Fission Yeast Eso1p Is Required for Establishing Sister Chromatid Cohesion during S Phase

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Sister chromatid cohesion is essential for cell viability. We have isolated a novel temperature-sensitive lethal mutant named eso1-H17 that displays spindle assembly checkpoint-dependent mitotic delay and abnormal chromosome segregation. At the permissive temperature, the eso1-H17 mutant shows mild sensitivity to UV irradiation and DNA-damaging chemicals. At the nonpermissive temperature, the mutant is arrested in M phase with a viability loss due to a failure to establish sister chromatid cohesion during S phase. The lethal M-phase arrest phenotype, however, is suppressed by inactivation of a spindle checkpoint. The eso1+ gene is not essential for the onset and progression of DNA replication but has remarkable genetic interactions with those genes regulating the G1-S transition and DNA replication. The N-terminal two-thirds of Eso1p is highly homologous to DNA polymerase ɛ of budding yeast and humans, and the C-terminal one-third is homologous to budding yeast Eco1p (also called Ctf7p), which is required for the establishment of sister chromatid cohesion. Deletion analysis and determination of the mutation site reveal that the function of the Eco1p/Ctf7p-homologous domain is necessary and sufficient for sister chromatid cohesion. On the other hand, deletion of the DNA polymerase ɛ domain in Eso1p increases sensitivity to UV irradiation. These results indicate that Eso1p plays a dual role during DNA replication. The C-terminal region acts to establish sister chromatid cohesion, and the N-terminal region presumably catalyzes translesion DNA synthesis when template DNA contains lesions that block regular DNA replication.

Virtually all eukaryotic cells propagate through a process called the cell cycle that consists of four distinct phases, G1, S, G2, and M, whose principal role is to carry out duplication of the chromosomes and subsequent faithful distribution into daughter cells. Commitment to the initiation of the cell cycle is made at a point in late G1 phase called start or restriction point. In the fission yeast, Schizosaccharomyces pombe, passage through start requires the execution of at least two regulatory systems, Res-Cdc10-Rep (Res1p-Cdc10p and Res2p-Cdc10p-Rep2p) transcriptional factor complexes and Cdc2p-Cig2p/Cyc17p cyclin-dependent kinase complex (reviewed in references 37 and 54). Res-Cdc10-Rep complexes activate cell cycle start-specific transcription genes, which contain a cis regulatory element called the M1α cell cycle box. One of those target genes is cdc18+, whose product is a key component of the preinitiation form of origin replication complex and plays a crucial role in loading origins with the replication machinery including DNA polymerases in cooperation with minichromosome maintenance proteins (reviewed in reference 35). Cdc2p-Cig2p activity is also required for origin firing, but its critical target(s) has not been identified yet. These initiation factors and replication factors are highly conserved throughout eukaryotes.

In order to ensure faithful transmission of the duplicated chromosomes to daughter cells, the duplicated sister chromatids are linked together along their entire region during S phase until mid-M phase (13). In fact, the disorder of this linkage, called sister chromatid cohesion, can result in viability loss (10, 14, 29, 42, 50). Sister chromatid cohesion is formed by a set of proteins that are highly conserved through evolution. In the budding yeast Saccharomyces cerevisiae, there is a multi-protein complex called cohesion which contains at least four subunits: Scc1p/Mcd1p (homologous to fission yeast Rad21p), Scc3p, Smc1p, and Smc3p (reviewed in reference 34). Similarly, cohesion of Xenopus laevis egg consists of five subunits, three of which are homologues of Smc1p, Smc3p, and Scc1p/Mcd1p/Rad21p (23). From late G1 until M phase, Scc1p/Mcd1p stays bound to chromosomes but is removed in anaphase by Esp1p (homologous to fission yeast Cut1p)-dependent cleavage and then degraded in G2 by the anaphase-promoting complex–cyclosome (29, 51). Fission yeast Mis4p, which is homologous to budding yeast Scc2p and also required for sister chromatid cohesion, is not a cohesion subunit and belongs to a distinct class of proteins called adherins, which are not degraded in G2 (10). The role of adherin seems to be to load cohesion complex onto chromosomes in G1 (50). On the other hand, budding yeast Eco1p/Ctf7p, the third class of factors essential for sister chromatid cohesion, appears to be required only for the establishment of cohesion during S phase and not for the loading of adherin or cohesion onto chromosomes or for the maintenance of cohesion after S phase (42, 50).

