

# Direct regulation of topoisomerase activity by a nucleoid-associated protein

Soumitra Ghosh<sup>1</sup>, Bratati Mallick<sup>1</sup> and Valakunja Nagaraja<sup>1,2,\*</sup>

<sup>1</sup>Department of Microbiology and Cell biology, Indian Institute of Science, Bangalore 560012, India and <sup>2</sup>Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore 560064, India

Received July 27, 2014; Revised August 21, 2014; Accepted August 22, 2014

## ABSTRACT

**The topological homeostasis of bacterial chromosomes is maintained by the balance between compaction and the topological organization of genomes. Two classes of proteins play major roles in chromosome organization: the nucleoid-associated proteins (NAPs) and topoisomerases. The NAPs bind DNA to compact the chromosome, whereas topoisomerases catalytically remove or introduce supercoils into the genome. We demonstrate that HU, a major NAP of *Mycobacterium tuberculosis* specifically stimulates the DNA relaxation ability of mycobacterial topoisomerase I (TopoI) at lower concentrations but interferes at higher concentrations. A direct physical interaction between *M. tuberculosis* HU (MthU) and TopoI is necessary for enhancing enzyme activity both *in vitro* and *in vivo*. The interaction is between the amino terminal domain of MthU and the carboxyl terminal domain of TopoI. Binding of MthU did not affect the two catalytic trans-esterification steps but enhanced the DNA strand passage, requisite for the completion of DNA relaxation, a new mechanism for the regulation of topoisomerase activity. An interaction-deficient mutant of MthU was compromised in enhancing the strand passage activity. The species-specific physical and functional cooperation between MthU and TopoI may be the key to achieve the DNA relaxation levels needed to maintain the optimal superhelical density of mycobacterial genomes.**

## INTRODUCTION

The large, circular chromosomal DNA of bacteria is packed into a condensed structure termed the nucleoid. The nucleoid is generally confined to a relatively small cytoplasmic space, but displays a higher order organization (1,2). This highly compact DNA organization is achieved by two principal mechanisms: a protein-mediated packaging of the

DNA and topological maintenance (3). The former shapes the conformation of chromosomes by direct binding to DNA, and the latter prevents entanglement and excess supercoiling by catalytically manipulating DNA. The major contributors of protein-mediated DNA packaging are the nucleoid-associated proteins (NAPs) (4–6). NAPs are abundant, small basic proteins that bind to the DNA and change the trajectory of the DNA molecule, and hence, influence both the chromatin structure and different DNA-dependent processes (6). The well-characterized members of this family include HU, FIS, H-NS, StpA, Dps, IHF, etc. in *Escherichia coli* (6). These proteins differ from each other in their DNA-binding properties, and functionally with respect to DNA organization and regulation of DNA-dependent activities (5,6). Importantly, not all bacterial species encode homologs of all of these proteins. HU is an abundant NAP, conserved among all the eubacterial species (7) and is traditionally considered the archetypal bacterial counterpart of eukaryotic histones (8). In enterobacteriaceae, including *E. coli*, HU is a heterodimer of two similar subunits, HU $\alpha$  and HU $\beta$ . In a number of other bacteria, HU exists as a homodimer encoded by a single gene (6,7). Being a sequence-independent DNA-binding protein, it plays an architectural role in compaction (9) and constraining negative supercoils (10). It also functionally participates in DNA transactions *viz.* replication, transcription, recombination and repair (11,12). Thus, not surprisingly, many genes that confer stress response, colonization of the host and virulence in various pathogenic bacteria are regulated by the HU protein (13–15).

In contrast to the action of NAPs, topoisomerases catalyze the topological inter-conversions in order to facilitate DNA transaction processes (16,17). The topoisomerases are a class of enzymes that maintain the supercoiling state of the DNA in the cell by DNA binding, cleavage, passage of intact strand(s) and religation of the broken strands (18). The enzymes are broadly classified into type I or II depending on whether they cleave single- or double-strand DNA in the first step of the reaction (18–20). Thus, the overall topological status of the genome is maintained by the combined action of NAPs and topoisomerases (3,6,21,22).

\*To whom correspondence should be addressed. Email: vraj@mcbliisc.ernet.in

The human pathogen, *Mycobacterium tuberculosis* (*Mtb*) encodes a single HU gene annotated as *hupB* (23). *Mycobacterium tuberculosis* HU (MtHU) has unique properties compared to its bacterial counterparts. Apart from a conserved amino terminal domain (NTD), it has an extra carboxyl terminal tail (CTD) rich in multiple repeats of basic amino acids (24). It binds DNA with a high affinity in a sequence and structure-independent manner (24). MtHU is essential for *Mtb* growth and inhibition of MtHU causes growth arrest (24,25). Overproduction of MtHU results in a highly condensed genome while inhibition of its DNA binding by small molecule inhibitors leads to decompaction of the nucleoid (24), indicating its important role in nucleoid organization in *Mtb*.

The *Mtb* genome encodes a single type I and a type II topoisomerase (23) to take care of all the DNA relaxation and supercoiling needs of the genome, respectively. In contrast, *E. coli* and a large number of eubacteria possess at least two type I and two type II topoisomerases to ensure that all topological issues are sorted out (18). Under representation of these essential enzymes in the *Mtb* genome raises an important question on the management of genome topology, critical for all downstream events. We showed that mycobacterial DNA gyrase is a potent decatenase carrying out the dual function of both negative supercoiling and DNA decatenation (26). In the absence of topoisomerase IV, which carries out efficient DNA relaxation in addition to decatenation, the genome has to find a mechanism to cope up with the load for DNA relaxation, so as to share the burden with TopoI. Based on the earlier observations of genetic cross talk between TopoI, gyrase and HU in *E. coli* (27,28), we considered such interactions in mycobacteria. Here, we show that a functional coupling between mycobacterial topoisomerase I and MtHU enhances the relaxation activity of the enzyme. MtHU specifically simulates the DNA relaxation activity of mycobacterial TopoI but not *E. coli* TopoI activity. A direct physical interaction between TopoI and MtHU seems to be necessary for the stimulation of enzyme activity. The enhancement of enzyme activity is independent of HU binding to DNA but dependent on the protein-protein interaction between the partner proteins.

