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Rfc5p regulates alternate RFC complex functions in sister chromatid pairing reactions in budding yeast

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Sister chromatid pairing reactions, termed cohesion establishment, occur during S-phase and appear to be regulated by Replication Factor C (RFC) complexes. For instance, RFCs that contain Ctf18p exhibit pro-establishment activities while those that contain Elg1p exhibit anti-establishment activities. It remains unknown whether Ctf18p-RFC and Elg1p-RFC functions are simply opposing or instead reveal complicated and non-parallel regulatory mechanisms. To better understand the nature of these novel pathways, we analyzed the small RFC subunit Rfc5p that is common to both Ctf18p-RFC and Elg1p-RFC. Despite this commonality, the data show that diminished Rfc5p function rescues *ctf7/eco1* mutant cell phenotypes, revealing that Rfc5p promotes anti-establishment activities. This rescue is specific to establishment pathways in that *rfc5-1* greatly accentuates growth defects when expressed in *scc2* (deposition), *mcd1/scc1* or *smc3* (cohesion maintenance) mutated cells. Our results reveal for the first time a role for small RFC subunits in directing RFC complex functions—in this case towards anti-establishment pathways. We further report that Pds5p exhibits both establishment and anti-establishment functions in cohesion. This duality suggests that categorizations of establishment and anti-establishment activities require further examination.

Introduction

A typical cell cycle invariably includes a DNA replication step to produce two exact copies of each chromosome. The Replication Factor C complex (RFC) is a five subunit complex consisting of one large subunit (Rfc1p) accompanied by four small subunits (Rfc2p-Rfc5p) and is required for DNA replication. RFC complexes hydrolyze ATP to open and then load PCNA or PCNA-like sliding clamps onto primer-template regions of DNA. In turn, sliding clamps maintain polymerase association with DNA and thus promote processive DNA replication.[1] All five of the canonical RFC subunits (Rfc1p-Rfc5p) and PCNA are essential for DNA replication during S phase. In addition to Rfc1p, there are three alternative large subunits (Ctf18p, Elg1p and Rad24p) that exhibit extensive sequence homology to Rfc1p and associate individually with each of the four small subunits Rfc2p-Rfc5p. Ctf18p-RFC both binds PCNA and exhibits PCNA loading/unloading activities.[2,3] Elg1p-RFC also binds PCNA, but the extent that this complex promotes sliding clamp association with DNA remains unknown.[4-6] Rad24p-RFC is unique in associating with the 9-1-1 sliding clamp complex composed of Rad17p, Mec3p and Ddc1p.[7-9] More recent evidence reveals that Rad24p-RFC can also bind and elicit PCNA unloading.[10] Notably, all four RFC complexes and both PCNA and Rad17p, Mec3p, Ddc1p sliding clamps function in DNA repair pathways.[11]

In order for the products of DNA replication to be segregated properly in mitosis, a multi-step cohesion pathway is required earlier during the cell cycle. During S-phase for instance, the products of chromosome replication, termed sister chromatids, become decorated with cohesin complexes that ultimately serve as a molecular glue that tethers the sisters together. Cohesins contain both structural components (Smc1p, Smc3p, Mcd1p/Scc1p and Scc3p/Irr1p) and accessory factors (Pds5p and Rad61p).[12,13] Before chromatid-associated cohesins can participate in sister chromatid pairing, however, they must be modified by Ctf7p/Eco1p—an aceytltransferase that targets Smc3p specifically during S-phase.[14-16] In the absence of this establishment step, sister chromatids remain cohesin-decorated but unpaired, resulting in massive chromosome mis-segregation and cell death.[17,18] Several models of the cohesin structures that maintain sister chromatid pairing remain actively debated.[19]

A series of findings now reveal that RFC complexes also exhibit critical roles in sister chromatid pairing reactions.[20-24] Early studies revealed that Ctf18p-RFC promotes sister

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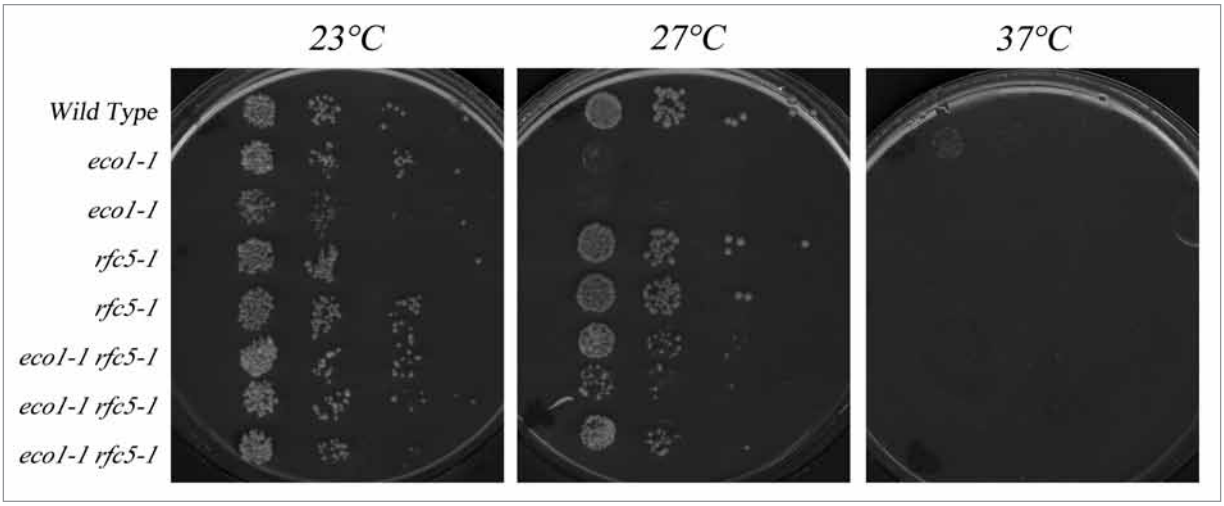

