Deacetylation of topoisomerase I is an important physiological function of \textit{E. coli} CobB

Qingxuan Zhou\textsuperscript{1,2}, Yan Ning Zhou\textsuperscript{3}, Ding Jun Jin\textsuperscript{3} and Yuk-Ching Tse-Dinh\textsuperscript{1,2,*}

\textsuperscript{1}Department of Chemistry and Biochemistry, Florida International University, Miami, FL 33199, USA, \textsuperscript{2}Biomolecular Sciences Institute, Florida International University, Miami, FL 33199, USA and \textsuperscript{3}Transcription Control Section, RNA Biology Laboratory, National Cancer Institute, National Institutes of Health, Frederick, MD 21702, USA

Received June 28, 2016; Revised March 28, 2017; Editorial Decision March 31, 2017; Accepted April 03, 2017

ABSTRACT

\textit{Escherichia coli} topoisomerase I (TopA), a regulator of global and local DNA supercoiling, is modified by \textit{N}\textsuperscript{\epsilon}\textsuperscript{-}-Lysine acetylation. The NAD\textsuperscript{+}-dependent protein deacetylase CobB can reverse both enzymatic and non-enzymatic lysine acetylation modification in \textit{E. coli}. Here, we show that the absence of CobB in a \textit{ΔcobB} mutant reduces intracellular TopA catalytic activity and increases negative DNA supercoiling. TopA expression level is elevated as \textit{topA} transcription responds to the increased negative supercoiling. The slow growth phenotype of the \textit{ΔcobB} mutant can be partially compensated by further increase of intracellular TopA level via overexpression of recombinant TopA. The relaxation activity of purified TopA is decreased by \textit{in vitro} non-enzymatic acetyl phosphate mediated lysine acetylation, and the presence of purified CobB protects TopA from inactivation by such non-enzymatic acetylation. The specific activity of TopA expressed from His-tagged fusion construct in the chromosome is inversely proportional to the degree of \textit{in vivo} lysine acetylation during growth transition and growth arrest. These findings demonstrate that \textit{E. coli} TopA catalytic activity can be modulated by lysine acetylation–deacetylation, and prevention of TopA inactivation from excess lysine acetylation and consequent increase in negative DNA supercoiling is an important physiological function of the CobB protein deacetylase.

INTRODUCTION

Posttranslational modification is a reversible mechanism used by cells for rapid adaptation to changes in external environment without requiring the synthesis of new RNA and protein to fulfill the needs of adaptation to diverse growth conditions (1–3). Posttranslational modifications can alter the protein function, conformation, stability and localization. \textit{N}\textsuperscript{\epsilon}-Lysine acetylation is one of the most abundant posttranslational modifications (4–6). Recently, proteomics studies have demonstrated the broad existence of lysine acetylation not only in eukaryotes, but also in diverse bacterial species including \textit{Escherichia coli} (7–10). In addition, compelling evidence revealed the significant roles of \textit{N}\textsuperscript{\epsilon}-Lysine acetylation in bacterial physiology (6,11,12) for the regulation of central metabolism enzymes, motility and chemotaxis gene expressions and stress resistance systems (12–16). Studies of \textit{E. coli} chemotaxis response regulator CheY, acetyl-CoA synthetase, RNA polymerase, regulators of capsule synthesis B (RcsB), RNase R, RNase II and \textit{N}-hydroxyarylamine \textit{O}-acyetyltransferase (NhoA) explored the role of lysine acetylation in the regulation of these proteins (13,17–20). Two distinct mechanisms of lysine acetylation have been reported in \textit{E. coli} (10). The first mechanism is enzymatic acetylation, which requires the Pka/YfiQ enzyme, a Gcn5-like acetyltransferase, or other acetyltransferases yet to be identified, to transfer the acetyl group from acetyl-coenzyme A (acCoA) to the \textit{ε}-amino group of a lysine (9,21). Both enzymatically acetylated-lysine and nonenzymatically acetylated-lysine modifications are reversed by the NAD\textsuperscript{+}-dependent sirtuin deacetylase CobB, a predominant lysine deacetylase in \textit{E. coli} that shows no preference for these two types of acetylated lysine sites (21).