## MATERIALS AND METHODS

### Cloning, expression and purification of proteins

The full-length MtHU and MtHU-NTD proteins were overexpressed in *E. coli* BL21 cells and the proteins were purified according to published protocols (24). The *hupB*-CTD was PCR amplified using specific primers (Frd: 5'GG CATATGGCTGTTAAGCGTGGTGTG 3', Rev: 5'GA CTCGAG TTTGCGACCCCGCCGAGC 3') from the *Mtb* genome and cloned into expression vector pET20b with a C-terminal His tag. *Escherichia coli* BL21 was used to express and purify the proteins. The MtHU-CTD overexpressing cells were re-suspended in a lysis buffer [10 mM Tris-HCl pH 7.5, 1000 mM KCl, 1 mM PMSF] and lysed by sonication. The lysate was centrifuged at 15 000 rpm for 20 min at 4°C and the supernatant was allowed to bind with Ni-NTA beads for 2 h at 4°C. The beads were washed with 10 column volumes of a lysis buffer containing 100 mM imidazole. The protein was eluted with a buffer containing 500

mM imidazole and 100 mM KCl. The eluted fractions were analysed on 15% SDS PAGE. The *Mycobacterium smegmatis* TopoI (MsTopoI), MtTopoI, MtTopoI-NTD MtTopoI-CTD and MtGyrase proteins were purified as described previously (29–33). The purity of the proteins was determined on SDS-PAGE and the concentrations were determined by Bradford assay.

### DNA relaxation and supercoiling assay

The DNA relaxation assays were as described before (29,30). Briefly, the negatively supercoiled pUC18 was incubated in the presence of mycobacterial TopoI (10 nM) and various amounts of wild-type or mutant MtHU proteins (as and when required) were added in 20 µl reaction containing an assay buffer (40 mM Tris-HCl (pH 8.0) 20 mM NaCl, 1 mM EDTA and 5 mM MgCl<sub>2</sub>) and incubated at 37°C for 30 min. After incubation, proteinase K (1 mg/ml) was added to the mixture and incubated for 15 min at 37°C. The samples were resolved on 1% agarose gel and stained with ethidium bromide. For DNA supercoiling reactions, relaxed pUC18 DNA was incubated with increasing concentrations of MtHU in the presence of a reaction buffer containing 35 mM Tris-HCl (pH 7.5) 5 mM MgCl<sub>2</sub>, 25 mM potassium glutamate, 2 mM spermidine, 1.4 mM ATP, 50 µg/ml BSA, 90 µg/ml yeast tRNA and 5% glycerol. The incubation was carried out at room temperature for 15 min and followed by addition of *Mtb* DNA gyrase holo enzyme and further incubated at 37°C for 30 min. The reactions were terminated by heat inactivation at 68°C for 10 min and the products were resolved on 1% agarose gel, analyzed after staining with ethidium bromide. All the assays were repeated minimum three times and representative gels are shown.

### Co-immunoprecipitation (Co-IP) assays

Two micrograms of purified MtTopoI and MtHU (and their variants) were incubated in 200 µl of a Co-IP buffer containing 10 mM sodium phosphate (pH 7.4), 50 mM NaCl, 0.5% Nonidet P40 at 4°C for 2 h on a cyclomixer. Ten microliters of affinity purified anti-MtHU antibody bound protein A sepharose beads were added to the protein mixture and incubated at room temperature for 2 h on a rocker. After incubation, the protein A beads were washed three times with the Co-IP buffer. The beads were re-suspended in SDS loading buffer (without β-mercaptoethanol) and boiled for 5 min. The extent of MtTopoI pull down by MtHU was analyzed on 8–15% gradient SDS PAGE and the proteins were visualized by silver staining.

The *in vivo* interactions between MtHU and MtTopoI were analyzed by the Co-IP technique according to the published procedures with some modifications (33). The exponentially growing cells of *Mtb* H37Ra were resuspended and lysed in a buffer containing 10 mM sodium phosphate (pH 7.4), 150 mM NaCl and 1 mM EDTA. Co-IPs were carried out by incubating 10 µg of DNase I treated *Mtb* total cell lysate with anti-HU antiserum in 100 µl Co-IP buffer for 3 h at 4°C. Ten microliters of slurry of protein A sepharose was added and incubation was continued for another 1 h. The beads were washed with Co-IP buffer three times and resuspended in an SDS PAGE sample loading buffer. The

proteins were resolved on 12% SDS PAGE and analyzed by western blotting.

### Glutaraldehyde cross linking

Two micrograms of MsTopoI and its variants were incubated with MthHU in a reaction buffer containing 10 mM sodium phosphate (pH 7.4) and 50 mM NaCl for 10 min on ice. Glutaraldehyde was added to a final concentration of 0.1% and incubated at room temperature for 10 min. The reactions were stopped by the addition of an SDS gel loading dye and resolved in 12% SDS PAGE.

### Surface plasmon resonance spectroscopy

The MthHU protein was immobilized onto a CM5 sensorchip (BIAcore 3000 system, GE Healthcare). Different concentrations of wild-type and truncated MtTopoI proteins were injected in HBS (10 mM sodium phosphate pH 7.4, 350 mM NaCl, 3 mM EDTA and 0.005% (v/v) polysorbate 20) at a flow rate of 20  $\mu$ l/min. The binding response was measured for 120 s after the end of the injection. The interactions between the MthHU and MtTopoI proteins were analyzed and steady-state binding was determined at each concentration. Following each injection cycle, chip surfaces were regenerated with an injection of 25 mM NaOH. Sensorgrams were processed by BIAevaluation software (GE Healthcare) to obtain kinetic parameters of protein–DNA interaction. The steady-state binding constants were determined by fitting the data to a 1:1 Langmuir isotherm (34).

### Electrophoretic mobility shift assays

The 5'-end-labeled 32-mer single-stranded oligonucleotides harboring a strong TopoI site (STS) were used as specific DNA substrates for EMSA experiments (33,35). Reactions containing 40 mM Tris-HCl (pH 8.0), 20 mM NaCl, 1 mM EDTA and 0.1 pmol oligonucleotides were incubated with varying concentrations of MthHU and fixed concentrations of MtTopoI for 15 min on ice. The protein–DNA complexes were resolved from free DNA in 8% non-denaturing polyacrylamide gel electrophoresis (PAGE) at 4°C using a 1 $\times$  TBE buffer (Tris-Borate-EDTA). The bands were visualized by a phosphorimager (model BAS 1800; Fujifilm).

