6126–6134 *Nucleic Acids Research, 2009, Vol. 37, No. 18* *Published online 19 August 2009*
doi:10.1093/nar/gkp656

The zinc finger of Eco1 enhances its acetyltransferase activity during sister chromatid cohesion

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Received May 19, 2009; Revised July 21, 2009; Accepted July 22, 2009

ABSTRACT

Eco1p/Ctf7p is an essential acetyltransferase required for the establishment of sister chromatid cohesion. Eco1p acetylates Smc3p and Mcd1p (Scc1p or Rad21p) to establish cohesion during S phase and in response to DNA damage, respectively. In addition to its acetyltransferase domain, Eco1p harbors a conserved zinc finger domain. The zinc finger has been implicated in the establishment of sister chromatid cohesion in S phase, yet its function on the molecular level and its contribution to damage-induced cohesion are unknown. Here, we show that the zinc finger is essential for the establishment of cohesion in both S phase and in response to DNA damage. Our results suggest that the zinc finger augments the acetylation of Eco1p itself, Smc3p and likely Mcd1p. We propose that the zinc finger is a general enhancer of substrate recognition, thereby enhances the ability of Eco1p to acetylate its substrates above a threshold needed to generate cohesion during DNA replication and repair. Finally our studies of the zinc finger led to the discovery that Eco1 is a multimer, a property that could be exploited to coordinate acetylation of substrates either spatially or temporally for establishment of sister chromatid cohesion.

INTRODUCTION

Faithful segregation of chromosomes during mitosis requires physical attachment of the chromatids from the time of their replication until their separation, a process known as sister chromatid cohesion. A four-subunit protein complex called cohesin is responsible for generating the physical cross-linking between the chromatids [recently reviewed in ref. (1–4)]. Generation of cohesion is a multi-step process that starts by cohesin loading onto chromosomes during G1 (5–8). However, cohesin is converted to its cohesive, active state only during S (9–12). This conversion, commonly referred to as cohesion establishment, is dependent on the essential cohesin auxiliary protein named Eco1p/Ctf7p (hereon referred to as Eco1p) (13–15). Strikingly, in the yeast *Saccharomyces cerevisiae* over-expression of Eco1p is essential and sufficient to induce genome-wide cohesion in G2/M, suggesting that limiting Eco1p activity restricts cohesion establishment during the cell cycle (16). The *S. cerevisiae* Eco1p is a 32 kDa polypeptide that bears two conserved major domains. A C_2H_2-type zinc finger that is located at the N-terminus and an acetyltransferase domain in the C-terminus (Figure 1) (17). Understanding the function of these two domains is important to elucidate the function of this critical cohesion regulator.

The acetyltransferase domain of Eco1p has sequence and structural similarities to other acetyltransferases of the GNAT family (17). *In vitro*, Eco1p can acetylate itself, as well as several cohesin subunits: Mcd1p (Scc1p or Rad21p), Smc3p and Scc3p (IRR1p) and the cohesin auxillary factor Pds5p (17). Yet, the linkage between Eco1p acetyltrasferase activity and cohesion establishment *in vivo* has been recognized and characterized on the molecular level only recently. Truncations of the acetyltransferase domain of Eco1p revealed that this domain activity is essential for cell viability (18). Furthermore, the K112 and K113 residues of the cohesin subunit Smc3p have been identified as targets of Eco1p *in vivo* and their acetylation has been shown to be critical for the establishment of cohesion during S phase (18–21).

Recently, the K84 and K210 residues of Mcd1p were also identified as likely targets of Eco1p in vivo. Eco1p acetylation of these Mcd1p residues is essential to establish cohesion in G2/M in response to DNA damage (22). Interestingly, Eco1p acetylation of Mcd1p and Smc3p are not interchangeable, but rather promote cohesion establishment only in their proper context of DNA repair and DNA replication, respectively. How Eco1p chooses its target under different conditions is unclear and requires

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a detailed understanding of the protein structure–function properties.

In contrast to the acetyltransferase domain, the function of Eco1p C_2H_2-type zinc finger, located in the N-terminus is much less defined. Generally, zinc fingers are one of the most abundant structural domains in proteins. It is a small, autonomous domain that folds into a compact structure around a zinc ion and can mediate both DNA binding and protein interactions (23–25). The significance of Eco1p zinc finger for cohesion was implicated by a genetic study in yeast that identified zinc finger mutations with reduced fidelity of chromosome transition (26,27).

Little is known about the biological or molecular functions of Eco1p zinc finger and its role may be associated with either or both characteristic roles of zinc finger domains. *In vivo*, Eco1p localizes to chromatin. This localization is thought to be mediated through interaction with the DNA polymerase clamp loader, PCNA and it depends on a PIP domain located outside the zinc finger domain (28). However, the interaction with PCNA does not rule out the possibility that the solitary zinc finger of Eco1p could directly bind to DNA as had been shown before (29). Alternatively, Eco1p zinc finger may promote protein–protein interactions. Out of its five characterized substrates, several studies have suggested that Eco1p directly interacts with Pds5p (30,31). However, no attempt has been reported to map the domain(s) that mediate this interactions, or furthermore, to define the specific contribution of the zinc finger to Eco1p protein–protein interactions. Therefore, the contribution of the zinc finger to protein interaction remains elusive. Taken all together, the molecular and biological functions of the Eco1p zinc finger are poorly understood. Here, we analyze cells carrying mutations in the Eco1p zinc finger by various assays to assess the contribution of the zinc finger to the functions of Eco1p in the establishment of sister chromatid cohesion, *in vivo*.