\textit{Escherichia coli} topoisomerase I, an enzyme encoded by the \textit{topA} gene, regulates global and local DNA supercoiling. The level of supercoiling affects DNA-centered processes, including DNA replication, transcription, recombination and transposition (22–25). The level of DNA supercoiling itself is constantly affected by transcription (26), as well as changes in nutrient availability and growth environment (27–30). The homeostatic state of unconstrained DNA supercoiling is maintained mainly by the relaxation action of topoisomerase I (TopA) and the supercoiling action of DNA gyrase (25,31). Transcription of \textit{topA} is increased when DNA is more negatively supercoiled while transcription of \textit{gyrA} and \textit{gyrB} is increased by DNA re-
laxation (32,33). In addition, the initiation of \textit{E. coli} top\lea\n transcription at multiple promoters (34,35) is dependent on multiple sigma factors including \(\sigma^{70}\), \(\sigma^{32}\) and \(\sigma^{\ast}\). The relaxation of transcription-driven negative supercoiling by TopA is important for the response to stress challenge including heat shock and oxidative stress (36–38). TopA function has also been shown to be important for survival following acid and antibiotics challenge (39–41). Posttranslational modification of TopA could be an additional mechanism for regulation of TopA activity and DNA supercoiling. In previous proteomics studies of protein acetylation in \textit{E. coli}, multiple lysines of TopA were found to be acetylated (7–9,42–44). Lysine acetylation of TopA was found to be sensitive to acetyl phosphate (acP) levels (9,43). Therefore, it appears that cation of TopAc could be an additional mechanism for regulation of transcription-driven negativesupercoiling by TopA. The frequency of observation of TopA acetylation and its cation of TopA catalytic activity could be important for survival following acid stress challenge including heat shock and oxidative stress (36–38). TopA function has also been shown to be important for survival following acid and antibiotics challenge (39–41). Posttranslational modification of TopA could be an additional mechanism for regulation of TopA activity and DNA supercoiling. In previous proteomics studies of protein acetylation in \textit{E. coli}, multiple lysines of TopA were found to be acetylated (7–9,42–44). Lysine acetylation of TopA was found to be sensitive to acetyl phosphate (acP) levels (9,43). Therefore, it appears that cation of TopAc could be an additional mechanism for regulation of transcription-driven negativesupercoiling by TopA.

\textbf{MATERIALS AND METHODS}

\textit{Escherichia coli} strains BW25113 [\(\Delta\text{(araD-araB)}\)567 \(\Delta\text{lacZ4787}::\beta\text{rnB-3}) \gamma^\ast\text{ rph-1} \Delta\text{(rhaD-rhaB)}\)568 \(\text{hsdR514})\]

JW1106-1 [\(\Delta\text{(araD-araB)}\)567 \(\Delta\text{lacZ4787}::\beta\text{rnB-3}) \gamma^\ast\text{ cobB779}::\text{kan rph-1} \Delta\text{(rhaD-rhaB)}\)568 \(\text{hsdR514})\]

(46) were provided by the Yale Coli Genetic Stock Center. The strain YN1434 containing a KanR cassette inserted immediately before the \(\text{topA}\) gene that encodes additional MRGSHHHHHHGS sequence at the N-terminus of the TopA protein was constructed using the phage lambda Red-mediated recombination system (47) with details provided in the Supplementary Information. pETOP plasmid is a derivative of pBAD/Thio (Invitrogen) expressing recombinant \(\text{E. coli}\) TopA (48).

ASKA plasmid pCA24N that encodes CobB with an N-terminal His tag (49) was provided by NBRP (NIG, Japan): \textit{E. coli}. The \textit{E. coli} cobB gene was amplified using the primers: 5\textprim{T}GAGCGTATGCAAGCCTCAGCTATGGGACAAAGAGTACTCGTACT-3\textprim{C} and 5\textprim{T}GGGATATTACCTCTGCTCATTGCCTGGGATGTCCTCGTACT-3\textprim{C} and ASKA plasmid pCA24NCobB as the template with Q5 High-fidelity DNA polymerase (New England Biolabs). The PCR product was cloned into pBAD/Thio to create plasmid pCobB using HiFi DNA assembly master mix (New England Biolabs). Cells were cultured in LB (Luria broth) with shaking at 200 rpm at 37\textdegree C. Then, the following final concentrations: carbenicillin 50 \(\mu\text{g/ml}\), kanamycin 50 \(\mu\text{g/ml}\).

\textbf{Chloroquine gel analysis of DNA supercoiling}

Plasmids were isolated from BW25113 or JW1106-1 transformants collected at the indicated growth stage using GeneJET Plasmid Mini prep Kit (Thermo Scientific). The superhelical density of the isolated plasmid DNA (1 \(\mu\text{g}\)) was compared using 2D electrophoresis analysis in 0.8\% agarose gels with TAE buffer (40 mM Tris–acetate, pH 8.0, 2 mM EDTA). The first dimension gel and buffer contained 3 \(\mu\text{g/ml}\) chloroquine, and electrophoresis was carried out at 3 V/cm for 16 h. The gel was soaked and equilibrated in the running buffer containing 25 \(\mu\text{g/ml}\) chloroquine for at least 5 h. For the second dimension electrophoresis, the gel was rotated 90\degree in the gel tank and electrophoresed at 1.5 V/cm for 20 h. To remove chloroquine, the gel was washed by shaking in TAE buffer for 2 h with the buffer replaced every 30 min. The gel was then stained with ethidium bromide and visualized with UV light (AlphaImager Mini, Protein-Simple).

