shi D, Li X, et al. Rtt101-Mms1-Mms22 coordinates replication-coupled sister chromatid cohesion and nucleosome assembly. EMBO Rep. 2017a;18 (8):1294–1305.
[41] Kueng S, Hegemann B, Peters BH, et al. Wapl controls the dynamic association of cohesin with chromatin. Cell. 2006;127(5):955–967.
[42] Rowland BD, Roig MB, Nishino T, et al. Building sister chromatid cohesion: smc3 acetylation counteracts an antiestablishment activity. Mol Cell. 2009;33 (6):763–774.
[43] Sutani T, Kawaguchi T, Kanno R, et al. Budding yeast Wpl1(Rad61)-Pds5 complex counteracts sister chromatid cohesion-establishing reaction. Curr Biol. 2009;19 (6):492–497.
[44] Sonoda E, Matsusaka T, Morrison C, et al. Scc1/Rad21/Mcd1 Is required for sister chromatid cohesion and kinetochore function in vertebrate cells. Dev Cell. 2001;1(6):759–770.
[45] Gard S, Light W, Xiong B, et al. Cohesinopathy mutations disrupt the subnuclear organization of chromatin. J Cell Biol. 2009;187(4):455–462.
[46] Robison B, Guacci V, Koshland D, et al. A role for the Smc3 hinge domain in the maintenance of sister chromatid cohesion. Mol Biol Cell. 2018;29(3):339–355.
[47] Guacci V, Koshland D. Cohesin-independent segregation of sister chromatids in budding yeast. Mol Biol Cell. 2012;23(4):729–739.

[48] Tong K, Skibbens RV. Pds5 regulators segregate cohesion and condensation pathways in Saccharomyces cerevisiae. Proc Natl Acad Sci. 2015;112 (22):7021–7026.
[49] Longtine MS, McKenzie III A, Demarini DJ, et al. Additional modules for versatile and economical PCR-based gene deletion and modification in saccharomyces cerevisiae. Yeast. 1998;14(10):953–961.
[50] Baudin A, Ozier-Kalogeropoulos O, Denouel A, et al. A simple and efficient method for direct gene deletion in Saccharomyces cerevisiae. Nucleic Acids Res. 1993;21(14):3329–3330.
[51] Wach A, Brachat A, Pohlmann R, et al. New heterologous modules for classical or PCR-based gene disruptions in Saccharomyces cerevisiae. Yeast. 1994;10 (13):1793–1808.
[52] Sikorski RS, Hieter P. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics. 1989;122 (1):19–27.
[53] D'Ambrosio C, Schmidt CK, Katou Y, et al. Identification of cis-acting sites for condensin loading onto budding yeast chromosomes. Genes Dev. 2008;22 (16):2215–2227.
[54] Parnas O, Zipin-Roitman A, Mazor Y, et al. The Elg1 clamp loader plays a role in sister chromatid cohesion. PLoS One. 2009;4(5):e5497.
[55] Elbatsh AMO, Haarhuis JHI, Petela N, et al. Cohesin releases DNA through asymmetric ATPase-driven ring opening. Mol Cell. 2016;61(4):575–588.
[56] BylundGO, Burgers PMJ, Replication protein A-directed unloading of PCNA by the Ctf18 cohesion establishment complex. Mol Cell Biol. 2005;25:5445–5455.
[57] Wood A, Krogan NJ, Dover J, et al. Bre1, an E3 ubiquitin ligase short article required for recruitment and substrate selection of rad6 at a promoter. Mol Cell. 2003;11(1):267–274.
[58] Hwang WW, Venkatasubrahmanyam S, Ianculescu AG, et al. A conserved RING finger protein required for histone H2B monoubiquitination and cell size control. Mol Cell. 2003;11 (1):261–266.
[59] Zhang W, Yeung CHL, Wu L, et al. E3 ubiquitin ligase Bre1 couples sister chromatid cohesion establishment to DNA replication in Saccharomyces cerevisiae. Elife. 2017;6:e28231.
[60] Luke B, Versini G, Jaquenoud M, et al. Report the cullin rtt101p promotes replication fork progression through damaged DNA and natural pause sites.. Curr Biol. 2006;16(8):786–792.
[61] Duro E, Vaisica JA, Brown GW, et al. Brief report Budding yeast Mms22 and Mms1 regulate homologous recombination induced by replisome blockage. DNA Repair (Amst). 2008;7(5):811–818.
[62] Zaidi IW, Rabut G, Poveda A, et al. Rtt101 and Mms1 in budding yeast form a CUL4 DDB1-like ubiquitin ligase that promotes replication through damaged DNA. EMBO Rep. 2008;9(10):1034–1040.
[63] Vaisica JA, Baryshnikova A, Costanzo M, et al. Mms1 and Mms22 stabilize the replisome during replication stress. Mol Biol Cell. 2011;22 (13):2396–2408.
[64] Rudra S, Skibbens RV, Lichten M. Chl1 DNA helicase regulates Scc2 deposition specifically during DNA-replication in saccharomyces cerevisiae. PLoS One. 2013;8(9):1–17.
[65] Billon P, Li J, Lambert JP, et al. Acetylation of PCNA sliding surface by Eco1 promotes genome stability through homologous recombination. Mol Cell. 2017;65(1):78–90.
[66] Song J, Lafont A, Chen J, et al. Cohesin acetylation promotes sister chromatid cohesion only in association with the replication machinery. J Biol Chem. 2012;287 (41):34325–34336.
[67] Orgil O, Matityahu A, Eng T, et al. A conserved domain in the scc3 subunit of cohesin mediates the interaction with both mcd1 and the cohesin loader complex. PLoS Genet. 2015;11(3):e1005036.
[68] Birnboim HC, Nasim A. Excision of pyrimidine dimers by several UV-sensitive mutants of S. pombe. Mol Gen Genet. 1975;136(1):1–8.
[69] Nasim A, Smith BP. Genetic control of radiation sensitivity in Schizosaccharomyces pombe. Genetics. 1975;79(4):573–582.
[70] Birkenbihl RP, Subramani S. Cloning and characterization of rad21 an essential gene of Schizosaccharomyces pombe involved in DNA double-strand-break repair. Nucleic Acids Res. 1992;20(24):6605–6611.
[71] Haering CH, Löwe J, Hochwagen A, et al. Molecular architecture of SMC proteins and the yeast cohesin complex. Mol Cell. 2002;9(4):773–788.
[72] Gruber S, Haering CH, Nasmyth K. Chromosomal cohesin forms a ring. Cell. 2003;112(6):765–777
[73] Murayama Y, Samora CP, Kurokawa Y, et al. Establishment of DNA-DNA Interactions by the Article Establishment of DNA-DNA Interactions by the Cohesin Ring. Cell. 2018;172(3):465–469.
[74] Sakai A, Hizume K, Sutani T, et al. Condensin but not cohesin SMC heterodimer induces DNA reannealing through protein-protein assembly. Embo J. 2003;22 (11):2764–2775.
[75] Eng T, Guacci V, Koshland D. ROCC, a conserved region in cohesin's Mcd1 subunit, is essential for the proper regulation of the maintenance of cohesion and establishment of condensation. Mol Biol Cell. 2014;25 (16):2351–2364.
[76] Kulemzina I, Schumacher MR, Verma V, et al. Cohesin rings devoid of Scc3 and Pds5 maintain their stable

association with the DNA. PLoS Genet. 2012;8(8): e1002856.
[77] Zhang N, Kuznetsov SG, Sharan SK, et al. A handcuff model for the cohesin complex. J Cell Biol. 2008;183 (6):1019–1031.
[78] Cattoglio C, Pustova I, Walther N, et al. Determining cellular CTCF and cohesin abundances to constrain 3D genome models. Elife. 2019;8:e40164.
[79] Shi D, Zhao S, Zuo M-Q., Zhang J, Hou W, Dong M-Q., Cao Q., Lou H. The acetyltransferase Eco1 elicits cohesin dimerization during S phase. J Biol Chem. 2020;295(22):7554–7565 doi:10.1074/jbc.RA120.013102
[80] Huis in't Veld PJ, Herzog F, Ladurner R, et al. Characterization of a DNA exit gate in the human cohesin ring. Science. 2014;346(6212):968–972.
[81] Gligoris TG, Scheinost JC, Bürmann F, Petela N, Chan KL, Uluocak P, Beckouët F, Gruber S, Nasmyth K, Löwe J. Closing the cohesin ring: structure and function of its Smc3-kleisin interface. Science. 2014;346(6212):963–967
[82] Skibbens RV. Condensins and cohesins - one of these things is not like the other! J Cell Sci. 2019;132(3): jcs220491.
[83] Heidinger-Pauli JM, Mert O, Davenport C, et al. Systematic reduction of cohesin differentially affects chromosome segregation, condensation, and DNA repair. Curr Biol. 2010;20(10):957–963.
[84] Davidson IF, Bauer B, Goetz D, et al. DNA loop extrusion by human cohesin. Science. 2019;1345(6471):1338–1345.
[85] Kim J, Shi Z, Zhang H, Finkelstein IJ, et al. Human cohesin compacts DNA by loop extrusion. Science. 2019;366(6471):1345–1349.
[86] Liu W, Biton E, Pathania A, Matityahu A, Irudayaraj J, Onn I. Monomeric cohesin state revealed by live-cell single-molecule spectroscopy. EMBO Rep. 2020;21(2): e48211 doi:10.15252/embr.201948211