### DNA cleavage and religation

DNA cleavage experiments were carried out by incubating a fixed concentration of MtTopoI with the 32-mer STS oligonucleotide in the presence of increasing concentrations of MthHU, in a buffer containing 40 mM Tris-HCl (pH 8.0), 20 mM NaCl and 1 mM EDTA at 37°C for 30 min to yield a 5'-end-labeled 19-mer cleavage product. The reactions were terminated with 45% formamide dye and heating at 90°C for 2 min. The samples were resolved on 12% denaturing PAGE using a 1 $\times$  TBE as a running buffer at 300 V and visualized as described earlier. To determine the effect of MthHU in MtTopoI-mediated intramolecular religation, cleavage reactions were carried out for 15 min followed by incubation with MthHU for 10 min. The religation reaction was initiated by the addition of 5 mM MgCl<sub>2</sub> and incubation for an additional 15 min. The reaction products were resolved on 12% denaturing PAGE.

### Strand passage assay

An assay for DNA strand passage has been designed and described recently (36). The nick-translated radiolabeled pUC18 plasmid DNA was incubated with 500 nM MtTopoI in an assay buffer [40 mM Tris-HCl (pH 8.0), 20 mM NaCl and 1 mM EDTA, 10% glycerol] for 15 min on ice. To determine the initial counts in the control reaction, an anti-topoI clamp closing antibody 1E4F5 (37) was added to trap the plasmid DNA in the enzyme cavity. Two times molar excess of MthHU or MthHUD17A mutant were added followed by the addition of 5 pmoles of 32-mer STS oligonucleotides. The reactions were incubated for 15 min at 37°C, 10 mM MgCl<sub>2</sub> was then added and incubated for another 30 min. At the end of the incubation, 1E4F5 antibody was added to the reactions, spotted on a pre-equilibrated nitrocellulose filter, followed by washing with a high salt buffer (40 mM Tris-HCl (pH 8.0), 1 M NaCl and 1 mM EDTA) thrice to remove the unbound DNA. The radioactivity retained on the filter was measured by a liquid scintillation counter. The strand passage ability was calculated by measuring the percentage reduction of the radioactive counts (cpm) upon completion of the reaction as described previously (36).

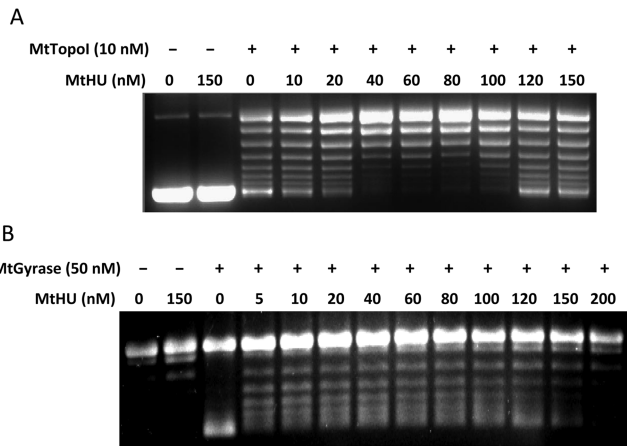
## RESULTS

### MthHU stimulates the DNA relaxation activity of MtTopoI

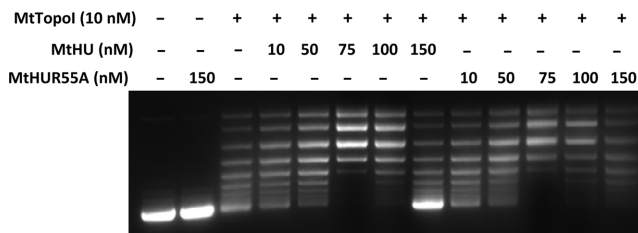
First, we addressed whether the DNA compacting protein HU and the enzymes that introduce topological changes functionally cooperate during their respective reactions. To evaluate whether MthHU influences the activity of MtTopoI, DNA relaxation assays were carried out with increasing concentrations of MthHU as described in Materials and Methods. At lower MthHU concentration, the DNA relaxation activity of MtTopoI increased, but at concentrations higher than 80 nM, TopoI activity was inhibited (Figure 1A). Next, the effect of MthHU was also tested on TopoI from *M. smegmatis* which has a high degree of similarity to MtTopoI (30). MthHU stimulated the relaxation activity of MsTopoI at lower concentrations and inhibited it at higher concentrations, as in the pattern seen with MtTopoI (Supplementary Figure S1). However, the supercoiling activity of MtGyrase was not stimulated at the concentration of HU that enhanced the TopoI activity. Notably, at higher (>150nM) concentrations of the NAP, the supercoiling activity was inhibited (Figure 1B). The inhibitory effect was stronger when the DNA was pre-incubated with MthHU. In the mobility shift assays used for evaluating MthHU binding to DNA, MtTopoI and MtGyrase had little effect (not shown).

### Stimulation of MtTopoI activity is independent of MthHU DNA binding

MthHU binds DNA with high affinity altering the trajectory of DNA molecules (24). The modulation of MtTopoI activity at lower concentrations and inhibition at higher concentrations of MthHU could be due to its DNA-binding activity. To evaluate, a coupled topoisomerase assay was carried out with a mutant of MthHU having reduced DNA-binding activity. In the dimeric structure of the DNA-binding domain



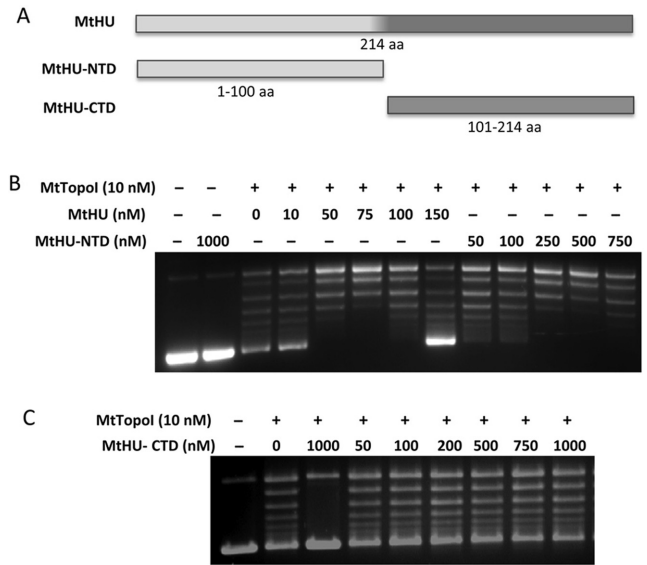
**Figure 1.** Effect of MthU on DNA relaxation activity of MtTopoI and supercoiling activity of MtGyrase. (A) DNA relaxation assays were carried out by incubating 200 ng of supercoiled pUC18 DNA and the indicated amounts of MthU in the absence (-) or presence (+) of 10 nM MtTopoI and the reaction products were analyzed as described in Materials and Methods. (B) For DNA supercoiling assays, 200 ng of relaxed pUC18 DNA was incubated with 50 nM MtGyrase in the presence of indicated amounts of MthU and the DNA products were analyzed as described in Materials and Methods.