In this report, we describe the fission yeast eso1+ gene, initially identified as the one responsible for a novel tempera-
ture-sensitive lethal cdc-like mutant that displays cell elongation and abnormal chromosome segregation. The eso1+ gene encodes an essential protein that contains two domains highly homologous to the budding yeast Rad30p or DNA polymerase η (18, 27, 40; reviewed in reference 53) and Eco1p/Ctf7p, respectively; the Eco1p/Ctf7p domain is essential for establishing sister chromatid cohesion, whereas the DNA polymerase η domain is involved in DNA repair as expected.

MATERIALS AND METHODS

Fission yeast strains, media, and genetic methods. The S. pombe mutant strains ade6-22, mis4-242, and rad21-K1 and the strain used for visualization of Cen1-green fluorescent protein (GFP) were described previously (11, 21, 47, 49). Strains were cultured in the complete medium YE or the minimal medium MM (EMM2/PM) (2). A nitrogen (ammonium chloride)-free derivative of MM (referred to as MM-N) was used to synchronize cells in G1. When necessary, minimal medium was supplemented with leucine (250 μg/ml for MM and 50 μg/ml for MM-N) and adenine sulfate (100 μg/ml). Transformations were performed using the lithium acetate procedure as described previously (38), and cells were spread on MMA plates (15). Double mutant strains were obtained by crossing single mutants followed by tetrad analysis. Flow cytometry was performed as described previously (48). UV irradiation was performed with a UV cross-linker (XL-1500 Spectronics Co. Ltd.). Cell numbers were determined with a particle counter (Z1; Beckman Coulter, Inc.). Other general genetic manipulations of S. pombe have been described previously (2, 31).

Libraries and vectors. The S. pombe genomic libraries were constructed by inserting restriction enzyme-digested wild-type (L572) genomic DNA into the pALK vector. The S. pombe cDNA library has been described previously (39, 45). The pALK vector used for genomic DNA expression was constructed by inserting an autonomously replicating sequence (ars1) and a leucine auxotrophic selection marker gene (LEU2 of S. cerevisiae) into the plBluescript II (SK+) vector (Stratagene) whose plasmid replication origin was derived from pBR322. The p. cDNA expression vector contains the LEU2 gene, ars1, and the simian virus 40 promoter to drive the expression of the insert. The pREP81-rad21 plasmid was described previously (49).

Isolation of eso1+ gene. The eso1+ gene was isolated by complementation of the temperature-sensitive eso1-H17 mutant. The h− eso1-H17 leu1-32 cells were transformed with S. pombe genomic libraries and selected on MMA plates first at 23°C for 24 h and then at 33°C for 4 days. Plasmid DNA clones were recovered from candidates and analyzed by Southern hybridization. The eso1+ cDNA was isolated from the cDNA library by colony hybridization and a 5′-RACE (rapid amplification of cDNA ends) PCR method.

Gene disruption. Gene disruption was performed by one-step gene replacement. The 1.2-kb EcoRI-PstI fragment of the eso1+ gene was replaced with a ura4+ cassette. The linear Sprl-XhoI fragment carrying the replaced gene was transformed into the h− h− ade6-M210ade6-M216 leu1-32lei1-32 ura4-D18/ ura4-α diploid strain, and stable Ura+ transformants were selected. The proper replacement of the one wild-type allele with the disrupted eso1 construct was confirmed by Southern blot analysis.

Deletion analysis and multicopy suppression analysis. The eso1+ cDNA clones variously truncated at the 5′ coding region were isolated from the cDNA library and used to construct a series of amino-terminal deletion mutants. A series of carboxyl-terminally truncated mutants were constructed by gradual deletion of a full-length eso1+ cDNA with exonuclease III. All deletion mutants constructed were confirmed for their structure by DNA sequencing and inserted into the pACL vector. The full-length eso1+ gene and its deletion mutants were transformed into h− eso1-H17 leu1-32 cells and plated on MPA. Plates were incubated at 25°C for 24 h and then at 32.5 or 36°C for 4 days or at 25°C for 6 days to determine the numbers of both complemented and stably transfected cells. The complementation (suppression) activities were calculated by dividing the number of colonies formed at the restrictive temperature by the number of colonies formed at 25°C. The ability of various cell cycle control genes to rescue the eso1-H17 mutant was determined similarly.

Nucleotide sequence accession number. The DDBJ-EMBL-GenBank accession no. for eso1+ is AB039861.

RESULTS

Isolation of the eso1-H17 mutant. To search for new genes regulating the cell cycle in fission yeast, we generated several temperature-sensitive cell division cycle (cdc) mutants (32, 46). One mutant names eso1-H17 (essential for S-chromatin organization; see below) was chosen for further study.

A diploid cell heterozygous for eso1+/eso1-H17 was not temperature sensitive and was indistinguishable in growth properties from wild-type diploid cells, indicating that the eso1-H17 allele is recessive. At the restrictive temperature, eso1-H17 mutant cells ceased proliferation with some cells elongated and others rounded (Fig. 1A), showing morphological heterogeneity. Upon a shift to the nonpermissive temperature, the eso1-H17 mutant cells were arrested with a broad peak of G2 DNA content. Cells were grown to mid-log phase at 23°C in MM medium and shifted up to 36°C. Cells were sampled at 2, 4, 6, and 8 h after the temperature shift and analyzed by flow cytometry.

Fig. 1. Phenotypes of the eso1-H17 mutant. (A) The eso1-H17 mutant shows an elongated cell morphology. Wild-type (h− leu1-32) and eso1-H17 (h− eso1-17 leu1-32) cells were inoculated on YE plates, incubated overnight at the indicated temperatures, and observed under the microscope. (B) The eso1-H17 mutant is arrested with a broad peak of G2 DNA content. Cells were grown to mid-log phase at 23°C in MM medium and shifted up to 36°C. Cells were sampled at 2, 4, 6, and 8 h after the temperature shift and analyzed by flow cytometry.

The eso1-H17 mutant was determined similarly.
to a mitotic delay. Thereafter, many cells showed cut (septation in the absence of nuclear division) and missegregation of chromosomes (Fig. 2B, eso1-H17 36°C 4-h panel).

**Arrest of eso1-H17 cells is suppressed by inactivation of a spindle assembly checkpoint.** Mitotic delay and arrest often arise by activation of a spindle assembly checkpoint. To determine whether the mitotic arrest-delay of eso1-H17 cells arose by this mechanism or not, we constructed an eso1-H17 Δmad2 double mutant strain and examined its behavior. Mad2p is not required for normal cell growth but is essential for spindle assembly checkpoint (16, 21). Unlike the eso1-H17 single mutant, the eso1-H17 Δmad2 double mutant was viable and continued to proliferate at 36°C (Fig. 2B, eso1-H17 Δmad2 36°C 4-h panel), indicating that the cell cycle arrest of the original eso1-H17 mutant was caused by the execution of a spindle assembly checkpoint. However, suppression of eso1-H17 by Δmad2 was incomplete, and its temperature sensitivity and missegregation phenotypes persisted to a certain extent in the double mutant (Fig. 2A and C). These results led us to conclude that Eso1p was essential for proper chromosome segregation but dispensable for the step of chromosome segregation per se. Since spindle assembly checkpoint was activated upon inactivation of Eso1p, this conclusion indicates that Eso1p may be required for the proper maintenance of chromosome structure.

**eso1-H17 cells are sensitive to DSB.** In addition to the mitotic defect, the eso1-H17 mutant was sensitive to UV irradiation (Fig. 3A) and exposure to methyl methanesulfonate (MMS) or bleomycin (Fig. 3B) even at the permissive temperature. By contrast, the mutant was slightly resistant to base-modifying 4-nitroquinoline-1-oxide (4NQO) (Fig. 3B). Since, unlike 4NQO, UV, MMS, and bleomycin induce double-strand breaks (DSB), these data show that the eso1-H17 mutant is sensitive to DSB.

Unlike damage checkpoint-deficient rad mutants, the eso1-H17 cells exposed to DSB-inducing chemicals were elongated with a single nucleus and did not show any detectable checkpoint defects (data not shown). These results suggest that the eso1-H17 mutant retains proper DNA damage checkpoint but is defective in either repair of DSB or recovery from DSB-caused cell cycle arrest or both.

**Eso1p is required during S phase.** To determine in which phase of the cell cycle Eso1p is required to function, eso1-H17 cells were synchronized to G1 by nitrogen starvation and then released to start the cell cycle in nitrogen-rich growth medium at the nonpermissive temperature. Cells were harvested every hour and assayed for viability by determining the number of colonies formed at the permissive temperature. The mutant cells entered and proceeded through the phase of DNA replication without any significant delay (Fig. 4A), but their viability decreased steeply between 3 and 6 h, the period in which DNA synthesis took place (Fig. 4B). Abnormal nuclear morphologies were frequently seen between 6 and 8 h, the period of mitosis (Fig. 4B). These results indicate that the function of Eso1p needs to be executed at least during S phase for proper cell cycling.

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**FIG. 2.** Loss of Eso1p function leads to spindle assembly checkpoint-dependent mitotic delay. (A) eso1-H17 cells quickly lost viability upon a shift to the nonpermissive temperature. Wild-type (h2 leu1-32), eso1-H17 (h2 eso1-H17 leu1-32), Δmad2 (h2 mad2::ura4 leu1-32), and eso1-H17 Δmad2 (h2 eso1-H17 mad2::ura4 leu1-32) cells were grown to mid-log phase at 25°C in YEL medium and shifted to 36°C. Cell aliquots were taken at 4 and 8 h after the temperature shift and plated on YEA at 25°C. (B) eso1-H17 cells are defective in chromosome segregation. Cells were grown to mid-log phase at 25°C in YEL medium and shifted to 36°C. Cell aliquots were taken at indicated times, fixed with glutaraldehyde, and stained with DAPI. Arrowheads indicate the cells showing a mitotic delay. Bar, 10 μm. (C) Temperature sensitivity of eso1-H17 cells is partially suppressed by deletion of the mad2p gene. The indicated cells were inoculated on YEA plates and incubated for 3 days at the indicated temperatures.
Premature sister chromatid separation occurs in *eso1-H17* cells. The demonstration of spindle assembly checkpoint-dependent mitotic arrest, abnormal chromosome segregation, and increased sensitivity to DSB-inducing reagents as the major phenotypes of the *eso1-H17* mutant led us to speculate that Eso1p might be involved in sister chromatid cohesion. To investigate this possibility, we used the Cen1-GFP system to visualize the behavior of the centromere DNA of chromosome I in live *eso1-H17* cells (11, 33). Cells were synchronized; released from G1 at the nonpermissive temperature; collected at 2, 4, and 6 h after release; and immediately observed under a fluorescence microscope. DNA replication occurred at around 4 h (Fig. 4A). Unlike wild-type cells, a large number of *eso1-H17* interphase cells had two split Cen1-GFP signals at 4 to 6 h, which proves defective sister chromatid cohesion (Fig. 4C). Photos of cells showing two-split Cen1-GFP dots are shown in Fig. 4D. We thus concluded that Eso1p was essential for the establishment of sister chromatid cohesion during S phase.

Eso1p function is not required for the maintenance of sister chromatid cohesion in G2-M phase. Sister chromatid cohesion is established during DNA replication and maintained until anaphase. To investigate whether Eso1p function is also required for cohesion maintenance, *eso1-H17* cells were grown to saturation in YEL medium. Under these conditions, most of the cells arrested growth with 2n DNA contents (Fig. 4E) with a low septation index (*eso1* mutant, 5.6%; *rad21* mutant, 9.4%), indicating that more than 80% of cells were arrested in G2 phase. The cells were exposed to 36°C, released 3 h later to resume cell cycling in fresh medium at 36°C, harvested every 30 min, and observed for mitosis. As expected, the majority of the *rad21-K1* cells that have a defective cohesin subunit (49) displayed abnormal mitosis as they proceeded through cell cycling, showing that cohesin complexes are also required for the maintenance of sister chromatid cohesion (14) (Fig. 4F, right graph). In contrast, the *eso1-H17* mutant cells progressed through M phase without significant abnormalities (Fig. 4F, left graph). Thus, Eso1p is not required for the maintenance of sister chromatid cohesion once it is established in S phase.

The *eso1* gene encodes a fusion between DNA polymerase and Eco1p/Ctf7p homologues. To clone the defective gene in *eso1-H17* cells, *S. pombe* genomic libraries were screened for those that rescued the temperature-sensitive lethality. Two non-overlapping clones, S1 and Bg1, could rescue the mutant up to 36°C, and further analysis showed that S1 contained the *eso1* gene (see below). Characterization of the multicopy suppressor gene on Bg1 will be described elsewhere.

The S1 clone contained a 9.4-kb SpeI fragment, the internal 6.7-kb SpeI-XhoI fragment of which was found to be active (Fig. 5A). The unique *ParI* site within this fragment was essential for the activity. Nucleotide sequencing revealed the presence of a single open reading frame across the *ParI* site. The predicted polypeptide was an 872-amino-acid protein with an estimated molecular mass of 99 kDa. The nucleotide and predicted amino acid sequences were confirmed by sequencing a corresponding *eso1* cDNA that was isolated subsequently.

The central region of the predicted Eso1p protein possesses two C2H2 zinc finger motifs (Fig. 5B and C). A database search revealed that the amino-terminal two-thirds of Eso1p is strongly related to Rad30p of *S. cerevisiae*. Rad30p protein, recently identified as DNA polymerase η, is a member of a damage-bypass replication protein family, which includes the UmuC and DinB proteins in *E. coli* and the Xeroderma pigmentosum variant gene product in *Homo sapiens* (19, 22, 26, 27, 40, 43; reviewed in reference 53). On the other hand, the carboxyterminal one-third of Eso1p shares significant homology with another protein family conserved among eukaryotes, which includes Eco1p/Ctf7p in *S. cerevisiae* and putative proteins from *Arabidopsis thaliana*, *Mus musculus*, and *H. sapiens* (42, 50). The genes encoding Rad30p and Eco1p/Ctf7p are not contiguous and present on chromosomes IV and VI, respectively, in budding yeast, and there is no Eco1p/Ctf7p-homologous region in the Xeroderma pigmentosum variant gene product in *H. sapiens*.

To obtain definitive evidence for the authenticity of the isolated gene and to confirm the function of *eso1*+, cells lacking the *eso1*+ gene were constructed by one-step gene replacement. The sequence in *eso1*+ corresponding to the carboxy-terminal half of Eso1p was replaced with the *S. pombe urad4*+ gene (Fig. 5A) and transfected into a diploid strain. Diploid cells disrupted for one *eso1*+ allele were identified and confirmed by genomic Southern hybridization. Tetrads analysis of the sporulated diploid cells revealed that only two spores were viable and that all viable spores were uracil auxotrophic (Fig. 5D). Microscopic observation showed that the Δ*eso1* spores germinated but arrested cell cycling after two to three divisions (Fig. 5E). Spore germination analysis showed that the Δ*eso1* cells resembled *eso1-H17* cells and displayed abnormal chromosomes (Fig. 5F). Furthermore, Δ*eso1/eso1-H17* diploid cells were still temperature sensitive for growth and failed to yield any haploid spores that could grow at 36°C (data not shown).
Based on these results, we concluded that the cloned gene was indeed eso1 itself.

The C-terminal region of Eso1p is necessary and sufficient for sister chromatid cohesion. To locate the functional domain of Eso1p, a series of amino- and carboxyl-terminal deletion mutants were constructed, and their ability to rescue the eso1-H17 mutant was determined. As shown in Fig. 6, the Rad30p-homologous region was totally dispensable, and the gene truncated in the entire amino-terminal region (ΔN597) retained nearly all its ability to suppress the mutant. On the other hand, the carboxyl-terminal region (C-terminal Eco1p/Ctf7p homologous region) was absolutely essential for function. Even a deletion of only 16 amino acids from the C terminus (ΔC16) largely abrogated the activity. Similarly, the C-terminal Eco1p/Ctf7p domain (ΔN597), but not ΔC16, suppressed the UV sensitivity of eso1-H17 cells (data not shown), indicating that the UV sensitivity of eso1-H17 cells results from poor establishment of sister chromatid cohesion. Furthermore, the eso1-H17 allele contained a point mutation that resulted in a change from glycine at position 799 to aspartic acid. These results indicate that loss of the function of the Eco1p/Ctf7p-homologous domain in Eso1p caused the eso1-H17 phenotypes.

To confirm the sufficiency of the Eco1p/Ctf7p-homologous domain for the establishment of sister chromatid cohesion, we tested whether the domain could rescue the eso1 null cell. Diploid cells in which one copy of eso1 was deleted were transformed with the eso1" gene deletion mutants described in Fig. 6. These transformants were then sporulated, and spores were allowed to germinate on appropriately supplemented minimal medium plates. Δeso1 cells harboring not only full-length eso1" but also the amino-terminal deletion mutants grew and formed colonies. Nuclear morphology (Fig. 7A), bleomycin sensitivity (Fig. 7B), and growth rate (Fig. 7C) of the Δeso1 cells rescued by ΔN458 or ΔN597 were indistinguishable from those of the Δeso1 cells rescued by full-length eso1". These results indicate that the C-terminal region of Eso1p is sufficient for sister chromatid cohesion and is fully functional without the N-terminal Rad30p-homologous region.

Deletion of the DNA polymerase η domain in Eso1p elevates UV sensitivity. As mentioned above, expression of the C-terminal Eco1p/Ctf7p-homologous region completely suppressed the defect of sister chromatid cohesion of cells lacking eso1". However, unlike those rescued by full-length eso1", the Δeso1 cells rescued by ΔN458 or ΔN597 were still sensitive to UV irradiation (Fig. 7B and D). Because, in the Δeso1 cells, not only the Eco1p/Ctf7p-homologous region but also about 30% of the Rad30p-homologous region was deleted, some of this ethanol and stained with DAPI, and those with abnormal chromosome structures (overcondensed chromosomes, cut, missegregation, etc.) were counted under the fluorescence microscope.

FIG. 4. Eso1p is required for the establishment of sister chromatid cohesion during S phase but not for its maintenance in G₂ and M phases. (A) DNA replication takes place without any significant delay in eso1-H17 mutant cells. Wild-type (h+ leu1-32) and eso1-H17 (h- eso1-H17 leu1-32) cells were grown to mid-log phase at 25°C and then nitrogen starved in MM-N medium for 24 h to be arrested in G₂. Cells were then released by transfer into MM medium preincubated at 36°C. Cell aliquots were taken every hour and analyzed for S-phase signals and wild-type control at 4 h. Bar, 5 μm. (E and F) Eso1p is not essential for the maintenance of cohesion in G₂ and M phases. Cells of eso1-H17 (h- eso1-H17 leu1-32) and rad21-K1 (h- rad21-K1 ura4-1 leu1-32) mutants were grown to saturation in YEL medium at 25°C and then released in fresh YEL medium preincubated at 36°C. Live cells were observed under the fluorescence microscope. (C) Frequencies of cells showing two split signals (1n 2n) were determined. As shown in Fig. 6, the Rad30p-homologous region was totally dispensable, and the gene truncated in the entire amino-terminal region (ΔN597) retained nearly all its ability to suppress the mutant. On the other hand, the carboxyl-terminal region (C-terminal Eco1p/Ctf7p homologous region) was absolutely essential for function. Even a deletion of only 16 amino acids from the C terminus (ΔC16) largely abrogated the activity. Similarly, the C-terminal Eco1p/Ctf7p domain (ΔN597), but not ΔC16, suppressed the UV sensitivity of eso1-H17 cells (data not shown), indicating that the UV sensitivity of eso1-H17 cells results from poor establishment of sister chromatid cohesion. Furthermore, the eso1-H17 allele contained a point mutation that resulted in a change from glycine at position 799 to aspartic acid. These results indicate that loss of the function of the Eco1p/Ctf7p-homologous domain in Eso1p caused the eso1-H17 phenotypes.

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region is likely to be required for the function of the DNA polymerase η domain (Fig. 5A). We thus concluded that loss of the DNA polymerase η domain elevates UV sensitivity and tentatively identified this domain as DNA polymerase η itself.

**eso1** + genetically interacts with adherin and cohesin. The structural and functional similarity of Eso1p to Eco1p/Ctf7p led us to test the possible genetic interactions between **eso1** + and the genes encoding adherin and cohesin subunits. The **eso1**-H17 mutant combined with **mis4**-242, a temperature-sensitive mutation of adherin (10), became more thermosensitive. Unlike **eso1**-H17 and **mis4**-242 single mutants, which grow at temperatures of 32.5 and 31.5°C, respectively, the double mutant failed to grow at 29°C (Fig. 8A) and barely grew at 27°C. Moreover, the **eso1**-H17 mutant was synthetically lethal with **rad21**-K1 mutation. Tetrad dissection of spores from the cross between **eso1**-H17 and **rad21**-K1 yielded no viable double mutant cells. Moreover, as shown in Fig. 8B, the **eso1**-H17 **rad21**-K1 double mutant rescued by the pREP81-**rad21** plasmid did not grow when **rad21** + in the plasmid was repressed.

| complementation (%) | 32.5°C | 36°C |
|---------------------|--------|------|
| full                | 872    |      |
| ΔN60                | 61     | 872  |
| ΔN339               | 340    | 872  |
| ΔN458               | 459    | 872  |
| ΔN597               | 598    | 872  |
| ΔC16                |        | 856  |
| ΔC90                |        | 782  |
| ΔC223               |        | 649  |
| ΔC396               |        | 476  |
| empty vector        | <0.03  | <0.03|

**FIG. 5.** Isolation and characterization of the **eso1** + gene. (A) Restriction map of the **eso1** + gene. The **eso1** + open reading frame is shown by an arrow, and the Eco1p/Ctf7p-homologous domain is shaded. The EcoRI-PstI region of **eso1** + was replaced with a **ura4** + gene cassette for generating an **eso1** null mutant. (B) Schematic illustration of Eso1p of *S. pombe* and Rad30p and Eco1p/Ctf7p of *S. cerevisiae*. The zinc finger motifs are filled. (C) Amino acid homologies between Eso1p, Rad30p, and Eco1p/Ctf7p. The predicted amino acid sequence of Eso1p is shown in a single-letter code and aligned with Rad30p and Eco1p/Ctf7p. Identical amino acids are boxed. The zinc finger motifs are underlined. (D) Cells with deletions of **eso1** + are lethal. Spores from **eso1** +/**eso1**::**ura4** + diploid cells were tetrad dissected on YEA plates and incubated at 30°C for 4 days. (E) Terminal phenotype of **Δeso1** cells. Cells that germinated from two independent **Δeso1** spores on a YEA plate were photographed. (F) DAPI staining of germinating **Δeso1** cells. **Δeso1** spores (Ura +) derived from **eso1** +/**eso1**::**ura4** + diploid cells were preferentially germinated in MM lacking uracil. Germinating cells were fixed with 70% ethanol, stained with DAPI, and photographed under the microscope.

**FIG. 6.** The carboxyl-terminal Eco1p/Ctf7p-homologous domain of Eso1p is essential to rescue the **eso1**-H17 mutant. N-terminally and C-terminally truncated **eso1** + genes were constructed and assayed for the ability to rescue the **eso1**-H17 mutant. The intact Eso1p is shown at the top (referred to as full). The Eco1p/Ctf7p-homologous domain is grey, and the zinc finger motifs are black. The amino acid numbers are shown at each mutant. The ability of the deletion mutants to complement **eso1**-H17 cells is shown in the right columns. For complementation activity, see Materials and Methods.
eso1 genetically interacts with G1-S regulators also. The execution of eso1 function during S phase suggests that eso1 function might be controlled by cell cycle start factors. We, therefore, examined the ability of various cell cycle start genes to rescue the eso1-H17 mutant. As shown in Table 1, the res1 and rep2 genes could partially suppress the temperature sensitivity. The cdc10, res2, and rep1 genes also exhibited weak suppression activities (data not shown). These genes encode a subunit of transcription factor complexes that regulate the cell cycle start-specific transcription of genes (reviewed in references 37 and 54). A major target gene of Res-Cdc10-Rep transcription factor complexes is cdc18, which encodes a component of the prereplicative complex. However, cdc18 itself had no detectable activity.

The cdc18 gene encodes a B-type cyclin that associates with Cdc2 kinase. Cig2p is thought to play an important role in the cell cycle start (24, 30), but this function is specific to Cig2p and is shared by the other B-type cyclins, Cdc13p and Cig1p (9). However, only cdc18 suppressed the mutant. These cell cycle start genes also rescued the rad21-K1 mutant (data not shown).

In addition to the cell cycle start regulators, pcn1 and cdc20, which encode proliferating cell nuclear antigen (PCNA) and a catalytic subunit of DNA polymerase ε, respectively, components of the DNA replication machinery, displayed partial suppression. The cdc20 cDNA having the activity contained only the carboxy-terminal one-third of the coding region devoid of the catalytic domain.

**DISCUSSION**

All the data presented show that eso1+, which we identified as a novel fission yeast cell cycle regulator, is a functional homologue of budding yeast ECO1/CTF7, which is required for the establishment of sister chromatid cohesion during S phase (42, 50). Like cohesin and adherin, Eso1p/Eco1p/Ctf7p seems to be evolutionarily conserved at least with respect to structure. cDNA sequence databases from higher eukaryotes contain proteins with significant amino acid homology to this family, though their function is presently unknown. However, there is a striking difference between Eso1p and other Eco1p/Ctf7p family members. Eso1p contains a sequence highly homologous to DNA polymerase η at its N-terminal side. DNA polymerase η performs DNA synthesis on a damaged template, a critical step in postreplication repair, and in fact synthesizes a DNA strand with correct bases on cis-syn thymine-thymine dimer-containing DNA templates (18, 25, 26; reviewed in reference 53). We found that deletion of the DNA polymerase η domain of Eso1p did not affect sister chromatid cohesion but elevated sensitivity to UV damage. This result is consistent with the possibility that the N-terminal region of Eso1p indeed has a DNA polymerase η activity.
Eso1p is essential for sister chromatid cohesion. Sister chromatids are separated precociously in the eso1-1 mutant though cohesin complexes stay bound to chromosomes (50). Similarly, we found no difference in the localization of Mis4GFPp and Rad21GFPp between wild-type and eso1-H17 mutant cells (K. Tanaka, unpublished data). Thus, the Eso1/Eco1/Ctf7 protein family is perhaps not required for the loading of adherin and cohesin onto chromosomes but has a role in connecting nascent sister chromatids that have been duplicated in S phase. Eso1 protein is present throughout the cell cycle at a constant level (K. Tanaka, unpublished data), indicating that, if it is, the activity is regulated at the posttranslational level. As shown in Fig. 6A, the truncation mutant ΔC16, in which G799, the residue mutated in eso1-H17, is intact, is partially active at 32.5°C but totally inactive at 36°C. This result suggests that the C-terminal region of Eso1p may physically interact with another protein(s). We found that eso1+ genetically interacts with pcnl+ (encoding PCNA) and cdc20+ (encoding a catalytic subunit of DNA polymerase ε). Similarly, POL30 (encoding PCNA) was isolated as a high-copy suppressor of the cif2-203 mutant in budding yeast (42). Interestingly, the Cdc20 protein truncated at the DNA polymerase domain retained eso1-H17 suppression activity. The carboxyl-terminal region of Cdc20p is essential for cell viability (8), and the DNA polymerase domain of polymerase ε is dispensable for growth in budding yeast (20). Although the molecular aspect of interactions of Eso1p with DNA polymerase ε and PCNA is totally unknown, Eso1p might colocalize with the replication machinery and thereby be promoted to interact with the nascent DNA loaded with adherin and cohesin. Polymerase ε and PCNA might have a role in colocalization and/or activation of Eso1p. The presence of the DNA polymerase η domain in Eso1p is also consistent with this possibility.

One of our remarkable findings is that the eso1-H17 mutant is partially suppressed by the cell cycle start genes, such as res1+, rep2+, and cig2+. The effect seems to be indirect because the expression of eso1+ is not regulated by the Res-Cdc10-Rep transcriptional activator complexes (T. Yonekawa, unpublished data). Res-Cdc10-Rep may act via the cig2 gene, since induction of its mRNA at the G1-S boundary fully depends on these transcriptional activators (28, 30, 36; K. Tanaka, unpublished data), but seemingly not via the rad21+ gene, because the expression of rad21+ gene is not Cdc10p dependent though it is cell cycle regulated with a peak during G1-S transition (4). Interestingly, only cig2+, not the cdc13+ or the cig1+ gene, was able to suppress the eso1 mutant though the cell cycle start function is not specific to Cig2p and is shared by other B-type cyclins (9). Cig2+ also rescues the temperature sensitivity of the rad21-K1 mutant. These results raise the possibility that the

The elevated DNA damage sensitivity, particularly to DSB, of the eso1 mutant due to defective sister chromatid cohesion is highly consistent with the fact that cohesin and adherin mutants are also sensitive to UV irradiation at the permissive temperature (10, 49). The DSB sensitivity of these mutants suggests that the recombinatorial repair system may require sister chromatid cohesion for its efficient execution. This is quite reasonable, because the distance between the two homologous sequences must influence the efficiency of recombinatorial repair. In fact, the rad21 mutant was originally isolated as a DSB repair-deficient mutant (3).

It is of considerable importance to elucidate how Eso1p interacts with adherin and cohesin and how Eso1p established sister chromatid cohesion during S phase. Up to now, little was known about the molecular basis of the establishment of sister chromatid cohesion during DNA synthesis. Adherin and cohesin are essential, but not in themselves sufficient, for sister chromatid cohesion.
Cig2 cyclin has a novel function as an activator of sister chromatin cohesion, though it is insufficient since Δcig2 cells are viable (5, 6, 36). Eso1p possesses a single Cdc2 kinase phosphorylation consensus sequence (SPKR; from position 505 to 508), but mutations that change the serine residue to alanine or aspartic acid did not significantly influence Eso1p activity, suggesting that Eso1p is unlikely to be a direct functional substrate of Cig2p-Cdc2p kinase (T. Yonekawa, unpublished data).

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