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Report

Budding Yeast Wpl1(Rad61)-Pds5 Complex Counteracts Sister Chromatid Cohesion-Establishing Reaction

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Summary

Sister chromatid cohesion, which is mediated by the cohesin complex, is vital for faithful segregation of chromosomes in mitosis and meiosis (reviewed in [1]). Cohesion is established during S phase, and this process requires the function of the acetyltransferase Eco1/Ctf7 [2–5]. The mechanism of the cohesion establishment is, however, still unclear. Here, we describe isolation and identification of genetic suppressors of budding yeast *eco1-1* temperature-sensitive mutant. By using a recently described microarray-based method [6], we successfully mapped 11 intergenic suppressor mutations in two genes, *wpl1* (also known as *rad61*) and *pds5*. Pds5 is a known accessory factor of cohesin complex [7–11], and we show that Wpl1/Rad61 protein forms a complex with Pds5 and colocalizes with cohesin on chromosomes, as its presumed human homolog Wapl [12, 13]. Impaired function of Wpl1-Pds5 complex makes Eco1 dispensable for cell survival. We also provide evidence that Wpl1 is required for efficient association of cohesin with G2 phase chromosomes and that Eco1 promotes dissociation of Wpl1-Pds5 from cohesin via acetylation of Smc3, a cohesin subunit. Taken together, the presented data suggest that Wpl1-Pds5 complex is inhibitory for cohesion establishment and that Eco1 establishes cohesion by hindering the function of Wpl1-Pds5 temporally in S phase.

Results and Discussion

Genetic Suppressors of Budding Yeast *eco1-1* Mutant

Among factors involved in sister chromatid cohesion establishment, Eco1 is the only known essential protein and has the biggest impact on cohesion [3, 4, 14–17]. To gain an insight to how Eco1 functions, we isolated genetic suppressors of the budding yeast temperature-sensitive (ts) *eco1-1* mutant. We spread about 10^9 cells of suppressor-free *eco1-1* mutant onto plates at 30°C and obtained 20 phenotypic revertants (*r1* to *r20*) that gained the ability to grow at the restrictive temperature due to a spontaneously occurred suppressor mutation (Figure S1 available online). Among them, 12 revertants showed suppression even at a higher temperature, and in the following study, we further analyzed these relatively strong revertants.

Though genetic suppressor provides a powerful tool to explore gene function and interaction, identification of suppressor mutations is a challenging task with conventional genetic methods. To overcome the problem, we employed a recently described approach to detect a single-nucleotide change in a genome by using a high-density DNA tiling array [6] (see Supplemental Experimental Procedures). The array analysis combined with subsequent genetic analysis allowed us to map the suppressor mutations of all of the strong revertants (Figures 1A–1C and Table S2). Six suppressors were located in the *wpl1/rad61* gene, five were located in the *pds5* gene, and the remaining one was an intragenic suppressor of the *eco1* gene itself. Some of the identified suppressors were cloned and reintroduced into the parental *eco1-1* strain, verifying that they were bona fide suppressor mutations (Table S2). *WPL1/RAD61* is a nonessential gene, and cells lacking Wpl1 are radiation sensitive [18, 19]. We found that WPL1 depletion also suppresses *eco1-1* cells (Figures 1D and S2A). In addition, we discovered that *wpl1* deletion, as well as *pds5* suppressor mutations (*pds5-r10* and *pds5-r14*), allowed growth of cells completely lacking Eco1 (Figures 1D, 1E, and S2A). Thus, Eco1's essential function for cell survival is dispensable in these suppressor mutants.

Suppressor Mutants Partly Restore Cohesion Defects in *eco1* Mutant

We examined whether the suppressor mutations restored sister chromatid cohesion in *eco1* mutant by analyzing the GFP-marked *URA3* locus [20] in metaphase-arrested cells. Δ*wpl1* and *pds5-r10* mutations greatly, though not perfectly, reduced the proportion of cells with clearly separated (>0.5 μm apart) two GFP-marked *URA3* loci in Δ*eco1* background (Figures 1F and S3A), indicating that they partly compensate for the loss of Eco1 function in sister chromatid cohesion. Note that, in cohesion-proficient cells, two *URA3* loci on sister chromatids are observed as a single focus of GFP signal. In this assay, we noticed that a significant fraction of Δ*eco1* Δ*wpl1* and Δ*eco1* *pds5-r10* cells showed closely separated GFP dots (<0.5 μm apart). This type of separation seems to represent cohesion loss only at a local chromosomal region rather than along the entire length of the chromosome because a GFP-marked centromere locus [21] showed a significantly smaller fraction of cells with closely separated GFP dots (Figure S3B). Note that centromeres are the major cohesin-binding sites and resist separation in nocodazole-treated cells [22].

We also found that Δ*eco1* Δ*wpl1* cells showed marked sensitivity to methyl methanesulfonate (MMS) and benomyl, DNA-damaging and microtubule-destabilizing reagents, respectively (Figure S5). Eco1's roles other than cohesion establishment during S phase [22–24] might not be restored by *wpl1* deletion.

Yeast Wpl1 and Pds5 Form a Complex

Pds5 protein physically interacts with cohesin in a salt-sensitive manner [8, 9, 11]. Human Pds5 forms a subcomplex with

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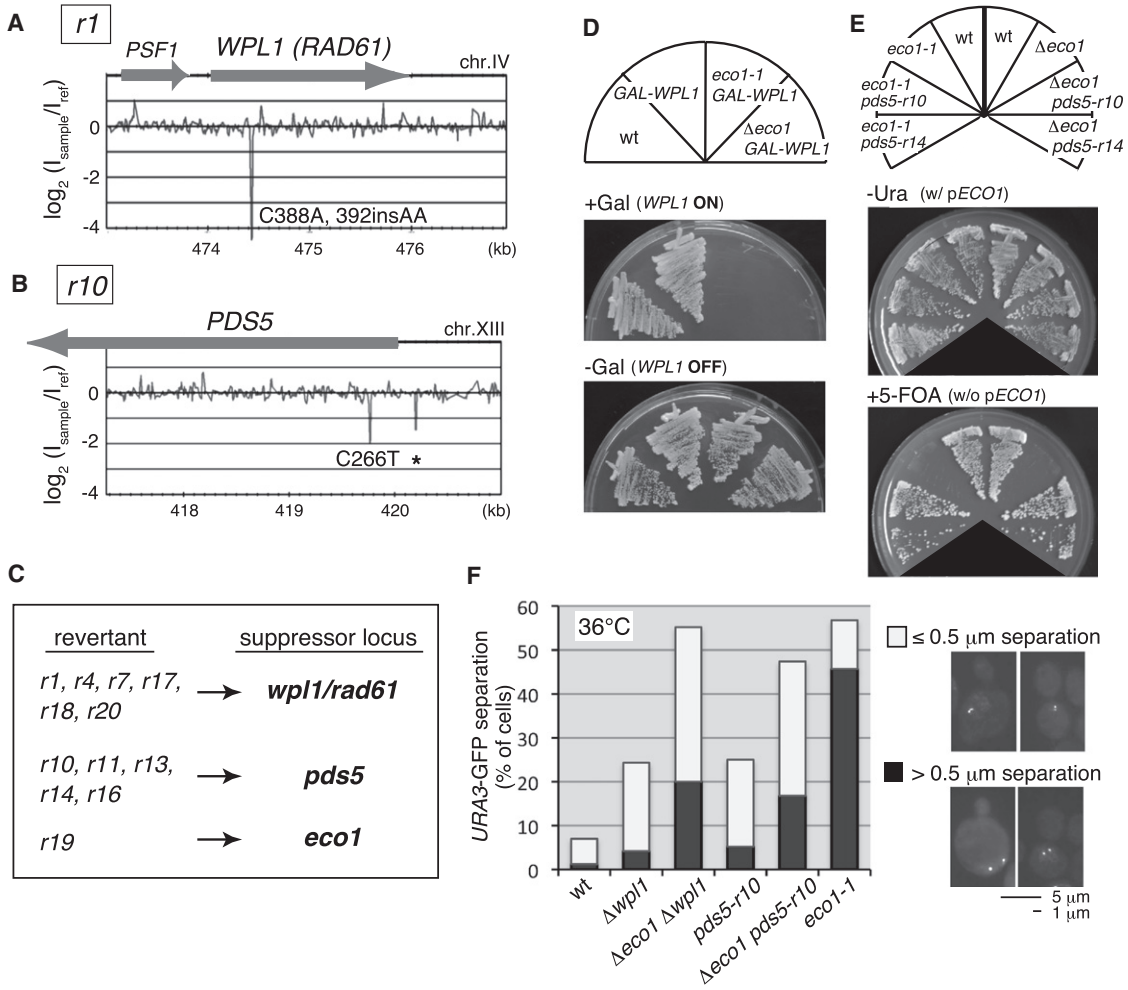

Figure 1. Identification of *eco1-1* Genetic Suppressors

(A and B) Mutations detected by microarray analysis. *WPL1 (RAD61)* locus in *r1* revertant (A) and *PDS5* locus in *r10* (B). Signal intensity on each probe in hybridization of a revertant genome (I_{sample}) was divided by that of a reference genome, *ECO1/eco1-1* diploid (I_{ref}), and the ratio, averaged within overlapping (about five) probes, was plotted in $\log_2$ scale. The x axis shows a position on a yeast chromosome in kb. Sharp depressions at the N termini of *WPL1/RAD61* and *PDS5* indicate the presence of mutations. The nature of the mutations revealed by sequencing is also indicated. Sequencing identified no mutation at the second depression, indicated by an asterisk in (B).

(C) Summary of the identified suppressor loci.

(D and E) Suppression of *eco1-1* and Δ*eco1* mutants by *WPL1* repression (D) and *pds5-r10* and *pds5-r14* suppressor mutations (E). Incubated at 30°C. Cells with a plasmid containing WT *ECO1* gene and *URA3* marker were used in (E).

(F) Sister chromatid cohesion assay with GFP-marked *URA3* locus. WT (K7100), Δ*wpl1* (ST258), Δ*eco1* Δ*wpl1* (ST286), *pds5-r10* (ST400), Δ*eco1 pds5-r10* (ST401), and *eco1-1* (ST352) strains were arrested in G2/M phase at 36°C, and premature separation of *URA3* locus on chromosome V, marked with GFP, was analyzed. Cells with closely (≤0.5 μm apart; white bars) and distantly (>0.5 μm; black) separated GFP signals were separately counted.

Wapl protein, which shows weak sequence similarity to Wpl1/Rad61 [12, 13]. We hence asked whether Wpl1 is associated with Pds5 and cohesin in budding yeast. FLAG epitope-tagged Wpl1 was coimmunoprecipitated with PK epitope-tagged Pds5 (Figure 2A), indicating that Wpl1 and Pds5 form a complex in vivo. A specific interaction between Wpl1 and Pds5 was confirmed by yeast two-hybrid analysis (Figure S4A). ChIP-on-chip analysis revealed that Wpl1 colocalized with cohesin and Pds5 along chromosomes (Figure S4B) [25]. Similar chromosomal localization of Wpl1 was also reported recently [26]. These results suggest that Wpl1 is the budding yeast ortholog of Wapl.

The notion that Wpl1 and Pds5 function as a single entity was supported also by analysis of *pds5-r10* mutant. We measured the amount of Pds5 associating with chromatin by the ChIP-qPCR method with three primer pairs designed at cohesin-binding sites. Wild-type Pds5 was bound to chromatin at all three loci in S phase, whereas the association was greatly reduced by *pds5-r10* mutation (Figure 2B). Importantly, chromatin association of Wpl1 was also diminished in *pds5-r10* cells, verifying that Wpl1 forms a complex with Pds5. Moreover, this strongly suggests that *pds5-r10* is a partial loss-of-function allele, which retains a minimum function to sustain cell growth.

Because Wpl1-Pds5 interacted with cohesin, we addressed whether a general defect in cohesion can suppress *eco1-1* mutant. However, a mutation in Scc1, a cohesin subunit [20], or Scc2, a factor required for cohesin loading [27], failed to rescue ts growth of *eco1-1* (Figures S2B and S2C). Together, our data indicate that impairment of the Wpl1-Pds5 complex