**Figure 2.** DNA relaxation by MtTopoI in the presence of wild-type and mutant (R55A) MthU. The standard relaxation reaction mixtures containing 200 ng of supercoiled pUC18 DNA and the indicated amounts of wild-type or mutant MthU were incubated in the absence (-) or presence (+) of 10 nM of MtTopoI. The DNA products were analyzed as described in Materials and Methods.

of MthU, Arg 55 is one of the key residues involved in the interaction with DNA (24). The mutant MthUR55A had ~100-fold reduced DNA-binding activity (24). The mutant protein was found to enhance the MtTopoI activity at concentrations similar to that of wild-type HU. However, the inhibition of relaxation activity seen at higher concentrations of the protein was significantly reduced with the mutant protein (Figure 2). These results indicate that the stimulation of MtTopoI was independent of MthU DNA binding, while inhibition could be a more general phenomenon due to the inaccessibility of TopoI to DNA at a higher concentration of MthU. If this is indeed the case, the inhibition of DNA gyrase would also be to a lower extent at higher concentrations of mutant HU. Mutant HU inhibited the supercoiling activity of gyrase to a lower extent compared to the wild-type (not shown). From all these results, it is evident that the DNA-binding ability of MthU is not required for MtTopoI activity stimulation but necessary for the inhibition of both supercoiling and relaxation reactions.

The MthU protein has two distinct structural domains, the NTD and CTD (Figure 3A and Supplementary Figure

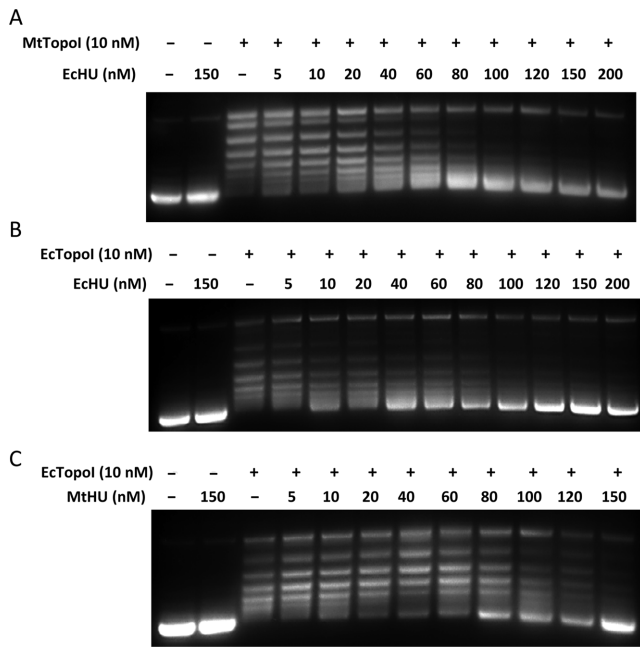


**Figure 3.** DNA relaxation by MtTopoI in the presence of truncated MthU proteins. (A) Schematic representation of the domain organization of MthU. Full-length MthU comprises 214 amino acids (aa). 1–100 aa constitute MthU NTD and 101–214 aa MthU CTD, respectively. MtTopoI-mediated DNA relaxation was monitored in the presence of full-length and truncated MthU proteins. Stimulation of the MtTopoI activity was seen with (B) MthU and MthU-NTD, whereas (C) MthU-CTD failed to stimulate the activity of MtTopoI.

S2) (24). The contribution of each domain in the stimulation of enzyme activity was determined by a coupled TopoI assay with truncated MthU proteins. For this, the individual domains were independently cloned, expressed and purified as described in Materials and Methods. Notably, MthU-NTD showed stimulation of MtTopoI-mediated relaxation activity, albeit at higher concentrations (Figure 3B). However, MthU-CTD had no effect on the DNA relaxation activity of MtTopoI (Figure 3C). From these results, it is apparent that MthU-NTD primarily contributes to the stimulation of relaxation activity.

### MthU specifically stimulates the mycobacterial TopoI

The characteristics of MthU-NTD are similar to *E. coli* HU (EchU) and other eubacterial HUs with respect to sequence and structure (Supplementary Figure S2) (24). Thus, it seemed likely that the EchU would also influence the relaxation activity of TopoI, given that MthU-NTD alone stimulated enzyme activity. When the effect of EchU on the DNA relaxation activity of MtTopoI was assessed, it did not stimulate the MtTopoI catalyzed reaction (Figure 4A). However, at high concentrations, EchU inhibited the relaxation activity (Figure 4A). Notably, EchU did not show any stimulation of *E. coli* TopoI (EcTopoI) mediated DNA relaxation activity (Figure 4B). In a reverse experiment, MthU also did not enhance EcTopoI activity but inhibited DNA relaxation at higher concentrations (Figure 4C). As described in the earlier section (Supplementary Figure S1), MthU enhanced the relaxation activity of MsTopoI, indicating that the enhancement of TopoI activity by MthU is specific to the mycobacterial system.

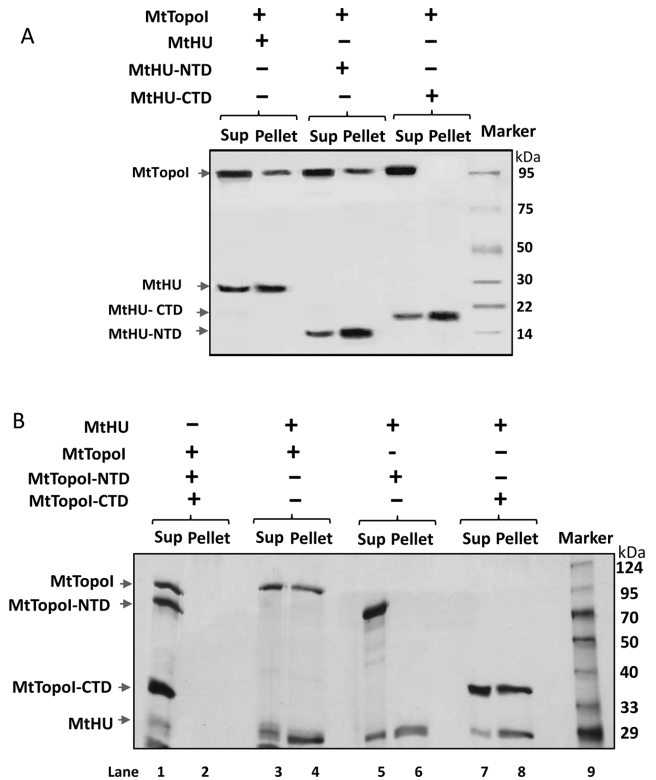


**Figure 4.** DNA relaxation activity of TopoI and HU from different bacterial species. (A) MtTopoI-mediated DNA relaxation was monitored in the presence of different concentrations of *E. coli* HU (EcHU). Effect of (B) EcHU (C) MthHU on DNA relaxation activity of EcTopoI. No stimulation of relaxation activity was seen with any of the above combinations.