Figure 1. *rfc5-1* mutation suppresses *ctf7*$^{eco1-1}$ mutant cell conditional growth. Growth of 10-fold serial dilutions of wildtype, *ctf7*$^{eco1-1}$ and *rfc5-1* single mutant strains compared to that of *ctf7*$^{eco1-1}$ *rfc5-1* double mutant strains (three independent isolates shown). Colony growth shown for cells on rich medium plates maintained at 23°C, 27°C and 37°C.

chromatid pairing reactions and is capable of binding the establishment factor Ctf7p/Eco1p in vitro.[17,20-22] More recent findings reveal that mutation in Elg1p-RFC also elicits sister chromatid pairing defects and that Elg1p binds Ctf7p/Eco1p in vitro.[23,24] Importantly, however, loss of *ELG1* rescues *ctf7/eco1* mutant cell defects, in stark contrast to the lethality resulting from loss of *CTF18* in *ctf7/eco1* mutant cells. A simple interpretation of these findings is that Ctf18p-RFC and Elg1p-RFC perform opposing pro- versus anti-establishment activities, respectively, and that the activities of these RFC complexes are directed by the associations of the unique large RFC subunit.[25] Large RFC subunits are commonly considered as directing alternate RFC complex functions in DNA replication and DNA repair pathways.

Despite the vast majority of studies that focus on the role of large RFC subunits in dictating RFC complex functions in DNA replication, repair and sister chromatid cohesion, there is limited evidence that small subunit assemblies are sufficient for RFC activity such as clamp unloading.[26] Here, we report on a novel and critical role for the small RFC subunit Rfc5p in directing anti-establishment activities of alternate RFC complexes and discuss new models regarding the regulatory mechanisms that drive RFC functions in vivo.

Results

Rfc5p directs RFC-dependent sister chromatid pairing regulation. Ctf18p-RFC is designated a pro-establishment factor in part based on observations that *CTF18* deletion is lethal when combined with *ctf7*^{eco1} mutations.[17] Conversely, Elg1p-RFC is categorized as an anti-establishment factor since *ELG1* deletion rescues *ctf7*^{eco1} mutant cell growth and cohesion defects.[23,24] RFC small subunits must also contribute to cohesion dependant activities given that mutations in *RFC4* or *RFC5* produce cohesion defects.[21,22] Despite these findings, analyses that test for the role of small RFC subunits in directing RFC complex function toward either pro- or anti-cohesion establishment are absent from the literature. We reasoned that diminishing Rfc5p function in *ctf7*^{eco1} mutant cells might produce a balanced reduction of Ctf18p-RFC and Elg1p-RFC and would thus be transparent to cohesion pathways. Alternatively, if small RFC subunits contribute in a biased fashion to cohesion regulation, then diminished Rfc5p function might elicit defects specifically in either establishment or anti-establishment activities. This difference is resolvable by testing for lethality or rescue of *ctf7*^{eco1} mutant cell phenotypes, respectively.[19] To differentiate between these possibilities, *ctf7*$^{eco1-1}$ mutant cells were crossed to *rfc5-1* cells, sporulated and the resulting diploids dissected. We obtained the predicted number of single mutant *ctf7*^{eco1} and *rfc5* cells and also of double mutant *ctf7*^{eco1} *rfc5* cells. Thus, the combination of *rfc5* and *ctf7*^{eco1} does not phenocopy the lethality of *ctf7 ctf18* double mutant cells. We then tested whether *rfc5-1* exacerbates or rescues conditional growth defects of *ctf7*^{eco1} mutant cells. Serial dilutions of log phase wild type, single and double mutant cell cultures were plated onto rich media and maintained at either 23°C, 27°C or 37°C. As expected, both *ctf7*^{eco1} and *rfc5* single mutant cells are inviable at 37°C. *ctf7*^{eco1} mutant cells exhibit extreme temperature sensitivity in that these cultures are inviable even at 27°C (**Fig. 1**). Surprisingly, the addition of the *rfc5* mutation rescues *ctf7*^{eco1} conditional lethality such that *ctf7 rfc5* double mutant cells exhibit growth at temperatures otherwise lethal for *ctf7*^{eco1} mutant cells (**Fig. 1**). We note that the *rfc5*-dependent rescue of *ctf7*^{eco1} mutant cell defects appears identical to that produced by deletion of *elg1* in *ctf7*^{eco1} mutant cells.[23] Moreover, the additional deletion of *ELG1* from *rfc5-1 ctf7*$^{eco1-1}$ mutant cells did not further enhance growth, suggesting that Elg1p and Rfc5p operate through a common pathway (data not shown). In combination, these studies suggest that the small Rfc5p subunit is a critical regulator of RFC complex function during sister chromatid pairing reactions.