\textbf{Assay of TopA relaxation activity in cell lysates}

Wild type and \(\text{Delta1 cobB}\) mutant strains were grown in LB at 37\textdegree C with shaking until OD\textsub{600} = 0.8. Cells were pelleted by centrifugation at 4\degree C and lysed by lysozyme treatment in lysis buffer (50 mM NaH\textsub{2}PO\textsub{4}, 0.3 M NaCl) on ice and three freeze-thaw cycles. The total soluble lysates were obtained as the supernatant fractions following centrifugation at 30,000 rpm at 4\degree C for 1 h. Protein concentrations were determined using the Bradford Protein Assay (Bio-Rad). TopA activity in cell lysates was assayed in a standard reaction volume of 20 \(\mu\text{l}\) (10 mM Tris–HCl, pH 8.0, 50 mM NaCl, 0.1 mg/ml gelatin, 6 mM MgCl\textsub{2}, 150 ng of supercoiled pBAD/Thio plasmid DNA). Purified recombinant TopA was added to relax the DNA substrate in control reactions. After 20 min incubation at 37\degree C, the reaction was stopped by addition of 4 \(\mu\text{ml}\) stop solution (50 mM EDTA, 50\% glycerol, 0.5\% (v/v) bromophenol blue). The DNA was electrophoresed in 0.8\% agarose gel with TAE buffer containing 1 \(\mu\text{g/ml}\) chloroquine. After the removal of chloroquine, the gel was stained with ethidium bromide and visualized with UV light.

\textbf{Western blot analysis of TopA protein in cell lysates}

Twenty microgram of total cell lysates in SDS gel sample buffer (2\% SDS, 62.5 mM Tris–HCl, pH 6.8) was heated at 100\degree C for 5 min. After separation by electrophoresis in 10\% SDS-polyacrylamide gel, the total proteins in the gel was subjected to western blot analysis with mouse monoclonal antibody against \textit{E. coli} TopA described previously (39). Signals were developed with ECL Plus reagents (Thermo Scientific), and detected with C-DigiT Blot Scanner (LI-COR). Bands were quantified using the Image Studio Digits Ver 4.0 software.

\textbf{Quantitative PCR of top\lea\n mRNA}

Wild type and \(\text{Delta1 cobB}\) cells were grown to \(\text{A}_{600}\) of 0.8. Total RNA was isolated using the Quick-RNA mini prep Kit.
Pull-down assays to study direct interaction between *E. coli* CobB and TopA

Purified recombinant TopA protein (5 nM) was mixed with purified N-terminal His-tagged CobB protein (10–50 nM) in pull-down buffer (10 mM HEPES, pH 7.4, 100 mM NaCl, 0.005% v/v Tween-20) overnight at 4°C. HisPur Cobalt Agarose resin (Thermofisher), pre-equilibrated in pull-down buffer, was then added to the CobB-TopA reaction and mixed at 4°C for 2 h. After centrifugation of the reactions, the resin was washed three times in pull-down buffer with 10 mM imidazole. The proteins bound to the resin were eluted in SDS sample buffer by boiling for 2 min. Eluates were electrophoresed in 10% polyacrylamide SDS gel, and TopA was detected by western blotting with monoclonal antibodies against *E. coli* TopA.

Purification and characterization of chromosomally encoded His-tagged TopA during growth transition and growth arrest

TB7 broth (10 g/l tryptone, 100 mM potassium phosphate pH 7.0) supplemented with 0.4% glucose was inoculated with overnight culture of strain YN1434 at 1:750 dilution. Cells were cultured with shaking at 37°C with shaking at 37°C for 4.5 h (1.5 l of culture at OD(600) = 0.7), 5.5 h (750 ml of culture at OD(600) = 1.7), 24 h (500 ml of culture at OD(600) = 1.9). His-TopA expressed from the chromosome was purified from the cell pellets using GE Ni sepharose columns with the protease inhibitor PMSF (1 mM) and CobB inhibitor nicotinamide (10 mM) added to the lysis buffer. Based on Coomassie blue staining of purified His-TopA, equal amounts of His-TopA (1 μg) purified from the three growth conditions were electrophoresed in 10% SDS gel and transferred onto nitrocellulose membrane. The His-TopA on the membrane was stained with the MemCode (Thermo Scientific) reversible protein stain kit before western blot analysis with antibodies against acetylated lysine. The signals were quantified using the Image Studio Digits Ver 4.0 software.

RESULTS

Loss of CobB led to increase in DNA supercoiling

To assess the effect of lysine acetylation on DNA supercoiling in *E. coli*, wild-type BW25113 and ΔcobB mutant JW1106 transformed with plasmid pBAD/Thio were grown at 37°C until OD(600) reached 0.8. The extracted DNA plasmids were electrophoresed in agarose gel in the presence of 3 μg/ml chloroquine in the first dimension and 25 μg/ml chloroquine in the second dimension to resolve the positively and negatively supercoiled DNA topoisomers. Plasmid pBAD/Thio from ΔcobB cells was more negatively supercoiled than DNA isolated from isogenic cobB+ cells (Figure 1). When the ΔcobB mutation was complemented in trans by cobB cloned into pCobB plasmid, the supercoiling level of the plasmid isolated from wild-type and ΔcobB mutant was identical (Figure 1). This result indicated that loss of CobB deacetylation function affects DNA supercoiling. Since TopA is the major activity for relaxation of negatively supercoiled in *E. coli* and CobB has been shown by a previous study to associate with TopA (45), the increase in negative DNA supercoiling in ΔcobB cell may be due to reduction in
TopA activity in the absence of CobB. Nonenzymatic lysine acetylation of TopA would require CobB for reversal of the post-translational modification. The influence of CobB on TopA activity and cell growth was investigated with further experiments.

**Loss of CobB reduced cellular TopA activity while TopA expression was increased**

We hypothesized that increased lysine acetylation on TopA in the \( \Delta \)cobB mutant led to reduction of TopA catalytic activity. The relaxation activities of TopA in total cell lysates of the wild type and \( \Delta \)cobB strains were compared in assay with negatively supercoiled plasmid DNA as the substrate. The results showed that TopA from \( \Delta \)cobB mutant cell lysate displayed reduced relaxation activity (Figure 2, top panel). The reaction buffer used in the Figure 2 experiments did not contain ATP so gyrase and topoisomerase IV present in the cell lysates would not be active on the plasmid DNA substrate under these experimental conditions. The reduction of TopA relaxation activity in the \( \Delta \)cobB mutant may account for the increased negative DNA supercoiling observed in Figure 1. Complementation by plasmid pCobB restored the TopA relaxation activity in the \( \Delta \)cobB mutant cell lysate (Figure 2, bottom panel). Overexpression of CobB in the wild-type \( \text{cobB} \) background did not result in higher relaxation activity. These results suggest that lack of normal physiological levels of CobB deacetylation activity could result in reduction of TopA catalytic activity, and increase of negative DNA supercoiling.

However, an alternate possibility to explain the reduction of TopA relaxation activity in the \( \Delta \)cobB mutant is that the intracellular level of TopA protein in this strain is lower, resulting in decreased relaxation activity in the total cell lysate. To examine this possibility, TopA protein and \( \text{topA} \) mRNA levels were analyzed. We first determined the TopA protein level in wild-type and \( \Delta \)cobB cells by western blotting analysis. Total soluble lysates obtained from wild type and \( \Delta \)cobB cells were processed in parallel and the same amount of total proteins was loaded onto an SDS-PAGE gel. Equal loading of total cellular proteins based on Bradford protein assay results was confirmed by Coomassie blue staining (Supplementary Figure S1). This allows us to compare the relative abundance of TopA from the same amount of total protein. TopA protein level was significantly increased in the \( \Delta \)cobB mutant, approximately 1.6-fold higher than that in wild-type cells (Figure 3A and B). Measurement of \( \text{topA} \) mRNA level by qPCR analysis also showed that \( \text{topA} \) transcription is higher (1.4–1.5-fold with \( \text{hctA} \) and \( \text{idhT} \) as internal references) in the \( \Delta \)cobB mutant than in wild-type cells (Figure 3C). These results showed that the reduction in the TopA catalytic activity in the \( \Delta \)cobB cell extract (Figure 2) was not due to decrease in the TopA protein level. The higher level of \( \text{topA} \) expression in the \( \Delta \)cobB mutant is likely due to the stimulation of \( \text{topA} \) transcription by the increase in negative supercoiling in the homeostatic regulation of DNA supercoiling (32, 53). However, even with the increased \( \text{topA} \) expression, there is a deficiency in the TopA catalytic activity affecting the level of DNA supercoiling in the \( \Delta \)cobB mutant.

**Overexpression of recombinant TopA partially compensated the delayed growth of the \( \Delta \)cobB mutant**

The growth kinetics of wild-type BW25113 and \( \Delta \)cobB mutant JW1106 transformed with pBAD/Thio plasmid was monitored continuously for 24 h in LB culture grown with vigorous shaking at 37°C. The \( \Delta \)cobB mutant strain grew much slower during the exponential phase when compared to the wild-type strain (Figure 4). The reduction of cellular TopA catalytic activity as well as increased negative supercoiling observed here for the \( \Delta \)cobB mutant might account in part for its slow growth phenotype. A high copy number plasmid pETOP derived from the pBAD/Thio plasmid...
that overexpresses recombinant TopA was introduced into both strains for growth rate analysis under the same conditions. While wild-type cells with either pBAD/Thio or pETOP displayed similar growth rates, the delayed growth of the ΔcobB mutant was partially rescued by the presence of pETOP (Figure 4). This result indicates that reduction of TopA activity when acetylation-deacetylation regulation is perturbed is of physiological significance. Overexpression of recombinant CobB from the pCobB plasmid also partially rescued the slow growth of the ΔcobB mutant, with greater degree of complementation in the presence of 0.0001% arabinose to further induce the expression of recombinant CobB (Figure 4).

Acetylation by acP decreases TopA catalytic activity

To examine the effect of lysine acetylation on TopA catalytic activity directly, we performed in vitro non-enzymatic lysine acetylation of TopA. Recombinant TopA expressed in a cobB+ genetic background, was purified and incubated with acetyl phosphate (acP). Increase in TopA lysine acetylation in the presence of 2 mM and 5 mM acP was observed by western blotting with antibodies against acetyllysine (Figure 5A). Assays with negatively supercoiled plasmid DNA as the substrate showed that the relaxation activity of TopA was reduced by ~4-fold following acetylation by 2 mM acP and >8-fold following acetylation by 5 mM acP (Figure 5B).
Lysine deacetylation by CobB can counter the effect of non-enzymatic lysine acetylation on TopA catalytic activity

Lysine acetylation is a reversible post-translational modification, and acetylation–deacetylation can take place at the same lysine residues. We showed evidence that TopA relaxation activity in ΔcobB cell lysate is reduced, and nonenzymatic acetylation of purified TopA resulted in a decrease of its catalytic activity. Hence, the effect of CobB lysine deacetylase on TopA inactivation by AcP-mediated acetylation was examined next. TopA was incubated with both 2 mM acP and purified CobB, so that acetylation and deacetylation can both take place. Notably, the acetylation level of TopA was reduced by CobB (Figure 6A). Accordingly, as shown in Figure 6B, the presence of CobB partially protected TopA catalytic activity from the inhibitory effect of acP-dependent lysine acetylation. These results indicate that CobB is important for maintaining TopA deacetylation and TopA catalytic activity.

TopA interacts directly with CobB in the absence of DNA

TopA has been demonstrated in a previous study (45) to be a CobB binding protein using a proteome microarray as well as bio-layer interferometry analysis. To confirm that TopA interacts directly with CobB in the absence of DNA, purified recombinant TopA and CobB proteins were mixed in binding buffer. HisPur Cobalt resin was used to pulldown TopA in complex with the N-terminal His-tagged CobB. Western blot analysis of the proteins bound to the cobalt resin with antibodies against TopA (Figure 7A) showed that the presence of His-tagged CobB allows binding of TopA to the Cobalt resin and co-elution with the His-CobB (Figure 7B and C).

Specific activity of His-TopA expressed from the chromosome is inversely proportional to degree of lysine acetylation during growth transition and growth arrest

To demonstrate directly the effect of in vivo lysine acetylation on the specific activity of TopA, we utilize E. coli strain YN1434 that expresses TopA with N-terminal His-tag from the chromosome. Cells were collected from cultures in buffered TB7 broth supplemented with 0.4% glucose following growth for 4.5 h (OD600 = 0.7), 5.5 h (OD600 = 1.7) and growth arrest at 24 h (OD600 = 1.9). Acetyl phosphate-dependent acetylation has been shown to occur mostly following entry into stationary phase under these experimental conditions (12,43). The His-TopA was purified with Ni-affinity chromatography and analyzed for level of lysine acetylation (Figure 8A) and specific activity using supercoiled plasmid DNA as substrate (Figure 8B). The results showed that the level of lysine acetylation found in the purified His-TopA first decreased as cells entered stationary phase at 5.5 h, and then increased in growth arrest at 24 h (Figure 8A). The specific activity of the purified His-TopA was found to be inversely proportional to the degree of lysine acetylation (Figure 8B). The His-TopA purified from the growth arrest (GA) culture had the lowest specific activity (~2-fold lower than OD600 = 0.7 culture, and 4-8-fold lower than OD600 = 1.7 culture). This further confirmed that TopA catalytic activity can be modulated in vivo based on the degree of lysine acetylation modification of the enzyme.

DISCUSSION

Bacterial DNA topoisomerase I (TopA) is involved in various essential DNA-centered processes. TopA is highly ef-
Evidence has been provided in this report that *in vitro* acP-mediated nonenzymatic lysine acetylation of TopA decreases its catalytic activity. TopA activity in the total cell lysate is significantly reduced as a result of the deletion of the *cobB* deacetylase gene, leading to an increase in negative supercoiling *in vivo*, even though *topA* mRNA transcription level detected by qPCR analysis is increased, and TopA protein expression level detected by western blot analysis is also increased. On the basis of these results, we propose that the reduction of TopA relaxation activity due to the missing deacetylase function in the *ΔcobB* mutant increases DNA negative supercoiling, which in turn upregulates *topA* gene transcription and TopA expression.

Lysine acetylation affects multiple cellular functions, including cell motility, carbon source utilization central metabolism, and stress responses (6, 13–16). Previous proteomics studies identified a large number of acetylated proteins in *E. coli* (7, 9, 44). In this report, we observe a slow growth phenotype in entering the exponential phase associated with the *ΔcobB* mutation. Nutrient should not be limiting in the LB medium under the growth condition of the experiment. The slow growth phenotype could potentially be due to the global effect of acetylation-deacetylation on a wide range of *E. coli* proteins. Interestingly, when the *ΔcobB* mutant is complemented with a high copy number plasmid expressing recombinant TopA, the slow growth phenotype is partially reversed. This could be due to the global effect of TopA via its influence on DNA supercoiling. TopA activity has been reported to be important for the removal of localized transcription-driven negative supercoiling at the ribosomal operon *rrnB* (58). Reduction in TopA catalytic activity as a result of the *cobB* deletion may lead to hyper-negative supercoiling at the highly transcribed rRNA loci as the cells exit the growth arrest to enter the exponential phase under the culture conditions used in this study.