### Physical interaction between MthHU and MtTopoI

The specific stimulation of mycobacterial TopoI by MthHU seen above suggests a role for protein–protein interaction between the two topology influencing proteins. To investigate, Co-IPs were carried out with the purified proteins. MthHU and MtTopoI were incubated together and immunoprecipitated with an anti-MthHU polyclonal antibody as described in Materials and Methods. The presence of MtTopoI in the MthHU pull-down fraction indicated a direct interaction between the proteins (Figure 5A). To map the site of interaction on MthHU, Co-IP experiments were also carried out with individual domains of MthHU. The NTD of MthHU could pull down MtTopoI, but no interaction was detected with MthHU-CTD (Figure 5A), indicating that the interaction surface of MtTopoI lies in the amino-terminal domain of MthHU. From these results and the data presented in Figure 3B, it is evident that physical interaction between MthHU-NTD and MtTopoI is required for the stimulation of TopoI activity.

Mycobacterial TopoI comprises an N-terminal domain (NTD) and a C-terminal domain (CTD) connected by a short linker region (31). The catalytic core resides in the NTD of mycobacterial TopoI while the CTD is involved in strand passage activity (31,36). To investigate the location of the protein–protein interaction interface on MtTopoI, Co-IP experiments were carried out with MthHU, full-length MtTopoI and its truncation constructs. The full-length protein and the C-terminal domain of MtTopoI co-precipitated with MthHU (Figure 5B), whereas the construct lacking the C-terminal domain failed to form a complex with MthHU (Figure 5B). A chemical cross-linking experiment also indi-

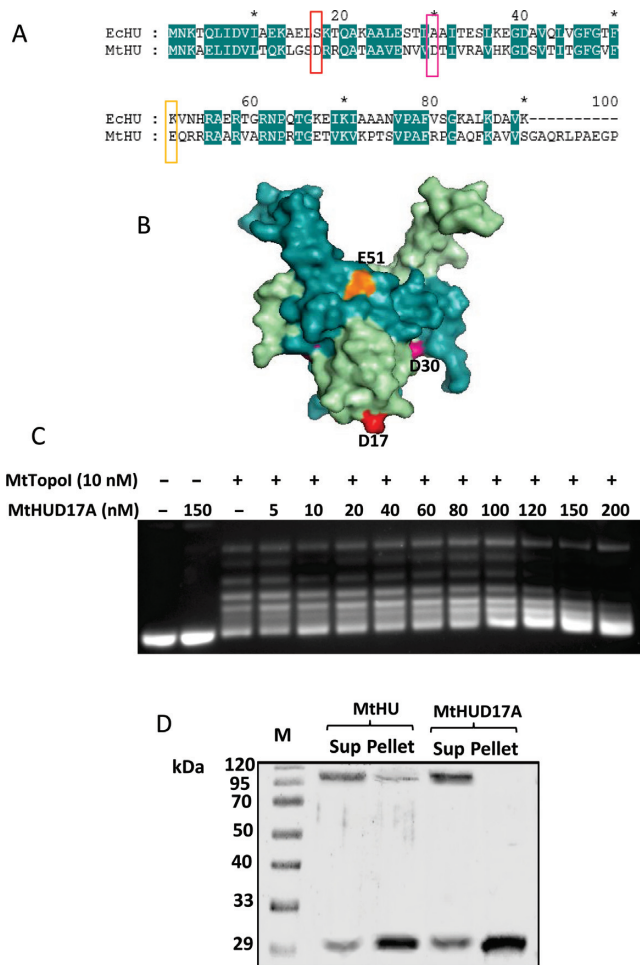


**Figure 5.** Physical interaction of MtTopoI and MthHU. Co-IP of MtTopoI and MthHU with the anti-HU antibody. (A) Co-precipitation of MtTopoI with full-length MthHU (lane 1,2) indicates a direct physical interaction. MtTopoI was seen in the pellet fraction along with the MthHU-NTD (lanes 3, 4), while the MthHU-CTD failed to pull down MtTopoI (lane 5, 6). (B) Identification of the MtTopoI domain involved in interaction with MthHU. The interaction of MthHU with full-length MtTopoI is shown in lane 3, 4. The MtTopoI NTD does not interact with MthHU (lane 5,6). The MtTopoI CTD is sufficient to interact with MthHU (lane 7, 8). MtTopoI, its NTD or CTD did not show cross-reactivity with the anti-HU antibody (lane 1, 2).

cated the association of full-length MtTopoI and its CTD with MthHU (Supplementary Figure S3A and B). Confirmation of this association was obtained by observing a direct physical interaction between MthHU and MtTopoI through an SPR assay as described in Materials and Methods. As shown in Supplementary Figure S4A, MtTopoI and its CTD showed an increase in RU compared to NTD when they were allowed to interact with MthHU immobilized on a sensor chip. Thus, it is apparent that the NTD of MthHU and the CTD of MtTopoI are involved in the physical interaction observed. The binding affinity of full-length MtTopoI to MthHU was determined to be  $3.5 \times 10^{-7}$  M by SPR (Supplementary Figure S4B). To further validate the interaction, immunoprecipitation of MthHU was carried out with *Mtb* H37Ra cell lysates. The presence of MtTopoI in the MthHU pull-down fraction suggested that these proteins interact *in vivo* as well (Supplementary Figure S4C).