Rfc5 supports cohesin maintenance pathways. While deletion of *ELG1* rescues *ctf7/eco1* establishment defects, it greatly

exacerbates the conditional growth observed in *smc1*, *smc3*, *mcd1/scc1* and *scc3/irr1* mutant cells (cohesion maintenance) and also in *scc2* and *scc4* (cohesin deposition) mutant cells.[23,24] Thus, it becomes important to test whether Rfc5p similarly supports cohesin maintenance pathways. *rfc5-1* and *mcd1-1/scc1* single mutant strains were crossed, the diploids sporulated and dissected to obtain wild type, single and double mutant cells. We obtained roughly the expected number of *rfc5-1 mcd1-1/scc1* double mutant cells, along with single mutant and wildtype strains. Serial dilutions of log phase strains were plated onto rich medium and grown at a range of temperatures. As expected, *rfc5-1* and *mcd1-1/scc1* single mutant cells exhibit robust growth at temperatures up to 30°C but are inviable when maintained at 37°C. In contrast, *rfc5-1 mcd1-1/scc1* double mutant cells are largely inviable at 30°C (**Fig. 2**). A single revertant *rfc5-1 mcd1-1/scc1* double mutant spores is currently under investigation. We repeated our analysis but this time using a different cohesin complex mutant strain, *smc3-5* cells. Serial dilutions of log phase strains obtained from sporulated diploids of *rfc5-1* crossed to *smc3-5* revealed that diminished Rfc5 adversely affects *smc3-5* mutant cell growth such that the double mutant cells are largely inviable at 32° (**Fig. 3**). We decided to include *elg1* deletion strains in the above crosses to aide in the comparison of *elg1* and *rfc5* effects. The results show that, similar to *rfc5-1*, deletion of *elg1* from *smc3-5* mutant cells greatly exacerbates the conditional growth phenotype. Even at the permissive temperature of 23°C, *elg1 smc3-5* double mutant cells exhibit diminished growth. This adverse effect was less detectable in *rfc5-1 smc3-5* double mutant cells (**Fig. 3**). These findings reveal the participation of Rfc5p in supporting cohesion maintenance pathways.

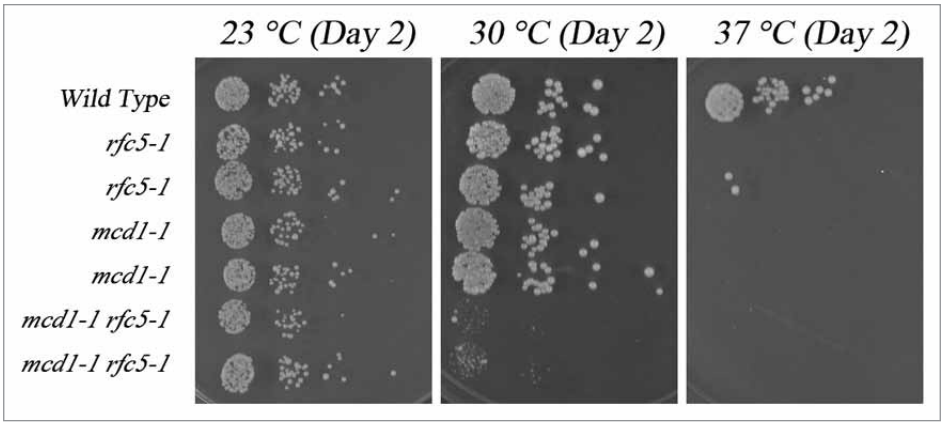

Figure 2. *rfc5-1* mutation exacerbates *mcd1-1* mutant cell conditional growth. Growth of 10-fold dilutions of wild type, *rfc5-1* and *mcd1-1* single mutant strains and *rfc5-1 mcd1-1* double mutant strains (two independent isolates shown). Colony growth shown on rich medium plates maintained at 23°C, 30°C and 37°C.

Rfc5 supports Scc2-dependent cohesin deposition pathways. The conversion of cohesins to a paired state (establishment) occurs only after cohesin deposition onto chromatin. Indeed, numerous studies document that deposition and establishment are temporally and genetically separable.[17,18,27,28] We decided to exploit these differences to test directly whether Rfc5p small subunit supports sister chromatid pairing accruing through cohesin deposition pathways. *rfc5-1* and *scc2-4* single mutant cells were mated, the diploids sporulated and wild type, single and double mutant strains isolated. We included *elg1* deletion strains in the matings for comparison. Serial dilutions of the resulting log phase cultures confirmed that both *rfc5-1* and *scc2-4* single mutant strains are inviable at 37°C but exhibit fairly robust growth at 30°C. However, the results show that both *scc2-4 elg1* and *scc2-4 rfc5* double mutant strains exhibit greatly exacerbated growth defects even at 30°C, with *elg1* deletion producing the more significant challenge to *scc2-4* mutant cell viability (**Fig. 4**). Thus, RFC complex functions are crucial very early in cohesion pathways and specifically during S-phase-dependent cohesin deposition and possibly as early as late G_1 phase.