MATERIAL AND METHODS

Yeast strains

Yeast strains used in this study are listed in Supplementary Table 1.

Site directed mutagenesis

Zinc coordinating residues in *ECO1* ORF were sequentially replaced by site directed mutagenesis (Promega) according to the manufacturer instructions. Primer information is listed in Supplementary Table 2.

Cell growth, synchronization and induction of expression by galactose

Exponentially dividing cultures were arrested in G1 by adding 15 nM alpha mating factor (bar1 strains, αF) (sigma), in S by adding 130 mM hydroxyurea (HU) (Sigma), and in G2/M by 15 μg/ml nocodazole (NZ) (Sigma). After supplementing the medium with reagent, cells were grown for 3–4.5 h and uniform cell cycle arrest was assayed by morphology. For expression of galactose induced genes cells were grown in YEP media with 3% glycerol (EMD, 30% v/v stock), 2% lactic acid (Fisher, 40% v/v stock pH 5.7) and 0.01 mg/ml adenine. Two percent galactose was added in mid-log and cells were grown for additional 2 h.

Microscopy

Fluorescence was observed using a Zeiss Axioplan 2 microscope (100× objective, NA = 1.40) with a Quantix CCD camera (Photometrics).

Immunoprecipitation

Yeast, asynchronous or synchronous, mid- to late-log cultures were collected by centrifugation and washed with water. Total 50–100 OD units of cell pellets were frozen in liquid nitrogen and kept at −80°C until use. For immunoprecipitation, cells were thawed on ice for 5 min and then resuspended in 350 μl chilled buffer IPB [50 mM Tris pH 7.6, 50 mM NaCl, 2.5 mM $MgCl_2$, 5 mM 2-mercaptoethanol and 0.4% NP-40, protease inhibitor cocktail (Roche)]. About two hundred fifty microliters glass beads (Sigma) were added and beat in a bead-beater for 3 pulses of 30 s at 4°C. The extract was clarified by centrifugation and diluted approximately 4× with IPB. Proteins tagged with HA or V5 were precipitated by anti-HA affinity matrix (Roche) or anti-V5 affinity matrix (Sigma), respectively, for 2 h at 4°C. For Mcd1p precipitation, rat anti-Mcd1 polyclonal antibody was added to the extract and the reaction was incubated for 2 h on ice. Antibodies were collected on protein G-agarose (Roche) for 1 h at 4°C. In all cases the beads were washed three times with 1 ml IPB for 5 min at room temperature. Proteins were eluted by adding 60 μl Laemmli buffer and boiling for 5 min. Samples were separated by polyacrylamide gel electrophoresis (SDS–PAGE), transferred to PVDF membrane and analyzed by western blot. The following antibodies were used for western blot, as indicated in the text: mouse anti-HA (Roche), mouse anti-V5 (Invitrogen), polyclonal rabbit anti-Mcd1, rabbit anti-acetyl-lysine (Cell Signaling).

Autoacetylation assay

Eco1 autoacetylation was preformed as previously described (18). Briefly, HA-tagged Eco1p was immunoprecipitated from cells as described above. After the final wash the antibody-bound protein was resuspended in 30 μl autoacetylation buffer (20 mM Hepes, pH 7.7, 50 mM KCl, 2.5 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 1 mg/ml BSA) 4.5 mg/ml Acetyl coA (Roche) was either included or excluded from the buffer as described in the text. The reaction was incubated for 30 min at either 23 or 37°C and stopped by addition of 30 μl Laemmli buffer. Proteins were eluted from the beads by boiling for 5 min. Proteins were separated by SDS–PAGE, transferred to PVDF membrane and analyzed by western blot.

DSB-induced cohesion assay

Damage-induced cohesion assay had been done as previously described (16). Briefly, YIO322 cells were

grown at 30°C and arrested in early S phase using hydroxyurea for 4 h 30 min. Cells were washed three times and resuspended in media containing nocodazole and grown at 30°C for 75 min. Cells were split into two Erlenmeyer flasks. One was kept at 30°C, while the second was shifted to 34°C to inactivate *eco1-203*. After 45 min cells were split again into two Erlenmeyer flasks and galactose was added to one flask at each temperature to induce DNA DSB by expressing HO endonuclease Mcd1p and eco1-zifp. After 1 h cells were shifted to 37.5°C for additional 45 min to remove S phase cohesion by inactivating *mcd1-1*. Samples were prepared for microscopy and sister chromatid cohesion was monitored.

RESULTS

Eco1 zinc finger is essential for cell viability

To understand the contribution of Eco1p zinc finger domain to the establishment of sister chromatid cohesion and assess its contribution to the acetyltransferase activity, we constructed a series of mutations in the zinc finger by sequentially replacing the zinc-coordinating residues with alanines (Figure 1). The predicted consequence of these substitutions is inability to stabilize the zinc ion within the structure, resulting in destruction of the domain's functional fold. To test the ability of the mutant proteins to support cell viability, we tested their ability to complement the activity of the temperature sensitive allele *eco1-203*. Either a wild type or zinc finger mutant allele were integrated at the URA3 locus under the control of galactose inducible promoter. Cells were grown at permissive temperature for *eco1-203* in non-inducing medium, serially diluted and plated with or without galactose and grown at various temperatures.

First we compared the biological activity of the different zinc finger alleles at the non-permissive temperature of 37°C (Figure 2A). As expected, at this temperature, cells that contain only the *eco1-203* allele are inviable but viability is restored when wild-type *ECO1* expression is induced. The zinc finger alleles show a significant decrease in viability that correlates with the number of mutations in the zinc finger. The mutant lacking all four zinc-coordinating residues (henceforth referred to as *eco1-zif*) reduces viability 10 000-fold similar to the inviability of cells expressing only *eco1-203* (Figure 2A). The partial function of alleles that only partially substitute zinc-coordinating residues may reflect partial folding and therefore, residual activity of the domain. Furthermore, Eco1p contains a histidine residue at position 62 just after histidine 57, which is one of the zinc coordinating residues (Figure 1). It is possible that this residue is compensating to some extent for the mutations introduced in histidine 53 and/or 57. To rule out the possibility that the mutations in the zinc finger affect the protein's stability, we confirmed by western blot that the expression level and stability of the mutant protein is comparable to wild-type Eco1p (Figure S1). No significant difference was found, indicating that the loss of viability of the cells carrying eco1-zifp results from lost of function rather than degradation of the protein (Figure 2).

Next we asked whether these zinc finger alleles retained any biological activity by examining their ability to complement *eco1-203* under less stringent conditions of 35°C. At this temperature, expression of all of our zinc finger alleles supported cell viability as well as wild-type *ECO1*. These results suggest that the zinc finger domain might only be required for Eco1p function at high temperature. Alternatively, the zinc finger domain is required for efficient Eco1 activity at all temperatures but overexpression compensates for the reduced activity of the mutant protein and support viability at lower temperatures.

To test the second possibility, we constructed a shut-off strain that contained the eoc1-zif allele or wild-type *ECO1*

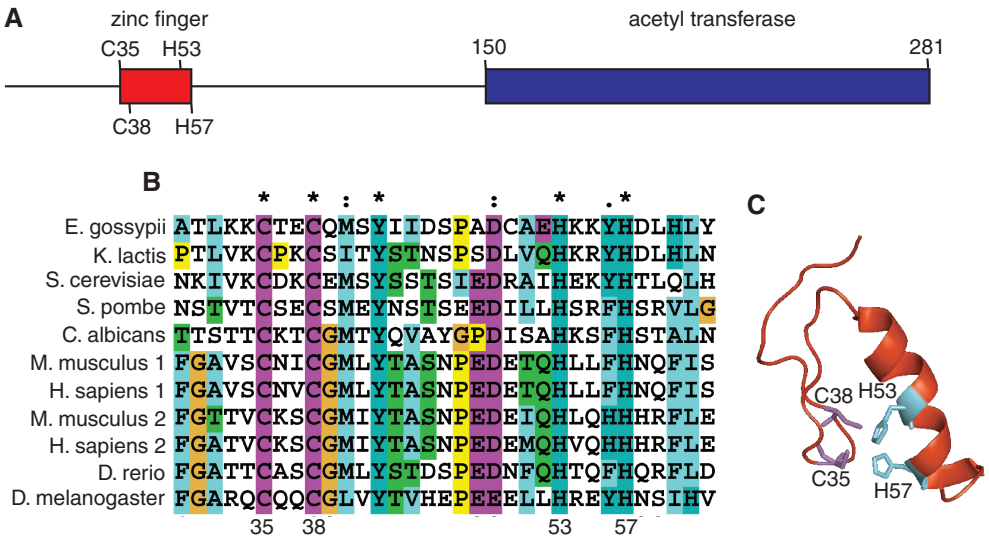

Figure 1. Eco1 zinc finger domain. (**A**) Map of Eco1 functional domains. Red and blue boxes represent the zinc finger and the acetyltransferase domains, respectively. Numbers indicate key residues in *S. cerevisiae*. (**B**) Multiple sequence alignment of Eco1p. When applicable, paralogs where included in the dataset. The conserved zinc coordinating Cysteines at position 35 and 38 and Histidines at position 53 and 57 are indicated. (**C**) Model of Eco1p zinc finger generated by Modeller (46). The zinc coordinating residues are color coded as in (B). *Consensus residues.

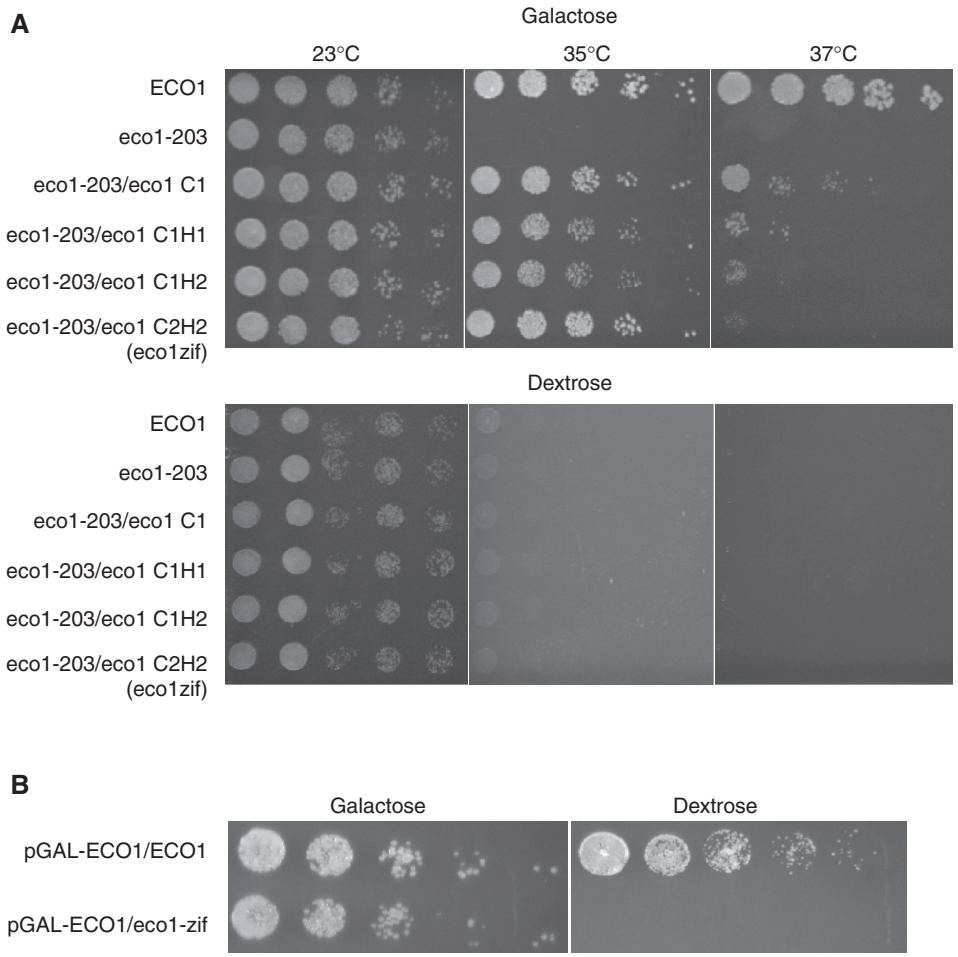

Figure 2. The zinc finger domain is essential for cell viability. (**A**) Cells carrying eco1-203 or both eco1-203 and either galactose-inducible *ECO1* or an eco1 zinc finger mutant allele were serially diluted and spotted on solid medium containing galactose (top panels) or dextrose (bottom panels) and grown at the indicated temperature. Strain used are YIO355 (eco1-203, GAL-*ECO1*), YIO35 (eco1-203), YIO35C1 (eco1-203, GAL-eco1 C35A), YIO35C1H1 (eco1-203, GAL-eco1 C35A, H53A), YIO35C1H2 (eco1-203, GAL-eco1 C35A, H53A, H57A) and YIO35C2H2 (eco1-203, GAL-eco1 C35A, H53A, H57A, C38A/*eco1-zif*). (**B**) Cells were carrying both galactose-induced *ECO1* and either *ECO1* or *eco1-zif* were serially diluted and spotted on solid rich medium containing galactose (left) or dextrose (right) and grown at 23°C. Strain used are YIO382 (GAL-*ECO1*, *ECO1*) and YIO381 (GAL-*ECO1*, eco1 C35A, H53A, H57A, C38A/*eco1-zif*).

under the native promoter at its endogenous locus and a second wild-type copy of *ECO1* allele under the control of galactose inducible promoter. These cells were constructed and grown in the presence of galactose at 23°C such that they were always expressing the *ECO1* allele. Then the expression of the galactose-induced *ECO1* allele was shut-off by adding dextrose to media. Western blot analysis validated the loss of the wild type Eco1p that was tagged by V5 epitope (Supplementary Figure S2). Upon addition of dextrose, cells with *eco1-zif* at the endogenous locus exhibited a 10 000-fold reduction of viability compared to cells with *ECO1* at the endogenous locus, a fold reduction similar to that seen for *eco1-203* at 35 or 37°C (Figure 2B). This result demonstrates that the zinc finger is critical for Eco1p activity even at low temperatures. Furthermore, taken together with the overexpression results, the zinc finger likely functions to augment an Eco1p activity as it can be partially replaced by producing more of eco1-zifp at low temperatures.

The zinc finger is essential for sister chromatid cohesion in S phase and damage response

Next, we wanted to test whether the lethality of cells expressing *eco1-zif* was due to a defect in sister chromatid cohesion (Figure 2). For this purpose, we constructed strains with the thermo-sensitive allele *eco1-203* and either a galactose-inducible *ECO1* or *eco1-zif* allele. Cultures of these strains were grown at the permissive temperature, arrested in G1 and then shifted to 37°C to inactivate *eco1-203*. Expression of either *ECO1* or *eco1-zif* was induced. Cells were released from G1 arrest and then allowed to progress to G2/M phase where they arrested. The level of sister chromatid cohesion in G2/M phase arrested cells was assessed by scoring one or two LacI-GFP dots at LacO array integrated in the Lys4 locus. As seen in Figure 3A, in the presence of *ECO1*, sister chromatids are seen as a single GFP spot indicating that the chromatids are held together. In cells lacking Eco1p activity, cohesion is not established and the

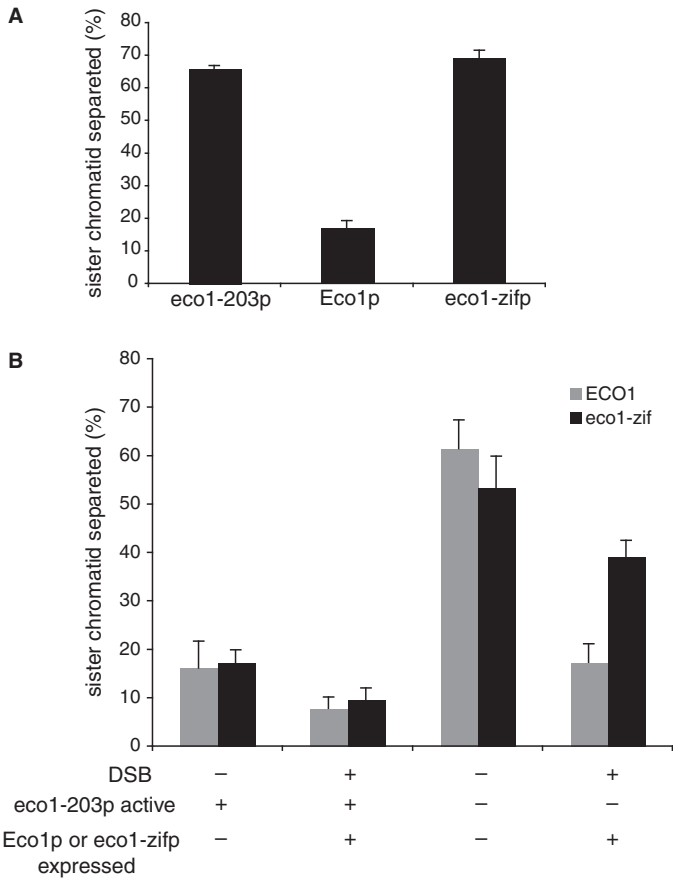

Figure 3. The zinc finger domain is essential for sister chromatid cohesion. (**A**) Cohesion assay in YIO35 (eco1-203), YIO355 (eco1-203, GAL-*ECO1*) or YIO35C2H2 (eco1-203, GAL-*eco1-zif*). $N = 3$, Error bars indicate standard deviation. (**B**) DSB-induced cohesion assay in YIO332 (eco1-203, GAL-*ECO1*) and YIO331 (eco1-203, GAL-*eco1-zif*). $N = 3$, Error bars indicate standard deviation.

separated chromatids appear as two distinct GFP spots. When eco1-zifp is expressed, the majority of the cells contain two GFP spots like the *eco1-203* allele. These results suggest that the zinc finger is critical to establish cohesion during S phase as expected for its critical role in cell viability.

Next, we asked whether the zinc finger was also required for DSB-induced cohesion. DSB induces de novo cohesion around the site of the DSB and additional cohesion genome-wide. Since DSB-induced cohesion also depends upon Eco1p, it might seem obvious that the zinc finger would be required for Eco1p activity in this context. However, an allele of Eco1p has already been identified that compromises cohesion after a DSB but not in S phase. Furthermore Eco1p acetylates distinct substrates for S phase and damage-induced cohesion. Given these distinct functions of Eco1p, it was important to test whether the zinc finger was critical for Eco1 function in DSB-induced cohesion.

To test this possibility, we used the previously described assay to monitor damage-induced cohesion in G2/M (16). Cells containing the thermo-sensitive alleles *mcd1-1* and *eco1-203* were arrested in S at permissive temperature. Cells were washed and were allowed to progress into G2 before temperature was shifted to 34°C to inactivate eco1-203p (but not mcd1-1p). At this point, galactose was added to express HO endonuclease and induce DNA DSB. In addition, galactose-induced expression of wild type Mcd1p and either Eco1p or eco1-zifp. Then, the temperature was further increased to 37.5°C to inactivate mcd1-1p and remove S phase cohesion and cells progress to M phase where they arrested. Cohesion was monitored as above, by one or two GFP spots at Lys4 (Figure 3B). Under these conditions, S phase cohesion was removed but sisters stay together only if cohesion was re-established in response to DSB. Induction of a DSB concurrent with the expression of a wild type Eco1p induced cohesion as indicated by a single GFP spot and as had been shown previously (16), indicating that under the experimental conditions the kinetics of Eco1p can support cohesion establishment in G2/M in respond to DNA damage. However, no sister chromatid cohesion was detected when eco1-zifp was expressed concomitant with the formation of the DSB (Figure 3B). These results show that the zinc finger is essential to both S phase and damage-induced cohesion. Moreover, the results support our conclusion that the zinc finger augments Eco1p activity. However, they also suggest, yet does not rule out that the zinc finger is dispensable for substrate specificity since it is required for cohesion in both S phase and damage respond, which involve acetylation of Smc3p and Mcd1p, respectively.

The zinc finger is important for acetyltransferase activity

Given that the zinc finger and the acetyltransferase activity are both required for S phase and DSB-induced cohesion, it seemed reasonable to test whether the zinc finger was important for Eco1p acetyltransferase activity. Eco1p can acetylate itself *in vitro*. To test if the zinc finger domain is required for Eco1p autoacetylation we preformed a previously described *ex vivo* autoacetylation assay (18). *eco1-203* cells were induced to express either Eco1-HAp or eco1-zif-HAp. The HA-tagged proteins were immunoprecipitated, washed and while attached to the beads resuspended in buffer with acetyl-coA and incubated at 37°C. The reaction was stopped and proteins were eluted from the beads and analyzed by SDS–PAGE. Their acetylation state was determined by western blotting using an anti-acetyl lysine antibody (Figure 4A). No autoacetylation was detected in the absence of tagged protein or without the addition of acetyl-coA indicating that the acetylation activity is specific to Eco1p. In the presence of acetyl-coA autoacetylation was detected when Eco1p was precipitated. However, no autoacetylation was detected for eco1-zifp, indicating that eco1-zifp is defective for acetyltransferase activity.

We next looked at acetylation of Smc3p K112 residue. Previous studies showed that acetylation of this residue is a marker for Eco1p activity *in vivo* but not essential for cohesion establishment. Cells bearing the thermo-sensitive allele *eco1-203* are grown at the permissive temperature, induced to express *ECO1* or *eco1* zinc

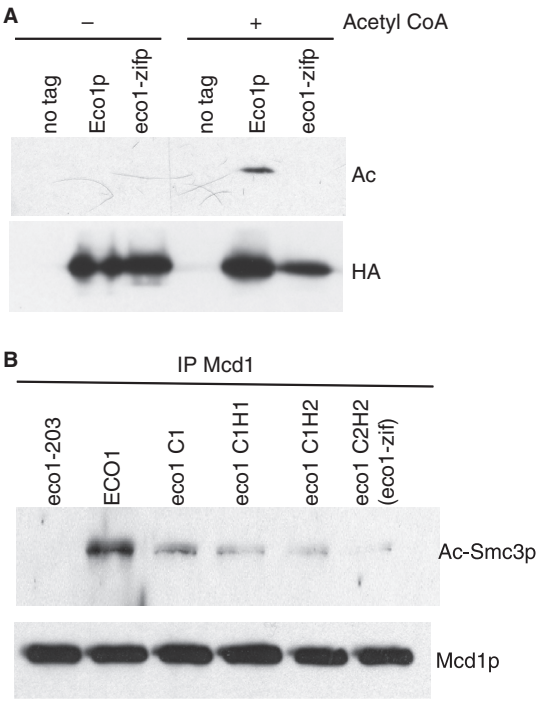

Figure 4. The zinc finger is essential for acetylation. (**A**) Immunoprecipitation with anti-HA from protein extracts made from YIO35 (eco1-203), YIO355 (eco1-203, GAL-*ECO1*-HA) and YIO35C2H2 (eco1-203, GAL-*eco1-zif*-HA). Precipitated proteins bound to the beads were incubated at 37°C with or without acetyl-coA and analyzed by western blot using anti-acetyl lysine antibody (upper panel) or anti-HA (lower panel). (**B**) Immunoprecipitation with anti-Mcd1 from protein extracts made from YIO35 (eco1-203), YIO355 (eco1-203, GAL-*ECO1*-HA), YIO35C1 (eco1-203, GAL-eco1 C35A-HA), YIO3C1H1 YIO351 (eco1-203, GAL-eco1 C35A, H53A-HA), YIO35C1H2 (eco1-203, GAL-eco1 C35A, H53A, H57A-HA) and YIO35C2H2 [eco1-203, GAL-eco1 C35A, H53A, H57A, C38A-HA (*eco1-zif*)]. The precipitated cohesin subunits Mcd1p and Smc3p were analyzed by western blot with anti-Mcd1 and anti-acetyl lysine, respectively.

finger alleles. Cells were lysed and the cohesin complex was immunoprecipitated and then analyzed for Smc3p acetylation using anti-acetyl antibody. The *eco1-203* has undetectable levels of Smc3p K112 acetylation even at the permissive temperature (Figure 4B, lane 1). Cells induced for *ECO1* restore high levels of Smc3p acetylation (Figure 4B, lane 2). In cells expressing the zinc finger mutants replacing one, two, three or four of the zinc coordinating residues Smc3p acetylation levels are significantly decreased with the lowest acetylation signal obtained when all four residues are replaced (Figure 4B). This reduction in acetylation is not due to differences in expression as the levels of wild-type and mutant proteins are very similar (Figure S3). Thus the zinc finger is required both for Eco1p autoacetylation and Smc3p K112 acetylation.

The augmentation of acetylation activity by the zinc finger might be explained by increasing accessibility to the substrate. Increased accessibility could be achieved by enhancing the binding between Eco1p and its substrates. However, the pattern of cohesin related proteins or general proteins that co-immunprecipitate with Eco1p and eco1-zifp is the same, so we have no evidence for compromised binding (Supplementary Figure S4). It had been reported previously that Eco1p presumably interacts with Pds5p (30,31). In that context, the significance of the current co-immunoprecipitation is in complementing the previous work and demonstrating this interaction *in vivo*. Moreover, we show here that Eco1p and cohesin are interacting *in vivo*. It is important to note that under our experimental conditions only a small fraction of Mcd1p was pulled down by Eco1p (data not shown) suggesting that only small sub-population of cohesion is associated with Eco1p *in vivo*. It is yet to be determined whether this interaction is mediated by Pds5 or by another cohesin subunit. A second way to promote accessibility to its cohesin substrates is by its localization to chromatin. Eco1p fractionates with chromatin. However, eco1-zifp also fractionates equally well with chromatin indicating that chromatin localization is independent of the zinc finger (Supplementary Figure S5). Localization of Eco1p to chromatin and replication fork involves interaction with several chromatin factors. Therefore, the current result cannot detect temporal and spatial malfunction of the mutant protein. In conclusion the zinc finger enhances acetyltransferase activity of Eco1p but the mechanism waits to be elucidated.

Eco1 is a multimer

Surprisingly, when eco1-zifp is immunoprecipitated from *eco1-203* cells and the ex-vivo autoacetylation assay is conducted at permissive temperature, activity is observed, similar to the levels after immunoprecipitation of Eco1p (Figure 5A). This could be explained in two ways. The eco1-zifp acetyltransferase might have robust acetyltransferase activity at 23°C. However if this were true eco1-zifp mutants would most likely be viable at 23°C, which they are not (Figure 2B). Alternatively, Eco1p may multimerize with itself. In this case heteromultimers could form between eco1-zifp and eco1-203p. Immunoprecipitation of eco1-zifp, brings along eco1-203p that are responsible for the robust autoacetylation activity.

Therefore, we tested the possibility that Eco1p is a multimer. To achieve this we constructed a yeast strain containing two, differentially tagged copies of *ECO1*. One copy was tagged with the V5 epitope (Eco1-V5p) under the gene's native promoter. The second one is under galactose-inducible promoter and tagged with HA (Eco1-HAp). We immunoprecipitated Eco1-V5p and looked for the presence of Eco1-HAp tagged protein. Figure 5B shows that Eco1-HAp is pulled down by Eco1-V5p. Furthermore, immunoprecipitation of the eco1-zif-HAp effectively co-precipitated Eco1-V5p (Supplementary Figure S6). This result explains the auto-acetyltransferase activity seen after immunoprecipitation of eco1-zifp from cells co-expressing eco1-zifp and eco1-203p at 23°C. Thus, Eco1p multimerizes *in vivo*, although the multimerization is independent of the zinc finger domain.

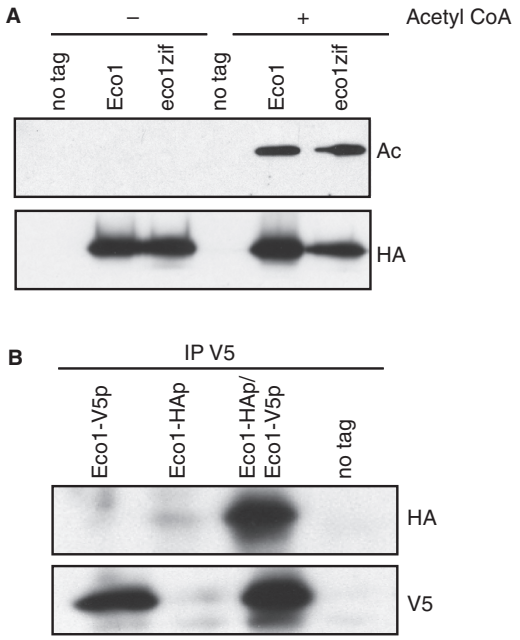

Figure 5. Eco1 is a multimer. (**A**) Immunoprecipitation with anti-HA from protein extracts made from YIO35 (eco1-203), YIO355 (eco1-203, GAL-*ECO1*-HA) and YIO35C2H2 (eco1-203, GAL-*eco1-zif*-HA). Precipitated proteins bound to the beads were incubated at 23°C with or without acetyl-coA and analyzed by western blot using anti-acetyl lysine antibody (upper panel) or anti-HA (lower panel). (**B**) Immunoprecipitation with anti-V5 from protein extracts made from 3136-3D (*ECO1*-V5), YIO312 (GAL-*ECO1*-HA), YIO30 (*ECO1*-V5, GAL-*ECO1*-HA,) and VG3131 (*ECO1*). Proteins were analyzed by western blot using antibodies against HA (upper panel) or V5 (lower panel).

DISCUSSION

Here we analyze the role of the Eco1p zinc finger by multiple approaches. Our results suggest that the zinc finger of Eco1p dramatically enhances the acetyltransferase activity. We show that disruption of the integrity of Eco1 zinc finger reduces the ability of Eco1p to autoacetylate itself *in vitro* and to acetylate Smc3p *in vivo*. Furthermore, the zinc finger is also likely necessary for Eco1p acetylation of Mcd1p since eco1-zifp mutant is defective for the establishment of DSB-induced cohesion, which specifically requires acetylation of Mcd1. Finally, the fact that over-expression of the zinc finger mutant eco1-zifp acetylates Smc3p to low but detectable levels *in vivo* suggests that Eco1p has basal acetyltransferase activity in the absence of the zinc finger. Taken together we suggest the zinc finger augments Eco1p acetyltransferase activity toward Eco1p substrates.

The enhancement of Eco1p acetyltransferase activity by its zinc finger has precedent. A mutation within Eco1p zinc finger domain (S41L) suppress the temperature sensitivity which result from a mutation in the catalytic domain (21). One model to explain how the zinc finger augments acetyltransferase activity is that the zinc finger functions in the substrate recognition. Indeed several histone acetyltransferases including p300/CBP and MOZ have zinc fingers that are required for their acetyltransferase activity. In some cases it has been shown that zinc finger of histone acetyltransferases mediates association with histones by interacting with sequences on the histone tail (32,33). One possibility is that the substrates share a common docking site, as had been shown for other acetyltransferases (34,35). However, we cannot find any shared amino acid sequence in the substrates proximal to acetylation sites. Furthermore, the zinc finger is required for Eco1-dependent acetylation of all its established acetylation sites in vivo, making this model less attractive.

As an alternative model, the zinc finger may orient substrates to maximize interactions with the functional residues of the active site with no discrimination between substrates. The zinc finger could dock on one site in the cohesin complex or on chromatin proximal to sites of cohesin enrichment. This proximity would enhance its local concentration and thereby its ability to acetylate multiple sites on different cohesin and cohesin-auxiliary substrates. However, we were unable to demonstrate interaction mediated by the zinc finger. Moreover, we did not find any DNA binding activity for the Eco1p (Onn and Koshland, unpublished observation), or were we to see any reduction in the ability of eco1-zifp to bind to chromatin *in vivo*. Therefore how the zinc finger stimulates Eco1p acetyltransferase activity for multiple disparate substrates remains an important but unanswered mechanistic question.

Here we show that the zinc finger is absolutely essential for cohesion establishment in S phase when Eco1p is expressed at endogenous levels but mostly dispensable when Eco1p is overexpressed. This implies that the augmentation of the acetyltransferase activity by the zinc finger is necessary to raise the basal level of transferase activity above a threshold necessary to establish cohesion. Previous studies from our laboratory have established that Eco1p acetylation of Smc3p is controlled during the cell cycle with Eco1p activity becoming limiting in G1 and G2/M. One way to turn up or down the acetyltransferase activity would be by modulating its zinc finger function, by modifications [e.g. (36,37)]. Moreover, the structure of Hat1 had reveled that the N-terminal domain induces conformational changes in the adjacent acetyltransferase domain suggesting that this kind of mechanism is feasible (38).

Our analysis of the Eco1 zinc finger led to the discovery that Eco1p is a multimer. Multimerization of other acetyltransferases has been reported (39). However, multimerization of acetyltransferases is not a structural prerequisite for activity and therefore, the function of Eco1p multimerization remains to be elucidated. Intriguingly, the ability of Eco1p to multimerize provides it with the potential to coordinate acetylation of multiple substrates either spatially or temporally as had been suggested before (39). Given its numerous cohesin targets *in vivo*, it is easy to imagine how this coordination could facilitate the establishment of sister chromatid cohesion. For example in embrace models, it could simultaneously acetylate Smc3p residues to promote establishment and Pds5p to immediately lock the embraced ring into a stable maintenance form (10,21,40–43). Alternatively as postulated in snap-like models, it could acetylate adjacent

cohesin complexes to promote oligomerization and hence cohesion (44,45). Dissection of these possibilities in the future will shade light on this highly complex process.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENTS

The authors thank Kate Lannon for technical help and the Koshland lab members for technical support and discussions.

FUNDING

Howard Hughes Medical Institutes grants (to D.K.). Funding for open access charge: Howard Hughes Medical Institute.

Conflict of interest statement. None declared.

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