According to a previous study, the intracellular concentration of acP in *E. coli* reaches at least 3 mM, and may be as high as 4.5 mM in wild-type cells (59). Nonenzymatic acP-dependent acetylation is the predominant mechanism of lysine acetylation in *E. coli* cells (9, 43). Our results showed that following *in vitro* acP-dependent acetylation, the activity of acP-acetylated TopA is reduced. Meanwhile, the NAD⁺-dependent sirtuin lysine deacetylase CobB can partially remove the lysine modification on TopA and maintain the TopA activity at a higher level. These results confirm our hypothesis that acetylation on TopA reduces its activity, and the deacetylation of TopA by CobB is of physiological significance.

We further established the correlation between degree of lysine acetylation and specific activity of topoisomerase I by purifying His-tagged TopA expressed from the chromosome following growth transition and growth arrest. Nonenzymatic acetylation of TopA by acetyl phosphate is expected to be highest at growth arrest following 24 h incubation in buffered TB7 media supplemented with glucose (12, 43). A relatively low level of relaxation specific activity was observed along with the high level of acetylated lysine for His-TopA purified from growth arrest cells. Further studies are needed to determine the basis for the decrease in TopA acetylation that was observed during the initial transition from exponential phase to stationary phase.
In previous proteomics studies, TopA was found to be acetylated at multiple lysine residues under various growth conditions (9,16). The locations of these acetylated lysines are shown (Figure 9) in the two available *E. coli* TopA crystal structures with ssDNA bound covalent to the active site, or non-covalently to the C-terminal domains (60,61). Most of the acetylated lysines are located in the solvent exposed positions. They would be accessible to the CobB deacetylase as substrate, and may influence initial association between TopA and chromosomal DNA for the formation of the catalytically competent TopA-DNA complex. The acetylation state of some of the TopA lysines could also potentially affect the enzyme conformational change required for the opening and closing of the gap between the 3′-OH and 5′-phosphotyrosine ends of the cleaved DNA single strand at the active site to allow passage of the complementary DNA single strand through the gap into the central hole of TopA. Lysine acetylation at the C-terminal domains of TopA may also influence its direct interaction with RNA polymerase during transcription elongation (41,62). The acetylation state of different TopA lysines could have differential effects on the protein-DNA or protein-protein interactions. The elucidation of the biochemical significance of individual TopA lysine acetylation sites would require future mechanistic and genetic studies.

The results presented here support modulation of TopA catalytic activity via lysine acetylation, and a physiological function of *E. coli* CobB in preventing TopA inactivation from excess lysine acetylation. There are questions that remain to be answered with regard to potential regulation of topoisomerase activity and DNA supercoiling as a global signal by acetylation-deacetylation in bacteria. Determination of the acetylation stoichiometry at specific TopA lysine residues under various growth conditions, including the growth conditions employed in this study, is currently incomplete. In addition to the confirmed lysine acetyltransferase Pka/YfiQ, there may be other *E. coli* acetyltransferases that remain to be identified and characterized. Lysine acetylation-deacetylation has been linked to stress response in bacteria (15), and may provide a more rapid mechanism for modification of TopA catalytic activity than the transcription response from the topA promoters.

**SUPPLEMENTARY DATA**

Supplementary Data are available at NAR Online.

**ACKNOWLEDGEMENTS**

The authors wish to acknowledge Yale CGSC and National BioResource Project (NIG, Japan): *E. coli* for providing strains and plasmids. We thank Shayna Sandhaus for assistance in editing the manuscript.

**FUNDING**

National Institute of Health (NIH) [R01 GM054226 to Y.T.], Y.N.Z., D.J.J. were supported by Intramural Research Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research. Funding for open access charge: Florida International University. Conflict of interest statement. None declared.

**REFERENCES**

1. Fan, J., Krautkramer, K.A., Feldman, J.L. and Denu, J.M. (2015) Metabolic regulation of histone post-translational modifications. *ACS Chem. Biol.*, 10, 95–108.
2. Su, X., Wellen, K.E. and Rabinowitz, J.D. (2016) Metabolic control of methylation and acetylation. *Curr. Opin. Chem. Biol.*, 30, 52–60.
3. Cain, J.A., Solis, N. and Cordwell, S.I. (2014) Beyond gene expression: The impact of protein post-translational modifications in bacteria. *J. Proteomics*, 97, 265–286.
4. Thao, S. and Escalante-Semerena, J.C. (2011) Control of protein function by reversible Nε-lysine acetylation in bacteria. *Curr. Opin. Microbiol.*, 14, 200–204.
22. Wang, Z. and Harshey, R.M. (1994) Crucial role for DNA
11. Bernal, V., Castano-Cerezo, S., Gallego-Jara, J., Ecija-Conesa, A., de
10. Wolfe, A.J. (2016) Bacterial protein acetylation: new discoveries
13. Li, R., Gu, J., Chen, Y.Y., Xiao, C.L., Wang, L.W., Zhang, Z.P., Bi, L.J.,
21. AbouElfetouh, A., Kuhn, M.L., Hu, L.I., Scholle, M.D., Sorensen, D.J.,
20. Zhang, Q.F., Gu, J., Gong, P., Wang, X.D., Tu, S., Bi, L.J., Yu, Z.N.,
9. Weinert, B.T., Iesmantavicius, V., Wagner, S.A., Scholz, C.,
5. Choudhary, C., Kumar, C., Gnand, F., Nielsen, M.L., Rehmans, M.,
12. Li, J.X., Chen, S., Li, Z., Wu, L., Yin, H., Zhao, Y., et al. (2012) DNA acetylation at site-specific
23. Tse-Dinh, Y.C. (1985) Regulation of the
14. Bernal, V., Castano-Cerezo, S., Gallego-Jara, J., Ecija-Conesa, A., de
24. Sahu, A.K., Becher, D., Antelmann, H., Mrksich, M., Anderson, W.F.
25. Zhao, W., Yao, Y. (2006) Regulation of the
26. Liu, L.F. and Wang, J.C. (1987) Supercoiling of the DNA template during transcription.
27. Goldstein, E. and Drlica, K. (1984) Regulation of bacterial DNA
28. Cheung, K.J., Badarinarayana, V., Selinger, D.W., Janse, D. and
29. Church, G.M. (2003) A microarray-based antibiotic screen identifies a regulatory role for supercoiling in the osmotic stress response of
Escherichia coli. Genome Res. 13, 206–215.
30. Balle, V.L. and Gralla, J.D. (1987) Changes in the linking number of supercoiled DNA accompany growth transitions in Escherichia coli. J. Bacteriol. 169, 4499–4506.
31. Lies, H.S. and Wolfe, A.J. (2014) Bacterial DNA supercoiling and [ATP]/[ADP] changes associated with a transition to anaerobic growth. J. Mol. Biol. 219, 443–450.
32. Zeichiedrich, E.L., Khodursky, A.B., Bacheller, S., Schneider, R., Chen, D., Lilley, D.M. and Cozzarelli, N.R. (2000) Roles of topoisomerases in maintaining steady-state DNA supercoiling in Escherichia coli. J. Biol. Chem. 275, 8103–8113.
33. Tse-Dinh, Y.C. (1985) Regulation of the Escherichia coli DNA
34. Menzel, R. and Tse-Dinh, Y.C. (1997) Regulation of Escherichia coli topA gene transcription: Involvement of a sigmaS-dependent promoter. J. Mol. Biol. 267, 481–489.
35. Qi, H., Menzel, R. and Tse-Dinh, Y.C. (1996) Effect of the deletion of the sigma 32-dependent promoter (P1) of the Escherichia coli topoisoamerase I gene on thermostolerance. Mol. Microbiol. 21, 703–711.
36. Weinstein-Fischer, D. and Altuviu, S. (2007) Differential regulation of Escherichia coli topoisomerase I by fis. Mol. Microbiol. 63, 1131–1144.
37. Weinstein-Fischer, D. and Elgrably-Weiss, M. and Altuviu, S. (2000) Escherichia coli response to hydrogen peroxide: A role for DNA supercoiling, topoisomerase I and fis. Mol. Microbiol. 35, 1413–1420.
38. Stewart, N., Feng, J., Liu, X., Chaudhuri, D., Foster, J.W., Drolet, M. and Tse-Dinh, Y.C. (2005) Loss of topoisomerase I function affects the RpoS-dependent and GAD systems of acid resistance in Escherichia coli. Microbiology, 151, 2783–2791.
39. Liu, J.F., Aedo, S. and Tse-Dinh, Y.C. (2011) Resistance to topoisomerase cleavage complex induced lethality in Escherichia coli via titration of transcription regulators PurR and FNR. BMC Microbiol. 11, 261.
40. Yang, J., Annamalai, T., Cheng, B., Banda, S., Tyagi, R. and Tse-Dinh, Y.C. (2015) Antimicrobial susceptibility and SOS-dependent increase in mutation frequency are impacted by E. coli topoisomerase I C-terminial point mutation. Antimicrob Agents Chemother, 59, 6195–6202.
41. Baeza, J., Dowell, J.A., Smallanan, M.J., Fan, J., Amador-Noguez, D., Khan, Z. and Denu, J.M. (2012) Stoichiometry of site-specific lysine acetylation in an entire proteome. J. Biol. Chem. 289, 20126–20138.
42. Kuhn, M.L., Zemaitaitis, B., Hu, L.I., Sahu, A., Sorensen, D., Minasov, G., Lima, B.P., Scholle, M., Mrksich, M., Anderson, W.F. et al. (2014) Structural, kinetic and proteomic characterization of acetyl phosphate-dependent bacterial protein acetylation. PLoS One 9, e94855.
43. Zhang, J., Sprung, R., Pei, J., Tan, X., Kim, S., Zhu, H., Liu, C.F., Grishin, N.V. and Zhao, Y. (2009) Lysine acetylation is a highly abundant and evolutionarily conserved modification in Escherichia coli. Mol. Cell Proteomics, 8, 215–225.
44. Liu, C.X., Wu, F.L., Jiang, H.W., He, X., Guo, S.J. and Tao, S.C. (2014) Global identification of CoB interactors by an Escherichia coli proteome microarray. Acta Biochim. Biophys. Sin. (Shanghai), 46, 548–555.
45. Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datsenko, K.A., Tomita, M., Wanner, B.L. and Mori, H. (2006) Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: The Keio collection. Mol. Syst. Biol., 2, 2006.0008.
47. Datsenko, K.A. and Wanner, B.L. (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. U.S.A.*, 97, 6640–6645.

48. Cheng, B., Shukla, S., Vasunilashorn, S., Mukhopadhyay, S. and Tse-Dinh, Y.C. (2005) Bacterial cell killing mediated by topoisomerase I DNA cleavage activity. *J. Biol. Chem.*, 280, 38489–38495.

49. Kitagawa, M., Ara, T., Arifuzzaman, M., Ioka-Nakamichi, T., Inamoto, E., Toyonaga, H. and Mori, H. (2005) Complete set of ORF clones of *Escherichia coli* ASKA library (a complete set of *E.coli* K-12 ORF archive): Unique resources for biological research. *DNA Res.*, 12, 291–299.

50. Zhou, K., Zhou, L., Lim, Q., Zou, R., Stephanopoulos, G. and Too, H.P. (2011) Novel reference genes for quantifying transcriptional responses of *Escherichia coli* to protein overexpression by quantitative PCR. *BMC Mol. Biol.*, 12, 18.

51. Peng, S., Stephan, R., Hummerjohann, J. and Tasara, T. (2014) Evaluation of three reference genes of *Escherichia coli* for mRNA expression level normalization in view of salt and organic acid stress exposure in food. *FEMS Microbiol. Lett.*, 355, 78–82.

52. Narula, G., Annamalai, T., Aedo, S., Cheng, B., Sorokin, E., Wong, A. and Tse-Dinh, Y.C. (2011) The strictly conserved Arg-321 residue in the active site of *Escherichia coli* topoisomerase I plays a critical role in DNA rejoining. *J. Biol. Chem.*, 286, 18673–18680.

53. Tse-Dinh, Y.C. and Beran, R.K. (1988) Multiple promoters for transcription of the *Escherichia coli* DNA topoisomerase I gene and their regulation by DNA supercoiling. *J. Mol. Biol.*, 202, 735–742.

54. Qi, H., Menzel, R. and Tse-Dinh, Y.C. (1999) Increased thermostability associated with topoisomerase I deletion and promoter mutations in *Escherichia coli*. *FEMS Microbiol. Lett.*, 178, 141–146.

55. Tse-Dinh, Y.C. (2000) Increased sensitivity to oxidative challenges associated with topA deletion in *Escherichia coli*. *J. Bacteriol.*, 182, 829–832.

56. Drolet, M. (2006) Growth inhibition mediated by excess negative supercoiling: the interplay between transcription elongation, R-loop formation and DNA topology. *Mol. Microbiol.*, 59, 723–730.

57. Peter, B.J., Arsuaga, J., Breier, A.M., Khodursky, A.B., Brown, P.O. and Cozzarelli, N.R. (2004) Genomic transcriptional response to loss of chromosomal supercoiling in *Escherichia coli*. *Genome Biol.*, 5, R87.

58. Masse, E., Phoenix, P. and Drolet, M. (1997) DNA topoisomerases regulate R-loop formation during transcription of the *rrnB* operon in *Escherichia coli*. *J. Biol. Chem.*, 272, 12816–12823.

59. Klein, A.H., Shulla, A., Reimann, S.A., Keating, D.H. and Wolfe, A.J. (2007) The intracellular concentration of acetyl phosphate in *Escherichia coli* is sufficient for direct phosphorylation of two-component response regulators. *J. Bacteriol.*, 189, 5574–5581.

60. Tan, K., Zhou, Q., Cheng, B., Zhang, Z., Joachimiak, A. and Tse-Dinh, Y.C. (2013) Structural basis for suppression of hypernegative DNA supercoiling by *E. coli* topoisomerase I. *Nucleic Acids Res.*, 43, 11031–11046.

61. Zhang, Z., Cheng, B. and Tse-Dinh, Y.C. (2011) Crystal structure of a covalent intermediate in DNA cleavage and rejoining by *Escherichia coli* DNA topoisomerase I. *Proc. Natl. Acad. Sci. U.S.A.*, 108, 6939–6944.

62. Cheng, B., Zhu, C.X., Ji, C., Ahumada, A. and Tse-Dinh, Y.C. (2003) Direct interaction between *Escherichia coli* RNA polymerase and the zinc ribbon domains of DNA topoisomerase I. *J. Biol. Chem.*, 278, 30705–30710.