Pds5 exhibits both pro- and anti-establishment activities. Pds5p binds cohesins and Pds5p activity is essential for maintaining cohesion until anaphase onset. In addition, several lines of evidence document that Pds5p also regulates cohesion by exhibiting anti-establishment activities.[29,30] Despite the importance of Pds5p in cohesion, the mechanism through which it performs anti-establishment activity remains unclear. We decided to capitalize on our findings that either *RFC5* or *ELG1* deletion rescue establishment mutant cell phenotypes but aggravate cohesin maintenance and deposition mutant cell phenotypes. To identify in which pathway Pds5p may function, *elg1* and *rfc5-1* single mutant strains were individually crossed to *pds5-1* single mutant cells and the resulting diploids sporulated. Serial dilutions of the resulting wild type, single and double mutant cells were plated onto rich medium and colony growth challenged at a range of temperatures. Diminished Rfc5p function neither rescues nor aggravates *pds5-1* mutant cell growth defects within the range of temperatures tested (**Fig. 5**). Surprisingly, however, the results show that deletion of *ELG1* suppresses *pds5-1* mutant cell growth defects even at 37° (**Fig. 5**). This current study, in combination with prior studies, reveal that *elg1* deletion suppresses defects that occur through establishment mechanisms.[23,24] These findings suggest that Pds5p exhibits an establishment activity in addition to a documented role as an anti-establishment factor.

Discussion

Historically, the role of Rfc1p-RFC complex has been relatively uni-dimensional: to load/unload PCNA sliding clamp at primer template sites to ensure processive DNA replication. With the identification of multiple large RFC subunits (Ctf18p, Rad24p and Elg1p) unique to alternate RFC complexes, this role expanded in numerous and important ways. First, the role of RFCs was extended to include DNA damage response and checkpoint function. Second, at least one RFC was found to

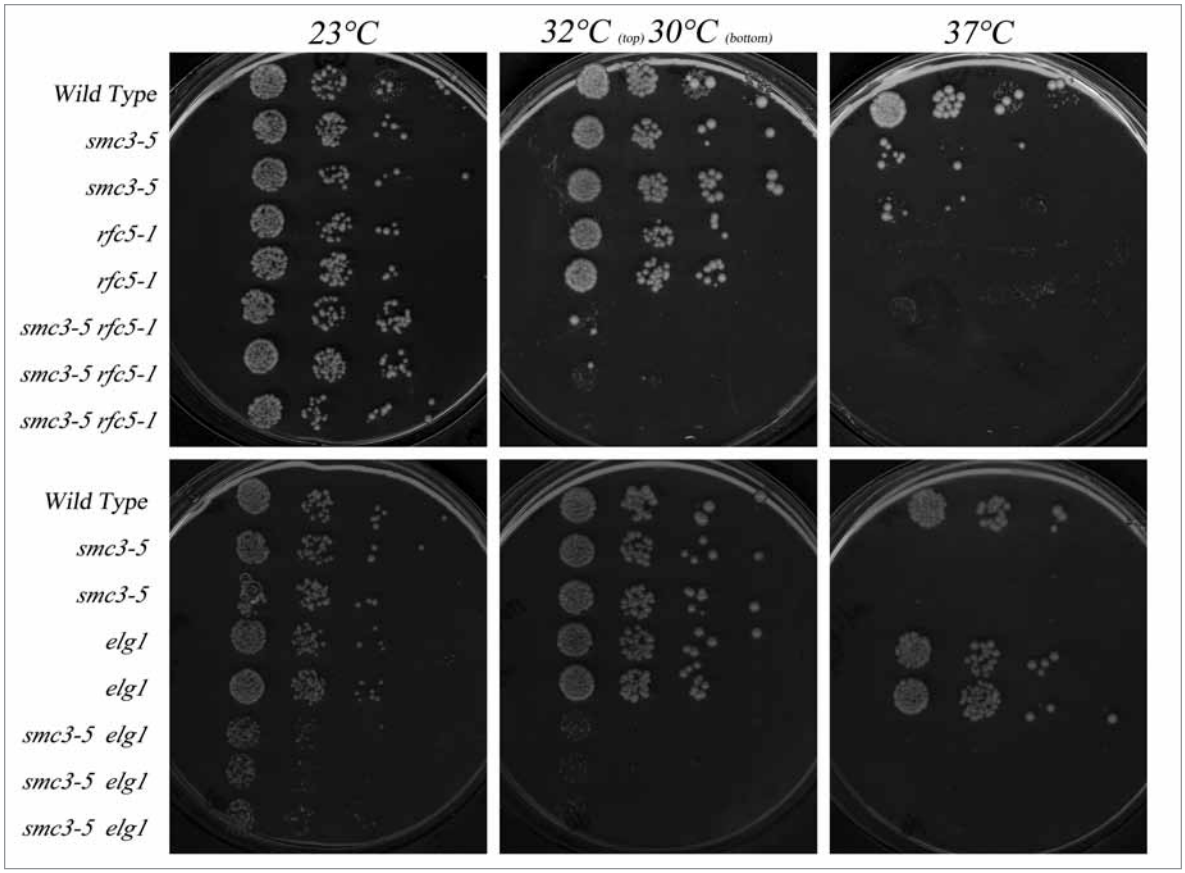

Figure 3. Growth analyses of *smc3-5* cohesion-defective strains in the presence of additional *rfc5-1* or *elg1* mutations. Top row: *rfc5-1* mutation exacerbates *smc3-5* mutant cell conditional growth. Growth of 10-fold dilutions of wild type, *rfc5-1* and *smc3-5* single mutant strains and *rfc5-1 smc3-5* double mutant strains (three independent isolates shown). Colony growth shown on rich medium plates maintained at 23°C, 30°C, 32°C and 37°C. Minor *E. coli* colonies also present in wild type patch. Bottom row: *elg1* deletion exacerbates *smc3-5* mutant cell conditional growth. Growth of 10-fold dilutions of wild type, *elg1*and *smc3-5* single mutant strains and *elg1 smc3-5* double mutant strains (three independent isolate shown). Colony growth shown on rich medium plates maintained at 23°C, 30°C and 37°C.

load/unload the 9-1-1 sliding clamp (Rad17p, Mec3p, Ddc1p) instead of PCNA.[11,31,32] Third, characterization of RFC complexes that incorporate Ctf18p revealed a pro-establishment activity crucial for sister chromatid pairing.[17,20,21] More recently, the role in cohesion expanded even further to include an Elg1p-RFC-dependent anti-establishment activity.[23,24] Up to this point, the various roles for RFC complexes were attributed to the identity of the unique large RFC subunit. Here, we challenge this notion and show for the first time that small RFC subunits such as Rfc5p play a critical role in determining RFC complex function. While our data is specific for sister chromatid cohesion reactions, we speculate that small RFC subunit-directed activities may be wide-spread and predict similar findings in regulating DNA replication and various DNA repair pathways.

Our finding that Rfc5p is capable of biasing entire RFC complex function with respect to cohesion suggests several mechanisms (**Fig. 6**). For instance, small subunits may contribute *directly* to biasing RFC complex function (**Fig. 6A**). Possible scenarios include that small subunits such as Rfc5p provide a cooperative binding site for cohesins or cohesin deposition-modified chromatin crucial for directing/recruiting RFC complex function. Small RFC subunits may also act directly in cohesion as sensors or signal transducers, consistent with Rfc5p function in checkpoint pathways.[33-35] Alternatively, Rfc5p may indirectly regulate RFCs by differentially regulate large subunit association in response to environmental conditions. In the current study, this particular allele of Rfc5p may inactivate the Elg1p-RFC anti-establishment complex but not the Ctf18p-RFC pro-establishment complex via differential binding (**Fig. 6B**). In either case, these scenarios highlight novel mechanisms through which small subunits may regulate RFC complex functions. While less likely, we further speculate that large RFC subunits may associate with some but not all small RFC subunits in differentiating between pro- and anti-establishment activities. In support of this latter model is evidence that Ctf18p binds Dcc1p and Ctf8p during cohesion establishment and that small RFC subunits appear to perform separable reactions in cohesion and DNA repair (**Fig. 6B**).[20-22,30,36] Lastly, RFC small subunit assemblies devoid of large subunits are competent to drive sliding clamp loading and this suggests that small subunits may be capable of forming a unique anti-establishment clamp loader/unloader

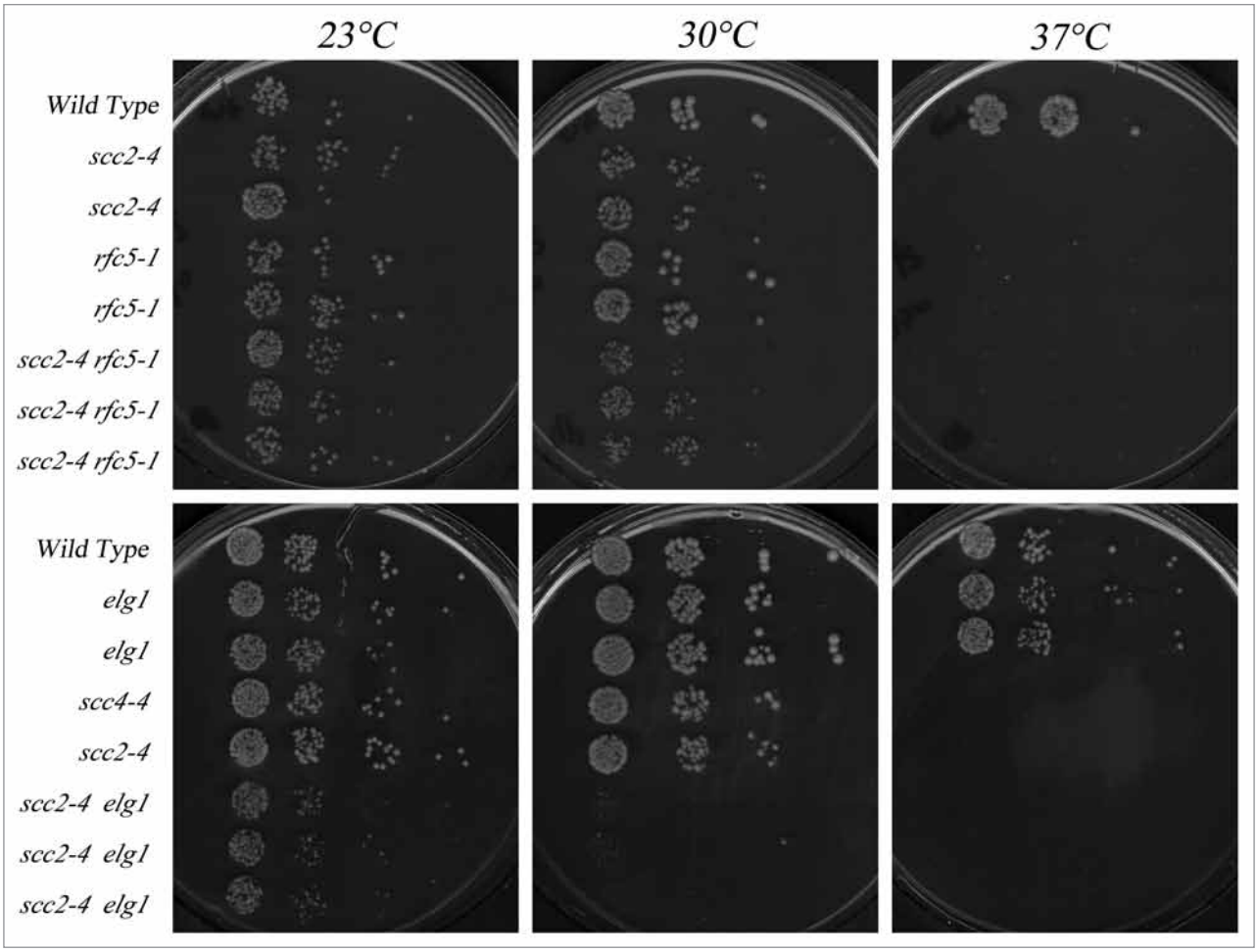

Figure 4. Growth analyses of *scc2* cohesion-defective strains in the presence of additional *rfc5-1* or *elg1* mutations. Top row: *rfc5-1* mutation exacerbates *scc2-4* mutant cell conditional growth. Growth of 10-fold dilutions of wild type, *rfc5-1* and *scc2-4* single mutant strains and *rfc5-1 scc2-4* double mutant strains (three independent isolates shown). Colony growth shown on rich medium plates maintained at 23°C, 30°C and 37°C. Bottom row: *elg1* deletion exacerbates *scc2-4* mutant cell conditional growth. Growth of 10-fold dilutions of wild type, *elg1* and *scc2-4* single mutant strains and *elg1 scc2-4* double mutant strains (three independent isolates shown). Colony growth shown on rich medium plates maintained at 23°C, 30°C and 37°C.

complex.[26] While the current study focuses on the small Rfc5p subunit in cohesion, these findings are likely to have broad implications regarding subunit contributions to DNA replication and directing alternate RFC complexes between various DNA repair pathways.

The mechanism through which Elg1p-RFC opposes Ctf7p/Eco1p activity is poorly understood. Our finding that deletion of *ELG1* suppresses the growth defects of *pds5* mutant cells suggests that Pds5p promotes establishment in coordination with Ctf7p/Eco1p activity. In support of this model are early studies that document an essential role for Pds5p in maintaining sister chromatid pairing.[37-39] A pro-establishment role is further supported by findings that Ctf7p/Eco1p physically binds Pds5p in vitro and that mutations of either is highly sensitive to changes in dosage of the other.[40] The combination of these findings is in stark contrast to the characterization of Pds5 as an anti-establishment factor.[29,41,42] The current study unambiguously points to the duality of pro- and anti-establishment regulation of sister chromatid pairing by Pds5p. We speculate that Pds5p functions in a multistep pathway in which its binding partners, modifications or other regulatory mechanisms switch Pds5p between pro- and anti-establishment activities. It is thus important that the combined impact of these studies direct new investigations aimed at elucidating the bi-functionality of factors involved in sister chromatid pairing reactions.

In several, but not all, instances we have uncovered evidence separating Elg1p function from that of Rfc5p. First, *elg1 smc3-5* double mutant cells exhibit diminished growth beyond that observed for *rfc5-1 smc3-5* double mutants. Second, *elg1* deletion produced more significant challenges to *scc2-4* mutant cell viability than the *rfc5-1* hypomorph. Third and most importantly, deletion of *ELG1* (but not expression of *rfc5-1* hypomorph) dramatically suppressed *pds5-1* mutant cell growth defects even at elevated temperatures. Despite these differences, both *elg1* and *rfc5-1* suppress *ctf7/eco1* mutant cell phenotypes to an identical extent. These findings obviate concerns that *rfc5-1* is simply mimicking *elg1* deletion. More importantly, they reveal the under-appreciated complexity of RFC complex function in

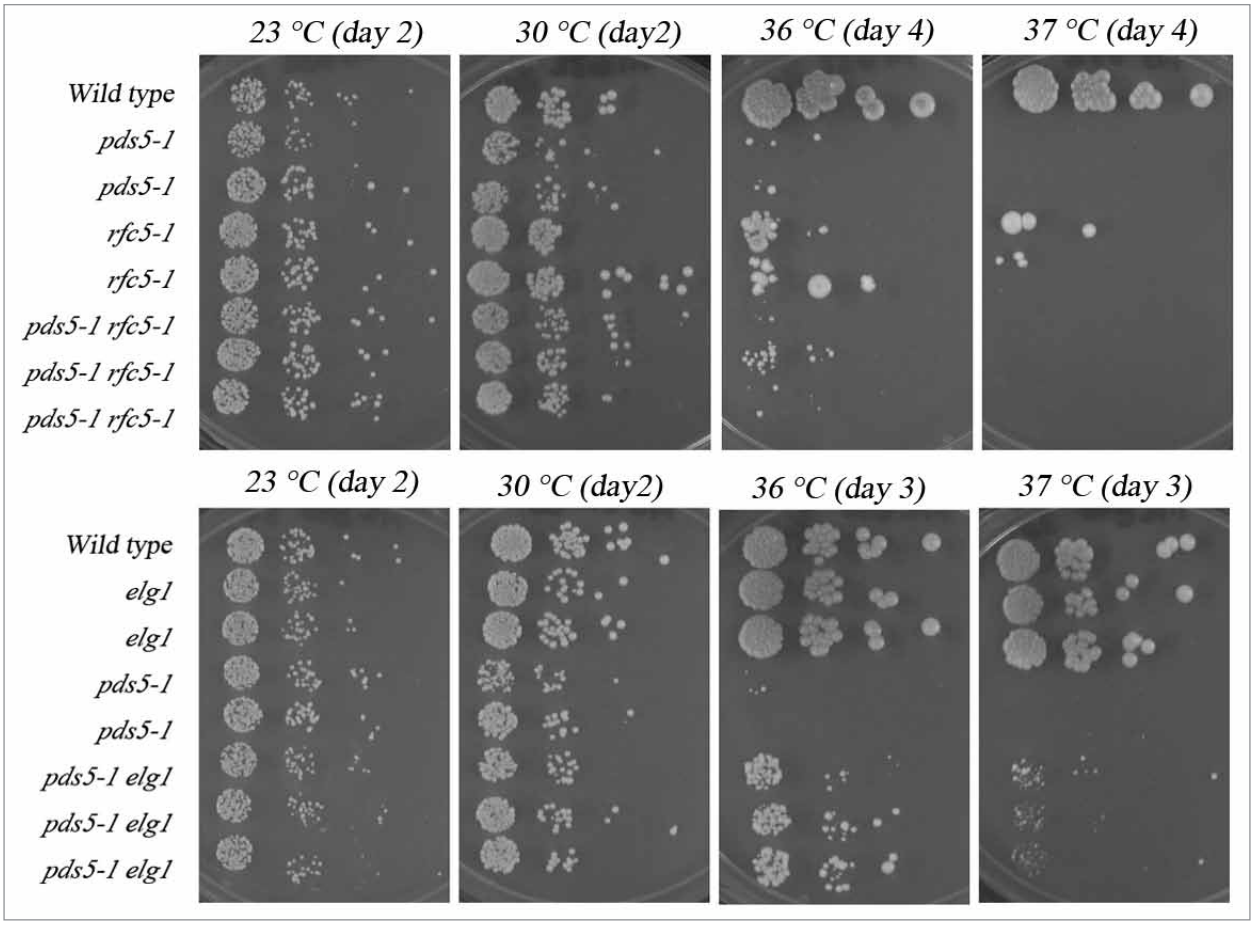

Figure 5. Growth analyses of *pds5-1* cohesion-defective strains in the presence of additional *rfc5-1* or *elg1* mutations. Top row: *rfc5-1* mutation neither suppresses nor exacerbates *pds5-1* mutant cell conditional growth. Growth of 10-fold serial dilutions of wildtype, *pds5-1* and *rfc5-1* single mutant strains and *pds5-1 rfc5-1* double mutant strains (three independent isolates shown). Colony growth shown for cells on rich medium plates maintained at 23°C, 27°C and 37°C. Bottom row: *elg1* deletion suppresses *pds5-1* mutant cell conditional growth. Growth of 10-fold serial dilutions of wildtype, *pds5-1* and *elg1* single mutant strains compared to *pds5-1 elg1* double mutant strains (three independent isolates shown). Colony growth shown for cells on rich medium plates maintained at 23°C, 27°C and 37°C.

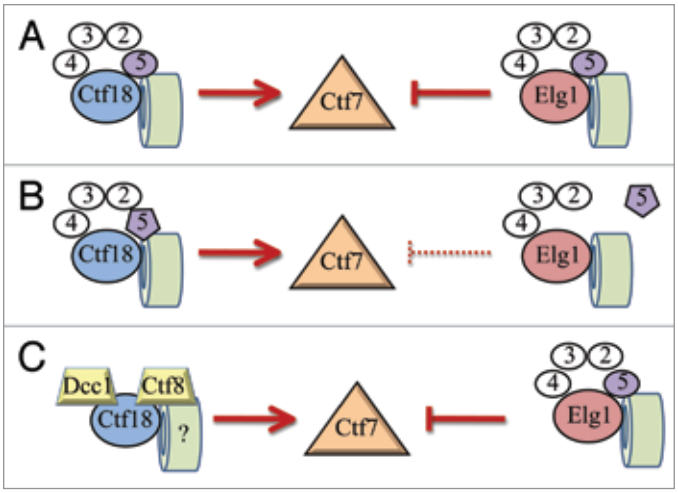

Figure 6. Possible roles for the small Rfc5p subunit in directing RFC complex function. (A) Contrasting roles of wildtype RFCs in regulating Ctf7p/Eco1p function. Ctf18p and Elg1p (large blue and salmon balls, respectively) both uniquely associate with Rfc2p-Rfc5p (small white balls, Rfc5p in purple) and also with PCNA (green cylinder) to regulate Ctf7p/Eco1p-dependent sister chromatid pairing. RFC arrangements as depicted previously.[44,45] (B) rfc5-1p mutant protein (purple small pentagon) may be deficient in binding Elg1p but not Ctf18p, suggesting a role for Rfc5p in specifying large subunit recruitment or activation. (C) A highly speculative model in which Ctf18p can independently bind Dcc1p and Ctf8p (independent of RFC small subunits) to promote cohesion. Alternatively, Rfc5p may play a sensory role—possibly in detecting chromatin contexts (possibly required for cohesin deposition) normally involved in cohesion establishment (not shown).

Table 1. Strains used in this study

YMM547	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	This study
YMM548	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rfc5-1:LEU*	This study
YMM549	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rfc5-1:LEU*	This study
YMM550	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 scc2-4*	This study
YMM551	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 scc2-4*	This study
YMM552	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rfc5-1:LEU2 scc2-4*	This study
YMM553	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rfc5-1:LEU2 scc2-4*	This study
YMM554	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rfc5-1:LEU2 scc2-4*	This study
YMM555	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	This study
YMM556	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rfc5-1:LEU2*	This study
YMM557	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rfc5-1:LEU2*	This study
YMM558	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 smc3-5*	This study
YMM559	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 smc3-5*	This study
YMM560	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rfc5-1:LEU2 smc3-5*	This study
YMM561	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rfc5-1:LEU2 smc3-5*	This study
YMM562	*Mata ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rfc5-1:LEU2 smc3-5*	This study
YMM563	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	This study
YMM564	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 elg1::KAN*	This study
YMM565	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 elg1::KAN*	This study
YMM566	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 scc2-4*	This study
YMM567	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 scc2-4*	This study
YMM568	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 scc2-4 elg1::KAN*	This study
YMM569	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 scc2-4 elg1::KAN*	This study
YMM570	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 scc2-4 elg1::KAN*	This study
YMM972	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	This study
YMM514	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rfc5-1:LEU2*	This study
YMM974	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rfc5-1:LEU2*	This study
YMM975	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 mcd1-1*	This study
YMM976	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 mcd1-1*	This study
YMM977	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 mcd1-1 rfc5-1:LEU2*	This study
YMM978	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 mcd1-1 rfc5-1:LEU2*	This study
YMM608	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	This study
YMM609	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 smc3-5*	This study
YMM610	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 smc3-5*	This study
YMM611	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 elg1::KAN*	This study
YMM612	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 elg1::KAN*	This study
YMM613	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 elg1::KAN smc3-5*	This study
YMM614	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 elg1::KAN smc3-5*	This study
YMM615	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 elg1::KAN smc3-5*	This study
YMM624	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	This study
YMM625	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 eco1-1:ADE2*	This study
YMM626	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 eco1-1:ADE2*	This study
YMM627	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rfc5-1:LEU2*	This study
YMM628	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rfc5-1:LEU2*	This study
YMM629	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 eco1-1:ADE2 rfc5-1:LEU2*	This study
YMM630	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 eco1-1:ADE2 rfc5-1:LEU2*	This study
YMM631	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 eco1-1:ADE2 rfc5-1:LEU2*	This study
YMM632	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	This study

All strains are contained within the W303 background unless otherwise noted.

Table 1. Strains used in this study

YMM633	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 pds5-1*	This study
YMM634	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 pds5-1*	This study
YMM635	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rfc5-1:LEU2*	This study
YMM636	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rfc5-1:LEU2*	This study
YMM637	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rfc5-1:LEU2 pds5-1*	This study
YMM638	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rfc5-1:LEU2 pds5-1*	This study
YMM639	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 rfc5-1:LEU2 pds5-1*	This study
YMM840	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	This study
YMM841	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 elg1::KAN*	This study
YMM842	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 elg1::KAN*	This study
YMM843	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 pds5-1*	This study
YMM844	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 pds5-1*	This study
YMM845	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 elg1::KAN pds5-1*	This study
YMM846	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 elg1::KAN pds5-1*	This study
YMM847	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 elg1::KAN pds5-1*	This study

All strains are contained within the W303 background unless otherwise noted.

cohesion pathways—and likely also those in DNA replication and repair.

Materials and Methods

Yeast strains and media. All strains used in this study are W303 background unless otherwise noted (**Table 1**). Diploid strains were sporulated in 0.3% potassium acetate and tetrads dissected on YPD media.[43] The genotypes of the resultant spores were analyzed for each wild type, single and double mutant spore recovered. Phenotypic analyses of isolated spores was performed as previously described.[23] Briefly, log phase cultures grown in rich liquid YPD medium were normalized and used to generate 10-fold serial dilutions of each strain. Each dilution series was plated on rich YPD agar plates and grown at a range of temperatures as indicated in each figure.

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