### An MthHU mutant with reduced affinity for MtTopoI

The NTD of MthHU has both acidic and basic amino acid residues distributed non-randomly. The basic amino acids are clustered in the beta strands to form a DNA-binding cleft (24). The acidic residues present on the outer surface



**Figure 6.** Mutational analysis of MthU to map the interaction site. (A) Sequence alignment of MthU-NTD and EchU proteins. The acidic residues which are unique in MthU are indicated with box. (B) Homodimeric structure of the MthU-NTD indicating the location of the surface-exposed acidic residues. (C) MtHUD17A mutation on MtTopoI-mediated DNA relaxation assay. The relaxation reaction mixtures containing 200 ng of supercoiled pUC18 DNA and the indicated amounts of the MthU mutant were incubated in the absence (–) or presence (+) of MtTopoI. (D) The absence of MtTopoI in pellet fraction of Co-IP experiment with the D17A mutant of MthU indicates the loss of interaction upon mutation.

of the dimerization helices could possibly be involved in protein–protein interaction. Given that the CTD of MtTopoI which binds the NTD of MthU has patches of basic amino acids, we reasoned that the resulting electrostatic attractions could be important in this interaction. Hence, to decipher the TopoI interaction surface on the MthU-NTD, alanine substitution was carried out for the acidic residues of the MthU-NTD. Based on the sequence analysis and crystal structure of the MthU-NTD, the surface exposed D17, D30 and E51 residues were mutated to Ala (Figure 6A and B). The resulting MthU mutants were assayed for their ability to stimulate MtTopoI activity. The MtHUD17A mutant failed to stimulate the DNA relaxation activity of MtTopoI (Figure 6C) while the rest of the mutants (D30A and E51A) showed activity comparable to that of a wild-type protein. The D17A mutation retained the dimerization state and did not affect the DNA-binding abil-

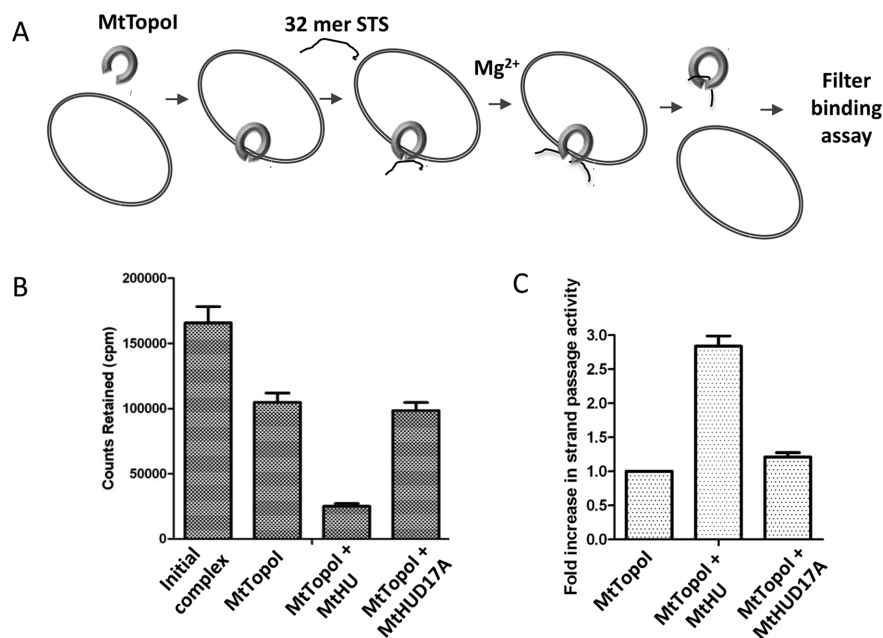
ity of MtHU (data not shown). However, the interaction of MtTopoI with the D17A mutant of MthU was significantly reduced (Figure 6D and Supplementary Figure S3C). In the MthU-NTD structure (Figure 6B), the three mutated surface-exposed acidic residues are distributed at different locations, far from each other. The mutational analysis indicated that D17 is one of the primary determinants of the interaction of MthU with MtTopoI. Taken together, these data establish that the stimulation of MtTopoI activity is via direct protein–protein interaction between two proteins.

### Interaction of MthU stimulates the TopoI-mediated strand passage activity

To elucidate the mechanism underlying HU-mediated stimulation of MtTopoI relaxation activity, the individual steps of the DNA relaxation reaction were monitored in presence of MthU. The relaxation reaction proceeds in sequential steps—binding of TopoI to DNA, cleavage, strand passage and religation (38). The effect of MthU in each step of the reaction was examined using the assays described earlier and in Materials and Methods (18,29,33,37). In the first step of the process, MthU did not affect the binding of MtTopoI to single-stranded 32 mer STS DNA at lower concentrations. Expectedly, at high concentrations of MthU, the binding of MtTopoI was inhibited (Supplementary Figure S5A). MtTopoI-mediated DNA cleavage and religation activities were also unaffected at low concentrations but at high concentrations of MthU, the activities were inhibited (Supplementary Figure S5B and C). Next, to determine the effect of MthU on the strand passage activity of MtTopoI, the enzyme was incubated with a radiolabeled relaxed circular form of pUC18 DNA (described in Materials and Methods) so that the DNA occupied the enzyme cavity (36). The occupancy of pUC18 DNA was ensured by using an anti-TopoI clamp-closing monoclonal antibody, which is shown to lock the TopoI clamp (37), thus trapping the plasmid DNA in the cavity of the enzyme. The TopoI–DNA complexes were incubated with MthU or MtHUD17A and STS oligonucleotides were added to form an enzyme–DNA gate (schematic in Figure 7A). The strand passage reaction would result in the escape of pUC18 DNA from the central cavity, measured by a filter-binding assay. The MtTopoI caused decreased retention of the labeled DNA on the filter, indicating the strand passage as demonstrated earlier (Figure 7B and C, (34)). The reaction in the presence of MthU caused a further reduction of the labeled DNA on the filter demonstrating enhanced strand passage. In contrast, the interaction-deficient mutant of MthU (MtHUD17A) did not show a marked reduction in the retention of pUC18 DNA with respect to the enzyme alone (Figure 7B), implying that the binding of MthU is indeed necessary to stimulate the strand passage activity of MtTopoI.

### DISCUSSION

Both HU and Topo I are key participants in bacterial chromosome organization (21,39). Mutations in either of them can cause perturbation in topology and nucleoid structure (24,28,40–42). The two proteins play different roles in the nucleoid organization process. TopoI is the major DNA re-



**Figure 7.** Assessment of MtTopoI-mediated strand passage activity in the presence of MthHU. (A) Schematic representation of the experimental work flow of the strand passage assay. Radiolabeled pUC18 DNA was incubated with 500 nM MtTopoI at 37°C followed by the addition of MthHU or MthHUD17A mutant. The cleaved DNA-enzyme gate for the passage of circular DNA was achieved when a 32-mer STS containing oligonucleotide was added. The plasmid DNA molecules, which retained in the enzyme cavity, were captured by a clamp-closing anti-topoI monoclonal antibody, and the weakly-bound salt-sensitive complexes were removed by washing with a high salt buffer. At the end of the reaction, radioactivity associated with each reaction was measured by a filter-binding assay. (B) Graph showing the amount of labeled pUC18 DNA (radioactive counts) retained after the completion of the strand passage reaction. The error bars represent standard deviation across three measurements. (C) The graph represents the fold increase in the strand passage activity of MtTopoI in the presence of MthHU or MthHUD17A.

laxation enzyme which removes the excess negative supercoils in the chromosome, whereas HU's effect on the chromosome is architectural as a result of its DNA compaction activity (5,6,43). We demonstrate the physical interaction between these two diverse topology modulators to functionally collaborate in the DNA relaxation process, which is essential to the organism. The interaction of the NTD of MthHU with the CTD of MtTopoI is necessary to modulate the superhelical DNA relaxation activity of MtTopoI. The absence of such direct stimulation of the EcTopoI activity either by MthHU or EcHU suggests that the observed mechanism may be of critical importance in mycobacteria.

There are several plausible mechanistic explanations which would account for these results. First, MthHU might reconfigure the DNA to produce an optimal substrate for TopoI. Earlier studies suggested that HU and other NAPs reconfigure the topology of the DNA *in vitro* (43,44). Such a reconfiguration could create a preferred substrate for TopoI, a mechanism employed by the single-strand DNA-binding protein during the stimulation of TopoI activity (45). The role of HU in constraining DNA supercoils to influence TopoI action has been suggested earlier (46). EcHU binding to DNA has been implicated in stimulation of decatenation activity of *E. coli* DNA gyrase (47). However, in the present study, the DNA-binding mutant of MthHU also showed a stimulation similar to that of a wild-type protein (Figure 2), indicating that the activity is independent of MthHU DNA binding. Notably, the stimulation activity of MthHU was confined to mycobacterial TopoI. In contrast, the inhibition of MtTopoI, EcTopoI and MtGyrase

was observed at higher concentrations of the protein. Given that these proteins are different, the inhibition seems to be a more general effect of decoration of HU all over the DNA, occluding the genome such that it is no longer accessible to TopoI. An alternative explanation is that MthHU stimulates the catalytic activity of MtTopoI by recruiting the protein to DNA via physical interaction. Thus, the interaction could shift the equilibrium of TopoI toward the DNA-bound state, increasing the effective rate of relaxation of the supercoiled substrate. Alternatively, MthHU might stabilize TopoI on DNA via a physical interaction and increase its processivity. The latter two mechanisms are not mutually exclusive, and the mechanism of stimulation may involve more than one mode. Yet another mode would be a direct role for MthHU in one or more steps of the reaction catalyzed by MtTopoI, given that the NAP binds to the enzyme even in the absence of DNA. The experiments described in Figure 7 and Supplementary Figure S5 support this possibility. Although MthHU had no influence on DNA binding, cleavage and religation steps of DNA relaxation by TopoI, it increased the intervening strand passage activity occurring between the two catalytic *trans*-esterification reactions of the enzyme by directly contacting the MtTopoI-CTD.

Given that both HU and TopoI are conserved across eubacteria and yet specific physical interaction is observed with the mycobacterial proteins but not between the *E. coli* counterparts raise evolutionary considerations. Both TopoI and HU from mycobacteria and other actinomycetes have distinctive features compared to other eubacterial homologs. Mycobacterial Topo I differs from other type IA

topoisomerases in many properties (29,35). The enzyme recognizes both single- and double-stranded DNA in a sequence-specific manner during the DNA relaxation reaction (33,35). The conserved N-terminal domain of the enzyme confers the site-specific binding, DNA cleavage and religation properties. The non-conserved C-terminal fragment lacking the typical DNA binding Zn<sup>2+</sup> finger motifs, exhibits non-specific DNA binding and enhances the processivity (31,36). The CTD of mycobacterial TopoI is highly flexible due to the presence of lysine-arginine rich basic patches. These basic patches in the CTD are necessary to promote strand passage during DNA relaxation (36). The flexible regions of the proteins are often stabilized by protein-protein interactions (48). The interaction of MtHU-NTD with TopoI CTD may provide additional stability to enhance the strand passage reaction thereby increasing the relaxation activity. The importance of electrostatic interactions between the two proteins is apparent from the loss of association and the resultant lack of stimulation of TopoI activity in an acidic residue (D17) mutant of MtHU. The absence of key residues in EchU and the basic stretches in EcTopoI could account for the lack of direct stimulation of EcTopoI activity. The presence of unique acidic residue in MtHU-NTD and basic patches in MtTopoI-CTD appears to contribute for the species-specific interaction between the interacting partners.

However, the functional collaboration observed between TopoI and HU could be a more general phenomenon likely to be found in other systems. Although in the biochemical experiments described here EchU did not stimulate EcTopoI, earlier data indeed point toward a functional interplay between the two proteins. In one of these studies, mutation in HU encoding genes resulted in a compensatory mutation in DNA gyrase leading to reduced supercoiling activity of the enzyme (49). In another report, a TopA mutation (*topA10*), could not sustain mutations in *hupA* and *hupB* (46). The synthetic lethality observed in the latter study and the isolation of suppressors having a mutation in gyrase in an HU knock-out strain indicate functional cross talk between TopoI and HU in *E. coli*. It may be noted that the *E. coli* genome harbors a large number of NAPs (6) and several topoisomerases (18), ensuring a division of labor and providing backup support to crucial functions. The underrepresentation of NAPs and topoisomerases in mycobacteria could have resulted in a more direct interaction between the proteins with the resultant and required increase in TopoI activity, as it is the only enzyme taking care of DNA relaxation in the *Mtb* genome.

The direct concentration-dependent modulation of MtTopoI activity by MtHU seen in the present studies would imply a precise control of the function of the TopoI *in vivo*. The enhancement of the activity at low concentrations of the NAP could be a prerequisite for the increase in TopoI activity behind the transcription or replication fork to attain the required level of topological homeostasis. Such a mechanism may be necessary in organisms where there are no back-up relaxases to take care of the extra load. On the other hand, the regions that are not actively transcribed would be the sites where nucleoid compaction is achieved by HU, and such sites are likely targets for TopoI inhibition. From the previous studies in *E. coli*, it is apparent that

there is an inverse correlation between HU levels and TopoI relaxation activity (46,50). However, HU levels estimated in these studies suggest that the concentration of the NAP *in vivo* may not reach the levels required for the inhibition of TopoI (46,50).

Being important enzymes in maintaining the cellular topological homeostasis, the activity of topoisomerases is subjected to modulation by a variety of mechanisms including interacting proteins (20). A number of DNA gyrase and topoisomerase IV modulatory proteins have been characterized which influence these Type II topoisomerases in a varied fashion (51–56). A direct interaction between *E. coli* RNA polymerase and TopoI has been observed (57). Recently, proteins involved in different cellular processes have been found to modulate mycobacterial TopoI activity (58–60). *Mtb* MazF protein, an RNA interferase and a component of the toxin-antitoxin system, physically interacts with Mt/Ms TopoI resulting in a mutual inhibition of both the enzyme activities (58). Two other enzymes, 3-methyladenine DNA glycosylase and ribokinase were shown to interact and inhibit MtTopoI function (59). However, in most of these cases, the exact mechanism of activation or inhibition is not elucidated. In a more recent study, the molecular mechanism of gyrase inhibition by a small protein modulator has been studied (61). YacG is shown to inhibit *E. coli* gyrase activity by a multifaceted mechanism. The direct control of gyrase activity is exerted by YacG through inhibition of the strand passage activity (61). In contrast, MtHU exerts its action by enhancing the strand passage activity of MtTopoI. These are a few examples that reveal the molecular mechanism of topoisomerase modulation by physically interacting topology modulatory proteins. Illustrations of such close functional associations between topoisomerases and interacting partners in the cell to influence the topological state of the genome and the consequent downstream events would be an emerging topic of study.

## SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

## ACKNOWLEDGEMENTS

We thank Adwait A. Godbole for MtTopoI overexpressing constructs, Rupesh Kumar for purified MtGyrase protein, Wareed Ahmed, M. N. Leelaram, Anuradha G. Bhat and other members of the laboratory for helpful discussions. M. S. Srilatha is acknowledged for the help and discussion in SPR experiments.

## FUNDING

Department of Biotechnology, Government of India (grant number COE205); Department of Science and Technology, Government of India for J. C. Bose Fellowship [to V.N.]. Funding for open access charge: J. C. Bose Fellowship [to V.N.] from Department of Science and Technology, Government of India.

*Conflict of interest statement.* None declared.



## REFERENCES

- Fisher, J.K., Bourniquel, A., Witz, G., Weiner, B., Prentiss, M. and Kleckner, N. (2013) Four-dimensional imaging of *E. coli* nucleoid organization and dynamics in living cells. *Cell*, **153**, 882–895.
- Umbarger, M.A., Toro, E., Wright, M.A., Porreca, G.J., Bau, D., Hong, S.H., Fero, M.J., Zhu, L.J., Marti-Renom, M.A., McAdams, H.H. *et al.* (2011) The three-dimensional architecture of a bacterial genome and its alteration by genetic perturbation. *Mol. Cell*, **44**, 252–264.
- Thanbichler, M., Wang, S.C. and Shapiro, L. (2005) The bacterial nucleoid: a highly organized and dynamic structure. *J. Cell. Biochem.*, **96**, 506–521.
- Browning, D.F., Grainger, D.C. and Busby, S.J. (2010) Effects of nucleoid-associated proteins on bacterial chromosome structure and gene expression. *Curr. Opin. Microbiol.*, **13**, 773–780.
- Dame, R.T. (2005) The role of nucleoid-associated proteins in the organization and compaction of bacterial chromatin. *Mol. Microbiol.*, **56**, 858–870.
- Dillon, S.C. and Dorman, C.J. (2010) Bacterial nucleoid-associated proteins, nucleoid structure and gene expression. *Nat. Rev. Microbiol.*, **8**, 185–195.
- Grove, A. (2011) Functional evolution of bacterial histone-like HU proteins. *Curr. Issues Mol. Biol.*, **13**, 1–12.
- Wong, J.T., New, D.C., Wong, J.C. and Hung, V.K. (2003) Histone-like proteins of the dinoflagellate *Cryptocodinium cohnii* have homologies to bacterial DNA-binding proteins. *Eukaryot. Cell*, **2**, 646–650.
- Aki, T. and Adhya, S. (1997) Repressor induced site-specific binding of HU for transcriptional regulation. *EMBO J.*, **16**, 3666–3674.
- Broyles, S.S. and Pettijohn, D.E. (1986) Interaction of the *Escherichia coli* HU protein with DNA. Evidence for formation of nucleosome-like structures with altered DNA helical pitch. *J. Mol. Biol.*, **187**, 47–60.
- Berger, M., Farcas, A., Geertz, M., Zhelyazkova, P., Brix, K., Travers, A. and Muskhelishvili, G. (2010) Coordination of genomic structure and transcription by the main bacterial nucleoid-associated protein HU. *EMBO Rep.*, **11**, 59–64.
- Kamashev, D. and Rouviere-Yaniv, J. (2000) The histone-like protein HU binds specifically to DNA recombination and repair intermediates. *EMBO J.*, **19**, 6527–6535.
- Mangan, M.W., Lucchini, S., Ó Cróinín, T., Fitzgerald, S., Hinton, J.C. and Dorman, C.J. (2011) Nucleoid-associated protein HU controls three regulons that coordinate virulence, response to stress and general physiology in *Salmonella enterica* serovar Typhimurium. *Microbiology*, **157**, 1075–1087.
- Priyadarshini, R., Cugini, C., Arndt, A., Chen, T., Tjokro, N.O., Goodman, S.D. and Davey, M.E. (2013) The nucleoid-associated protein HU beta affects global gene expression in *Porphyromonas gingivalis*. *Microbiology*, **159**, 219–229.
- Wang, G., Lo, L.F. and Maier, R.J. (2012) A histone-like protein of *Helicobacter pylori* protects DNA from stress damage and aids host colonization. *DNA Repair*, **11**, 733–740.
- Espeli, O. and Marians, K.J. (2004) Untangling intracellular DNA topology. *Mol. Microbiol.*, **52**, 925–931.
- Gellert, M. (1981) DNA topoisomerases. *Annual Rev. Biochem.*, **50**, 879–910.
- Champoux, J.J. (2001) DNA topoisomerases: structure, function, and mechanism. *Annual Rev. Biochem.*, **70**, 369–413.
- Maxwell, A. and Gellert, M. (1986) Mechanistic aspects of DNA topoisomerases. *Adv. Protein Chem.*, **38**, 69–107.
- Vos, S.M., Tretter, E.M., Schmidt, B.H. and Berger, J.M. (2011) All tangled up: how cells direct, manage and exploit topoisomerase function. *Nat. Rev. Mol. Cell Biol.*, **12**, 827–841.
- Travers, A. and Muskhelishvili, G. (2005) Bacterial chromatin. *Curr. Opin. Genet. Dev.*, **15**, 507–514.
- Wang, J.C. (2002) Cellular roles of DNA topoisomerases: a molecular perspective. *Nat. Rev. Mol. Cell Biol.*, **3**, 430–440.
- