

DNA polymerase 5 acetylation by Eso1 is essential for *Schizosaccharomyces pombe* viability

ZHIMING CHEN¹, HONGSHI CAO², YINGQIANG LU¹, QIANG REN¹ and LIANKUN SUN¹

¹Department of Forensic Pathology, Basic Medical College, and ²Department of Neurosurgery, The First Hospital, Jilin University, Jilin 130021, P.R. China

Received October 23, 2015; Accepted July 24, 2017

DOI: 10.3892/ijmm.2017.3192

Abstract. Eco1/Eso1 protein plays an important role in chromosome segregation, DNA repair and gene regulation. *Eco1* mutation induces Roberts syndrome clinically and rDNA transcription disorders *in vivo*. In this study, we examined the role of Eso1 protein binding to polymerase 5 (Pol5) and the acetylation of Pol5 protein in the regulation of *Schizosaccharomyces pombe* (*S. pombe*) viability. Immunoprecipitation and mass spectrometry assays identified Eso1 protein binding to Cdc2, Pol5 and Cdc21, as well as other proteins. Pol5 protein specifically bound to Eso1 protein, but not to the Rad30 part or Rad30 part plus the additional zinc finger domain of Eco1 protein. Mass spectrometry data further identified several acetylation or trimethylation modification sites in the lysine residues of the Pol5 protein. However, the mutation of the Pol5 K47 site to arginine was lethal to *S. pombe*. Eso1 protein was able to acetylate Pol5 protein and mediate *S. pombe* viability. On the whole, our data indicate that the Eso1 interaction with Pol5 which acetylates Pol5 protein is essential for *S. pombe* viability.

Introduction

Eco1/Eso1 protein plays an important role in chromosome segregation, DNA repair and gene regulation (1-3). Eco1/Eso1 protein mediates the formation of the cohesion complex during DNA replication, chromosome separation and cell mitosis from the metaphase to the anaphase transition of the cell cycle. The cohesion complex is critical for the faithful chromosome segregation between two sister chromatids at the anaphase (4), consists of 4 highly conserved proteins (Smc3, Smc1, Scc3 and Scc1) (5,6), and functions to entrap the chromatids by forming a topological ring (7) during the cell cycle. Eco1/Eso1 protein is an acetyltransferase and is able to acetylate

the two conserved lysine sites in the Smc3 protein, enabling Smc3 to form the cohesion complex (8-11). A previous study revealed that mutations of *ESCO2*, the human ortholog of Eco1, were associated with a human developmental disorder known as Roberts syndrome (12). The role of Eco1/Eso1 in the contribution to chromosome structure and organization was then proposed (13). For example, our previous genetics screening data also demonstrated an intimate association between cohesion and chromatin separation (14). Recently, rRNA transcription and protein translation defects occurred in budding yeast that carry Roberts syndrome-related *Eco1* mutations and in cells from patients with Roberts syndrome (15,16). These data indicate that Eco1/Eso1 protein may play an important role in *Schizosaccharomyces pombe* (*S. pombe*) viability. However, the molecular mechanisms through which Eco1 protein functions in *S. pombe*, remain to be defined. For example, determining how and which protein Eco1/Eso1 protein binds to or interacts with, and any additional functions of Eco1/Eso1 protein may shed light into this matter.

In the present study, we first purified Eso1 protein using a tandem affinity purification (TAP) tag antibody and immunoprecipitated whole cell extracts from the wild-type *S. pombe* strain to identify Eso1 binding partners. We then focused on one of its interacting proteins, polymerase 5 (Pol5), as the Pol5 protein provides a conserved function in rDNA transcription in yeast (17,18). In addition, Myb-binding protein 1a (Mybbp1a), a human homologue of Pol5, has been shown to function in rDNA transcription and processing, and to be associated with early embryonic development and carcinogenesis (19,20). Subsequently, we assessed how Eso1 interacts with Pol5 and examined alterations in Pol5 protein acetylation, and the effects of these on *S. pombe* viability. This study aimed to provide insightful information regarding the interaction of Eso1 with Pol5 and the acetylation of Pol5 protein, and the effects of these on *S. pombe* viability. Future studies are also required in order to investigate whether and how Eso1 acetylates the Pol5 K47 site for *S. pombe* survival.

Materials and methods

Strains and culture media. The *S. pombe* strains used in this study are listed in Table I and were cultured in standard conditions, as previously described (21). For *S. pombe* transformation, the lithium acetate protocol was utilized, as described

Correspondence to: Dr Zhiming Chen or Dr Liankun Sun, Department of Forensic Pathology, Basic Medical College, Jilin University, Xinjiang Street 126, Changchun, Jilin 130021, P.R. China
E-mail: chenzm@jlu.edu.cn
E-mail: sunlk@jlu.edu.cn

Key words: Eso1, polymerase 5, acetylation, rDNA transcription, tandem affinity purification, *Schizosaccharomyces pombe*

in a previous study (22). pDblet (acquired from Baumann's Lab, Stowers Institute, Kansas City, MO, USA) was used as a plasmid vector for the expression of Pol5 with or without K47 mutations, and 0.1%(w/v) 5-fluoroorotic acid (5FOA) was used to select against ura + *S. pombe* cells, which is supposed to contain transformed pDblet plasmids.

Mutagenesis and protein tagging. DNA sequences corresponding to Pol5 cDNA were amplified using standard PCR from the *S. pombe* genomic DNA. The primers used are listed in Table II. The resulting PCR product was then cloned into a pCloneNat1 vector [kind gift from Gregan's Lab, Max F. Perutz Laboratories, Vienna Biocenter (VBC), Vienna, Austria]. To create the Pol5 K47N and Pol5 K47R mutant strains, a site directed mutagenesis kit (QuikChange II; Agilent Technologies, Santa Clara, CA, USA) was employed at the corresponding residues using the primers listed in Table II. The resulting plasmids, pCloneNat1-pol5 K47N and pCloneNat1-pol5 K47R, were linearized with the restriction enzyme, *Bst*BI, by digesting nucleotides at the start codon, and then transferred into the diploid *S. pombe* strains. The tetrad analysis of the expected mutation strains was then confirmed by DNA sequencing and used in this study. These wild-type and mutated Pol5 with TAP, HA or FLAG tag were then tagged according to a previously study (23) and online protocol (http://mendel.imp.ac.at/Pombe_tagging/).

Cloning and expression of GST-Eso1 protein. The full-length Eso1 cDNA and its different truncated forms, including Rad30 homologue fragment (1-519 or M519*), Rad30 fragment plus the additional second zinc-finger domain (1-568 or R568*) and Eco1 homologue fragment (520-871) were amplified from the genomic DNA of *S. pombe* using PCR primers (Table II). The PCR product containing the *Bam*HI and *Xma*I restriction sites were cloned into the pGEX-4T-1 vector (kind gift from Gerton's Lab, Stowers Institute) in a manner that produces an N-terminally GST tagged Eso1 protein and its different truncated forms, respectively. The resulting plasmids were transferred into *E. coli* Bl21 (DE3) cells (# 200131; Agilent Technologies), and were amplified and DNA sequence-confirmed. To express the N-terminally GST tagged Eso1 protein and its different truncated forms, plasmids were transferred into *E. coli* Bl21 (DE3) cells and 0.3 mM of IPTG was added to induce the expression of Eso1 protein in DE3 cells at 25°C for 3 h. The glutathione resin was then utilized to pull-down and purify these GST fused proteins, as previously described (24).

TAP purification and liquid chromatography-mass spectrometry (LC-MS/MS) analysis. Trichloroacetic acid (TCA)-precipitated proteins from cultured *S. pombe* strains were resuspended in 30 μ l of buffer containing 100 mM of Tris-HCl pH 8.5, 8 M of urea and 5 mM of Tris(2-carboxylethyl)-phosphine hydrochloride (Pierce, Rockford, IL, USA), and alkylated with 10 mM of iodoacetamide (Sigma, St. Louis, MO, USA). Subsequently, a two-step digestion procedure was applied. In brief, 0.5 μ g of endoproteinase Lys-C (Roche Applied Science, Indianapolis, IN, USA) was added to each sample and incubated for at least 6 h at 37°C. The samples were then diluted with 2 M of urea in 100 mM of Tris-HCl pH 8.5 and 2 mM of calcium chloride. Subsequently, the samples were digested with 0.5 μ g of trypsin (Promega, Madison, WI, USA) at 37°C overnight with shaking.

The following, the reaction was quenched by the addition of 5% formic acid, and the peptide mixtures were then loaded onto a 100- μ m fused silica microcapillary column packed with 8 cm of reverse-phase material (Aqua; Phenomenex, Torrance, CA, USA) for LC-MS/MS analysis in a Deca-XP ion trap mass spectrometer equipped with a nano-LC electrospray ionization source (Thermo Fisher Scientific, Waltham, MA, USA). The full MS spectra were recorded on the peptides over a 400-1,600 m/z range. Mass spectrometer scan functions and HPLC solvent gradients were controlled by the XCalibur data system (Thermo Fisher Scientific). RAW files were extracted into ms2 file format using RAW Xtract v.1.0. The protein spectra were searched against the Swiss-Prot database using the Mascot software program (<http://www.matrixscience.com/>).

Immunoprecipitation and western blot analysis. *S. pombe* cells from a 100-ml culture grown into a 0.8 OD-595 were pelleted and immediately frozen in liquid nitrogen. The *S. pombe* cell pellets were then suspended in 1 ml of lysis buffer containing 50 mM of Tris pH 7.5, 150 mM of NaCl, 0.1% NP-40, 1 mM of DTT, 10% glycerol and a protease inhibitor tablet. The cells were lysed by adding glass beads followed by bead-beating for 60 sec 5 times with 1 min intervals on ice. The supernatant was then separated by centrifugation at 14,000 rpm at 4°C for 20 min, and subjected to co-immunoprecipitation assay with an anti-FLAG antibody for Eso1-FLAG protein and an anti-HA antibody for Pol5-HA protein, respectively. Specifically, the supernatant was added with 30 μ l of anti-FLAG or anti-HA affinity gel (#E6779; EZview™ Red Anti-HA Affinity Gel; Sigma-Aldrich, Shanghai, China) and incubated at 4°C overnight. The following day, the mixtures were washed 5 times with wash buffer containing 50 mM of Tris pH 7.5, 150 mM of NaCl and 1% Triton X-100; they were then eluted in 2X SDS buffer containing 10 mM of Tris pH 7.5, 1 mM of EDTA and 1% SDS. The elutes were loaded onto a 4-12% Bis-Tris gel for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the proteins were transferred onto a nitrocellulose membrane (Whatman, Piscataway, NJ, USA). For western blot analysis, the membrane was blocked with 5% skimmed milk for 1 h at room temperature and blotted with primary antibodies [anti-FLAG antibody (1:3,000; #F3165; Sigma), GST antibody (1:3,000; Abmart, Berkeley Heights, NJ, USA), a-HA antibody (1:10,000; #sc-7392; Santa Cruz Biotechnology, Santa Cruz, CA, USA)] against HA and FLAG tags (Sigma-Aldrich) respectively, as previously described (24). The glutathione resin bound with variant forms of Eso1 was incubated with whole cell extracts of *S. pombe* expressing C-terminally HA-tagged Pol5 at 4°C overnight, and the resulting samples were subjected to western blot analysis with the anti-HA tag antibody (Sigma-Aldrich).

In vitro acetylation assay. Purified GST-tagged Eco1p and HA affinity gel bound with Pol5-HA was added with a HAT buffer containing 50 mM of Tris pH 8.0, 5% glycerol, 0.1 mM of EDTA, 50 mM of KCl, 1 mM of DTT, 1 mM of PMSF and 10 μ M of Acetyl-CoA. This was followed by incubation at 30°C for 60 min, as previously described (24). The samples were then subjected to western blot analysis with anti-acetylated lysine antibody for acetylation levels and HA antibody as a loading control.

Table I. Yeast strains used in this study.

Name	Genotype	Source
ZCA002	h- leu1-32 ura4-D18 his3-D1 ade6-M216	Our stock
ZCA005	h- leu1-32 ura4-D18 his3-D1 ade6-M216 ESO1-TAP::KAN	Our stock
ZCA006	h- leu1-32 ura4-D18 his3-D1 ade6-M216 ESO1-FLAG::KAN	Our stock
ZCB001	h- leu1-32 ura4-D18 his3-D1 ade6-M216 POL5-HA::NAT	This study
ZCB002	h- leu1-32 ura4-D18 his3-D1 ade6-M216 ESO1-FLAG::KAN POL5-HA::NAT	This study
ZCB056	h- leu1-32 ura4-D18 his3-D1 ade6-M216 POL5-TAP::KAN	This study
ZCB057	h+/h- leu1-32 ura4-D18 his3-D1 ade6-M216/M210 POL5/POL5K47N::NAT	This study
ZCB058	h+/h- leu1-32 ura4-D18 his3-D1 ade6-M216/M210 POL5/POL5K47R::NAT	This study
ZCB060	h? leu1-32 ura4-D18 his3-D1 ade6-M216/M210 POL5K47R::NAT p-pdblet-POL5::URA	This study

Table II. Primers used in this study.

Primers	Nucleotide sequences
Wild-type	5'-atatctcgagttgagaacgttcccatctac-3'
Pol5	5'-atatggatccatccttgggcttggt-3'
Pol5 K47N	5'-cgttaaacggtttgaccaatggtctttctagtgtcg-3'
mutated	5'-cgaccactagaaagaccattggtcaaacggttaacg-3'
Pol5 K47R	5'-ttcgttaaacggtttgaccgaggtctttctagtgtcg-3'
mutated	5'-gcgaccactagaaagacctcggtcaaacggttaacgaa-3'
Pol5	5'-tacaacctcgattgtgag-3'
mutated	

Pol5, polymerase 5.

Results

Eso1 protein interaction with Pol5 protein in *S. pombe*. To identify proteins that can physically interact with Eso1 protein, Eso1 cDNA was tagged with a TAP tag at the C-terminus and this fusion protein was expressed under its endogenous promoter (25) using a protocol we have previously described (25). The proteins binding to Eso1-TAP protein were then purified using immunoprecipitation and mass spectrometry followed by TAP. Our data revealed that Eso1 protein was able to bind to Cdc2, Pol5 and Cdc21, as well as other proteins (Fig. 1A). The following experiments focused on Pol5 protein due to its function in rRNA synthesis and cell proliferation. The specificity of their binding was confirmed by immunoprecipitating the whole cell extracts of *S. pombe* from the wild-type strain, the strain that only expressed C-terminally FLAG-tagged Eso1, and the strain that expressed both C-terminally HA-tagged Pol5 and FLAG-tagged Eso1 with anti-HA or FLAG antibodies. Following western blot analysis using anti-FLAG or α -HA antibody, their binding and interaction (i.e., the proper expression of Eso1-FLAG and Pol5-HA) were directly confirmed from the indicated strains (Fig. 1B). Specifically, Pol5-HA protein could only be detected from whole cell extracts of strains that express both HA-tagged Pol5 and FLAG-tagged Eso1, indicating the specificity of their interaction.

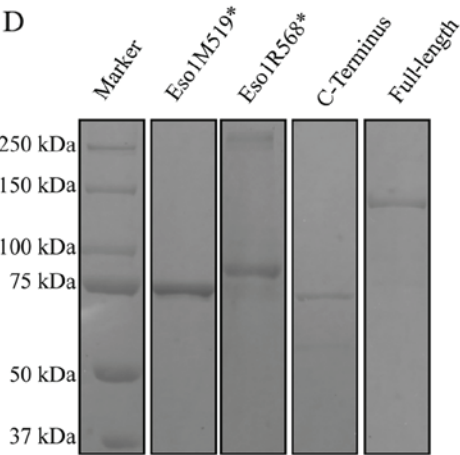
However, given the characteristics of the fusion Eso1 protein in *S. pombe*, which expresses both budding yeast Eco1 and Rad30 homologue (26), our present data was unable to distinguish the binding of Pol5 protein to Eco1 protein from the binding of Pol5 to the Rad30 homologue of Eso1 protein. Thus, we amplified DNA sequences of Rad30 homologue fragment (1-568 or R568*), Rad30 fragment plus the additional second zinc-finger domain (1-519 or M519*), Eco1 homologue fragment (520-871) and the full length Eso1 cDNA using genomic DNA (Fig. 1C). We then inserted these amplicants into pGEX-4T-1 to produce the expected form of Eso1 protein with the N-terminal GST-tag (Fig. 1D). Following GST pull-down assays and western blot analysis with anti-GST antibody, the correct expression of Eso1 proteins were confirmed (Fig. 1D). These proteins were then utilized to pull-down Pol5 in the whole cell lysis of *S. pombe* expressing C-terminally HA-tagged Pol5 for western blot analysis with anti- α -HA antibody. We found that the results were consistent with our first set of data, which showed that Pol5 protein binds to Eso1 protein from either Eso1-FLAG and Pol5-HA co-immunoprecipitation or mass spectrometry analysis following the purification of Eso1 by TAP (Fig. 1); Pol5-HA was able to pull-down the full-length recombinant GST-Eso1 protein. Strikingly, Pol5-HA could have also pulled-down the Eco1 homology part of Eso1, but not the Rad30 part or Rad30 part plus additional zinc finger domain of Eco1 protein (Fig. 1E), suggesting that the Eco1 homology part of Eso1 mediated binding to the Pol5 protein.

Mass spectrometry identification of Pol5 protein acetylation sites. C-terminal TAP tagging and Pol5 protein expression *in vitro* was established according to a protocol described in a previous study (23). Proteins were purified using an anti-TAP tag antibody and separated using SDS-PAGE. A prominent protein band of approximately 120 kDa was found after silver staining (Fig. 2A), which was in agreement with the predicted molecular weight of Pol5 protein plus TAP tag. Possible post-translational modifications of this purified Pol5 protein were then analyzed using mass spectrometry, and several acetylation or trimethylation modification sites in the lysine residues of Pol5 protein were identified (Fig. 2B). The Pol5 K47 residue attracted our attention, because there was a 100% modification ratio (although the number of the total spectrum count was low); the DNA sequence alignment of *S. pombe*

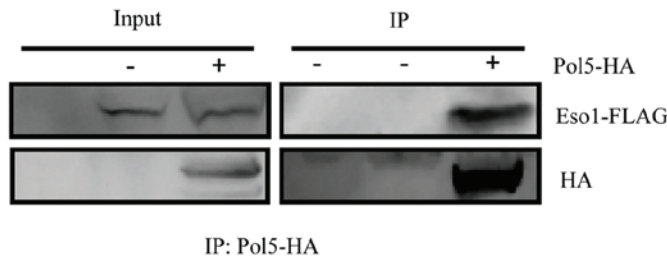
A

Name	Peptide Count	Distributed Spectral Count	Sequence Coverage (%)
Eso1	56	1119	28.2
Cdc2	2	2	11.78
Pol5	2	3	3.55
Cdc21	2	3	3.07
Cdc15	1	2	1.4
Rad25	4	10	20
Rad24	4	5	20.74

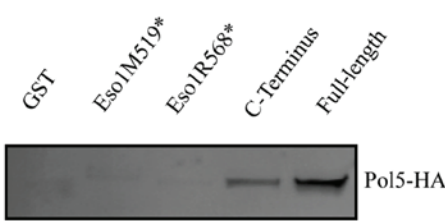
D



B



E



C

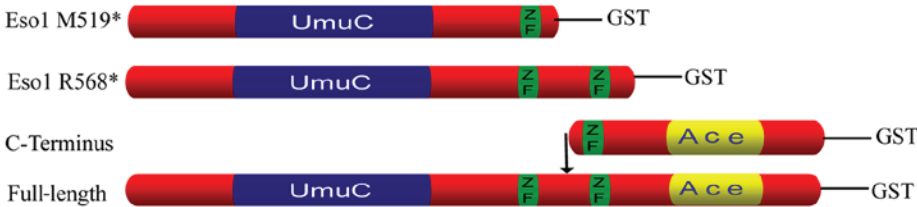


Figure 1. Eso1 interaction with Pol5 in *Schizosaccharomyces pombe* (*S. pombe*). (A) Immunoprecipitation and mass spectrometry identification of Eso1 binding partners in *S. pombe*. (B) Immunoprecipitation-western blot analysis: 50 μ g of whole-cell lysis from *S. pombe* expressing C-terminally FLAG-tagged Eso1 and HA-tagged Pol5 were immunoprecipitated using an anti-HA antibody, and subjected with western blot analysis with an anti-FLAG antibody. (C) Illustration of different forms of GST-fusion *S. pombe* Eso1 protein purified from *E. coli*. Different truncated forms of GST-fusion Eso1 protein were constructed and expressed as indicated. The * symbol indicates the induced stop codon at Eso1 cDNA to construct a truncated Eso1 protein (1-568 and 1-519); the arrow indicates the position of the start site of the C-terminal truncation (520-871) in Eso1 cDNA. (D) Coomassie staining of SDS-PAGE gel. GST-Eso1 and its different truncated forms were expressed and detected by SDS-PAGE and Coomassie staining. (E) Immunoprecipitation. Whole-cell lysis extracted from *S. pombe* expressing C-terminally HA-tagged Pol5 was immunoprecipitated using GST beads bound to GST-fused Eso1 protein or different truncated forms of GST-fused Eso1 protein, as indicated. Data indicated that only the Eso1-GST and C-terminus Eso1 protein were able to pull down the Pol5-HA protein.

and *S. cerevisiae* indicate that K47 residue is well conserved in budding yeast (Fig. 2C).

Acetylation of Pol5 protein K47 residue mediated S. pombe viability. The effects of Pol5 K47 acetylation on *S. pombe* cells were assessed, and *pol5-K47R/pol5+* heterozygous diploids were prepared. Following sporulation, only two spores in each diploid grew (Fig. 3A). DNA sequencing analysis was then performed. The results revealed that all viable spores contained a wild-type (non-mutated) allele of the *Pol5* gene, indicating that *S. pombe* expressing mutant Pol5 K47R was unable to survive. Moreover, since arginine (R) is structurally similar to lysine (K), which does not undergo acetylation change, we found that arginine substitution of lysine led to the absence of the acetylation of Pol5 K47; this resulted in the lethality to

S. pombe. In addition, our data also revealed that Pol5 K47N acetyl-mimetic mutant *S. pombe* cells grew normally (Fig. 3A).

Furthermore, we constructed the shuffle plasmid by integrating the Pol5 coding region cDNA plus the 200-bp upstream and downstream sequences into the pDblet vector. The transformation of this plasmid into *S. pombe* fully rescued the growth of Pol5 K47R mutants (Fig. 3). Subsequently, 5FOA was added into *S. pombe* to select against the expression of Ura4+ in pDblet-Pol5 plasmid-transferred *S. pombe*. Consistent with the data on Pol5 K47R mutation without pDblet-Pol5 plasmid, our data revealed that *S. pombe* growth was limited and lethal (Fig. 3B).

Eso1 acetylation of Pol5 protein K47 residue mediates S. pombe viability. We explored whether Eso1 protein is able

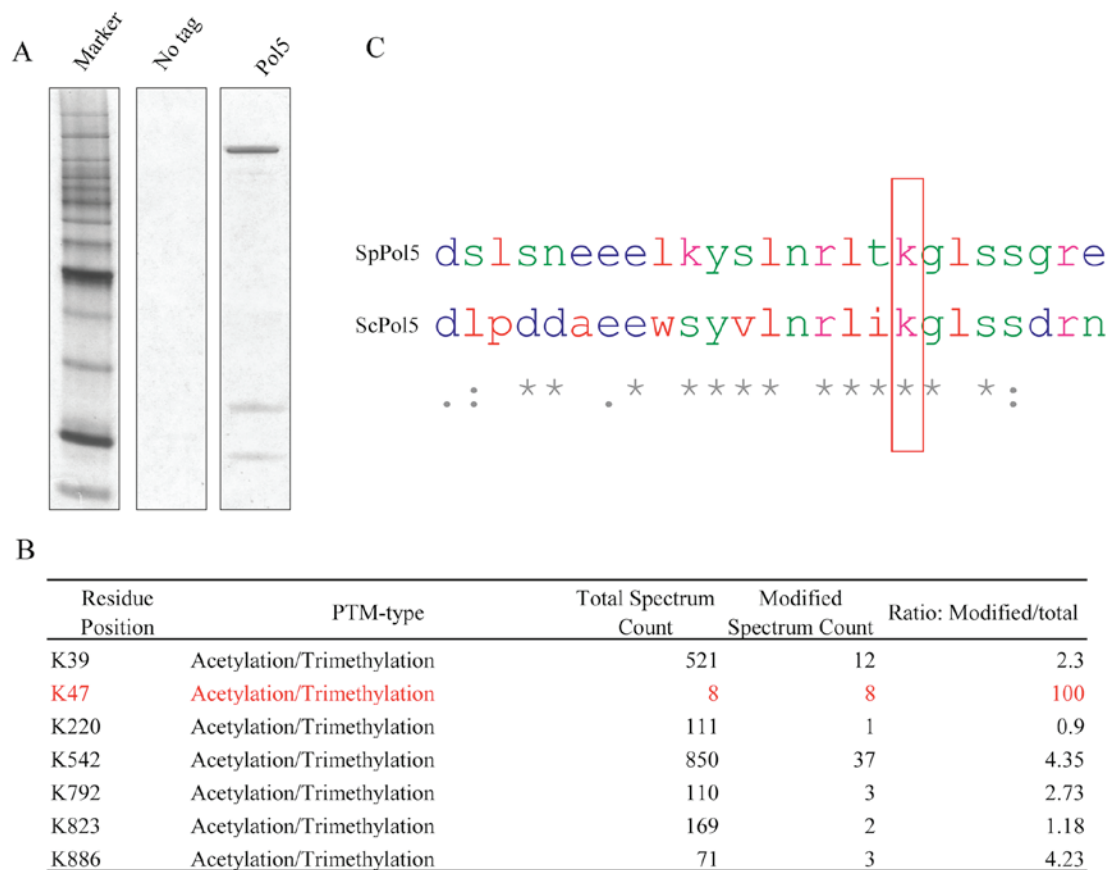


Figure 2. Tandem affinity purification (TAP) and characterization of Pol5 protein from *Schizosaccharomyces pombe* (*S. pombe*). (A) TAP-tagged Pol5 cDNA was transferred into *S. pombe*, and whole cell lysis was extracted from *S. pombe*. Pol5 protein was then purified by the TAP system. Purified proteins were separated by SDS-PAGE and visualized by silver staining. (B) Illustration of potential post-translational modification sites in Pol5 protein. (C) Mass spectrometry identification of the Pol5 K47 site. The acetylated lysine residue of Pol5 protein in *S. pombe* is conserved to K53 of Pol5 in budding yeast (a red box). In the alignment, asterisks indicate identity, while the colons and dots indicate similarity between amino acids.

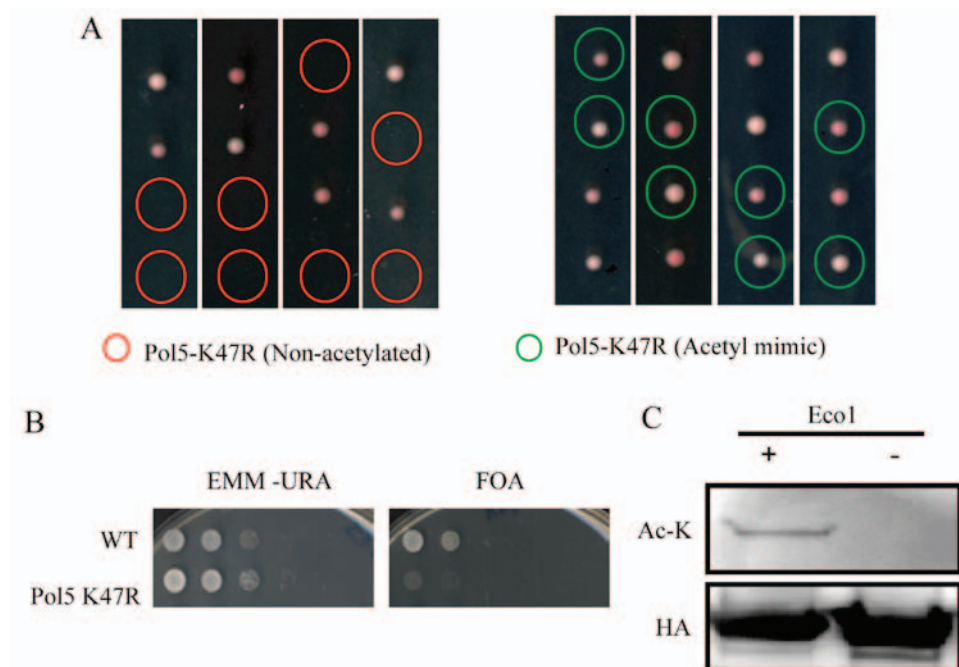


Figure 3. Lethality of non-acetylated K47 residue mutation in Pol5 protein in *Schizosaccharomyces pombe* (*S. pombe*). (A) POL5K47R/POL5 and POL5 K47N/POL5 heterozygous diploids were sporulated, and the viability of each tetrad was analyzed. POL5 K47R mutant spores are labeled with red circles, and POL5 K47N mutant spores are labeled with green circles. (B) Wild-type (WT) and Pol5 K47R cells containing pDblet-pol5 plasmids were plated on EMM-URA and YES 5'FOA plates. (C) Pol5 was acetylated by EcoI *in vitro*. Pol5-HA was immunoprecipitated on an HA affinity gel from whole cell extracts of *S. pombe* cells expressing C-terminally HA tagged Pol5. The beads were then incubated with or without EcoI in HAT buffer present with acetyl coenzyme A.

to acetylate Pol5 using acetylation assay following a protocol described in a previous study (27). We found that Pol5-HA protein was immunoprecipitated with EZview™ Red Anti-HA Affinity Gel from *S. pombe*. This was then incubated with the recombinant Eco1 protein purified from *E. coli* via the GST tag. Pol5 protein acetylation was detected by western blot analysis with an anti-acetyl-lysine antibody following the addition of Eco1 protein (Fig. 3C).

Discussion

In the present study, we first performed immunoprecipitation and mass spectrometry assays to identify several candidate proteins that bind to Eso1 protein (i.e., Cdc2, Cdc21, Cdc15, Rad24, Rad25 and Pol5). Functionally, Cdc2, as a serine/threonine kinase, is a highly conserved protein and a key player in the regulation of cell cycle progression (22,28). Cdc21 is a member of the MCM family of nuclear proteins, which can regulate DNA replication and cell cycle progression (29). Cdc15 is also involved in the formation of the cytokinetic contractile ring (28). Moreover, Rad24 and Rad25 are cell cycle checkpoint proteins that belong to the highly conserved 14-3-3 protein family (29-31), which regulates cell cycle arrest and DNA damage repair in response to UV light-induced DNA damage (24-27). Pol5 protein functions in yeast rDNA transcription (17,18) and the human homologue of Pol5, Mybbp1a, demonstrating functions in rDNA transcription, early embryonic development and tumorigenesis (19,20). Thus, given the important functions of Eso1 protein in cells, including the sister chromatid cohesion complex and mediation of DNA repair (2), it seems reasonable to hypothesize that the binding of Eso1 to these proteins illustrates the functions of Eso1 protein.

Furthermore, we focused on the interaction of Pol5 protein with Eso1 protein to assess their regulation of *S. pombe* viability. We performed a co-immunoprecipitation assay to further confirm the binding of Eco1 to Pol5 protein and found that Pol5 protein only bound to Eso1 protein, but not to the Rad30 part or Rad30 part, plus the additional zinc finger domain of Eco1 protein, indicating that their binding was specific. Upon their binding, Eso1 protein was able to acetylate Pol5 protein. Our confirmative evidence indicated that there were several acetylation sites in Pol5 protein lysine residues (Fig. 2B) and that arginine substitution of lysine led to the lack of Pol5 K47 acetylation, which resulted in the lethality of *S. pombe*; whereas for Pol5 K47N, the acetyl-mimetic mutant had normal *S. pombe* proliferation; thus, the Eso1 acetylation site in Pol5 protein could be at the Pol5 K47. However, at present, there are no Pol5 K47 acetylation antibodies available; thus, a definitive proof would require the involvement of a mass-spec analysis of the acetylation on Pol5 before and after incubation with Eso1 protein. Future studies are required to further verify the Eso1 acetylating Pol5 K47 site, although our present data suggest that Pol5 protein acetylation was essential in the regulation of *S. pombe* viability.

In conclusion, previous studies have revealed the essential role of Eco1/Eso1 protein in the formation of the cohesion complex between the two sister chromatids, and that the acetyl-mimetic mutation, Smc3 K47N, made Eco1 protein dispensable. In the present study, we found that the *S. pombe* carrying non-acetyltable mutations of Pol5 protein (i.e., Pol5 K47R) was unable to survive, indicating the essential role of Pol5 acetylation

in *S. pombe*. However, this study was not able to rule out whether other acetyltransferases, apart from Eso1, acetylate Pol5 protein. Future studies are warranted to verify Eso1, particularly the acetylate Pol5 K47 site; although the present study suggested so.

Acknowledgements

The authors would like to thank Dr Juraj Gregan of Max F. Perutz Laboratories, Department of Chromosome Biology, University of Vienna, Vienna, Austria; and the Stowers Proteomics and Molecular Biology groups, MO, USA. This study was supported by a grant from the National Natural Science Foundation of China (#81672122), and Jilin Provincial Science and Technology Agency (#20160414048GH).

References

1. Skibbens RV, Corson LB, Koshland D and Hieter P: Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery. *Genes Dev* 13: 307-319, 1999.
2. Tanaka K, Yonekawa T, Kawasaki Y, Kai M, Furuya K, Iwasaki M, Murakami H, Yanagida M and Okayama H: Fission yeast Eso1p is required for establishing sister chromatid cohesion during S phase. *Mol Cell Biol* 20: 3459-3469, 2000.
3. Unal E, Heidinger-Pauli JM and Koshland D: DNA double-strand breaks trigger genome-wide sister-chromatid cohesion through Eco1 (Ctf7). *Science* 317: 245-248, 2007.
4. Nasmyth K and Schleiffer A: From a single double helix to paired double helices and back. *Philos Trans R Soc Lond B Biol Sci* 359: 99-108, 2004.
5. Hirano T: Chromosome cohesion, condensation, and separation. *Annu Rev Biochem* 69: 115-144, 2000.
6. Nasmyth K: Disseminating the genome: Joining, resolving, and separating sister chromatids during mitosis and meiosis. *Annu Rev Genet* 35: 673-745, 2001.
7. Koshland DE and Guacci V: Sister chromatid cohesion: The beginning of a long and beautiful relationship. *Curr Opin Cell Biol* 12: 297-301, 2000.
8. Zhang J, Shi X, Li Y, Kim BJ, Jia J, Huang Z, Yang T, Fu X, Jung SY, Wang Y, *et al.*: Acetylation of Smc3 by Eco1 is required for S phase sister chromatid cohesion in both human and yeast. *Mol Cell* 31: 143-151, 2008.
9. Unal E, Heidinger-Pauli JM, Kim W, Guacci V, Onn I, Gygi SP and Koshland DE: A molecular determinant for the establishment of sister chromatid cohesion. *Science* 321: 566-569, 2008.
10. Rolef Ben-Shahar T, Heeger S, Lehane C, East P, Flynn H, Skehel M and Uhlmann F: Eco1-dependent cohesin acetylation during establishment of sister chromatid cohesion. *Science* 321: 563-566, 2008.
11. Feytout A, Vaur S, Genier S, Vazquez S and Javerzat JP: Psm3 acetylation on conserved lysine residues is dispensable for viability in fission yeast but contributes to Eso1-mediated sister chromatid cohesion by antagonizing Wpl1. *Mol Cell Biol* 31: 1771-1786, 2011.
12. Vega H, Waisfisz Q, Gordillo M, Sakai N, Yanagihara I, Yamada M, van Goslign D, Kayserli H, Xu C, Ozono K, *et al.*: Roberts syndrome is caused by mutations in ESCO2, a human homolog of yeast ECO1 that is essential for the establishment of sister chromatid cohesion. *Nat Genet* 37: 468-470, 2005.
13. Dorsett D: Cohesin: Genomic insights into controlling gene transcription and development. *Curr Opin Genet Dev* 21: 199-206, 2011.
14. Chen Z, McCrosky S, Guo W, Li H and Gerton JL: A genetic screen to discover pathways affecting cohesin function in *Schizosaccharomyces pombe* identifies chromatin effectors. *G3 (Bethesda)* 2: 1161-1168, 2012.
15. Bose T, Lee KK, Lu S, Xu B, Harris B, Slaughter B, Unruh J, Garrett A, McDowell W, Box A, *et al.*: Cohesin proteins promote ribosomal RNA production and protein translation in yeast and human cells. *PLoS Genet* 8: e1002749, 2012.
16. Xu B, Lee KK, Zhang L and Gerton JL: Stimulation of mTORC1 with L-leucine rescues defects associated with Roberts syndrome. *PLoS Genet* 9: e1003857, 2013.
17. Yang W, Rogozin IB and Koonin EV: Yeast POL5 is an evolutionarily conserved regulator of rDNA transcription unrelated to any known DNA polymerases. *Cell Cycle* 2: 120-122, 2003.

18. Shimizu K, Kawasaki Y, Hiraga S, Tawaramoto M, Nakashima N and Sugino A: The fifth essential DNA polymerase phi in *Saccharomyces cerevisiae* is localized to the nucleolus and plays an important role in synthesis of rRNA. *Proc Natl Acad Sci USA* 99: 9133-9138, 2002.
19. Mori S, Bernardi R, Laurent A, Resnati M, Crippa A, Gabrieli A, Keough R, Gonda TJ and Blasi F: Myb-binding protein 1A (MYBBP1A) is essential for early embryonic development, controls cell cycle and mitosis, and acts as a tumor suppressor. *PLoS One* 7: e39723, 2012.
20. Hochstatter J, Hölzel M, Rohrmoser M, Schermelleh L, Leonhardt H, Keough R, Gonda TJ, Imhof A, Eick D, Längst G, *et al*: Myb-binding protein 1a (Mybbp1a) regulates levels and processing of pre-ribosomal RNA. *J Biol Chem* 287: 24365-24377, 2012.
21. Sabatinos SA and Forsburg SL: Molecular genetics of *Schizosaccharomyces pombe*. *Methods Enzymol* 470: 759-795, 2010.
22. Gregan J, Rabitsch PK, Rumpf C, Novatchkova M, Schleiffer A and Nasmyth K: High-throughput knockout screen in fission yeast. *Nat Protoc* 1: 2457-2464, 2006.
23. Cipak L, Spirek M, Novatchkova M, Chen Z, Rumpf C, Lugmayr W, Mechtler K, Ammerer G, Csaszar E and Gregan J: An improved strategy for tandem affinity purification-tagging of *Schizosaccharomyces pombe* genes. *Proteomics* 9: 4825-4828, 2009.
24. Xiong B, Lu S and Gerton JL: Hos1 is a lysine deacetylase for the Smc3 subunit of cohesin. *Curr Biol* 20: 1660-1665, 2010.
25. Chen Z, Cao H, Guo W and Lu Y: Identification of two forms of the Eso1 protein in *Schizosaccharomyces pombe*. *Cell Biol Int* 38: 682-688, 2014.
26. Madril AC, Johnson RE, Washington MT, Prakash L and Prakash S: Fidelity and damage bypass ability of *Schizosaccharomyces pombe* Eso1 protein, comprised of DNA polymerase eta and sister chromatid cohesion protein Ctf7. *J Biol Chem* 276: 42857-42862, 2001.
27. Lu S, Goering M, Gard S, Xiong B, McNairn AJ, Jaspersen SL and Gerton JL: Ecol is important for DNA damage repair in *S. cerevisiae*. *Cell Cycle* 9: 3315-3327, 2010.
28. Fankhauser C, Reymond A, Cerutti L, Utzig S, Hofmann K and Simanis V: The *S. pombe* cdc15 gene is a key element in the reorganization of F-actin at mitosis. *Cell* 82: 435-444, 1995.
29. Ford JC, al-Khodairy F, Fotou E, Sheldrick KS, Griffiths DJ and Carr AM: 14-3-3 protein homologs required for the DNA damage checkpoint in fission yeast. *Science* 265: 533-535, 1994.
30. Ozoe F, Kurokawa R, Kobayashi Y, Jeong HT, Tanaka K, Sen K, Nakagawa T, Matsuda H and Kawamukai M: The 14-3-3 proteins Rad24 and Rad25 negatively regulate Byr2 by affecting its localization in *Schizosaccharomyces pombe*. *Mol Cell Biol* 22: 7105-7119, 2002.
31. van Heusden GP and Steensma HY: Yeast 14-3-3 proteins. *Yeast* 23: 159-171, 2006.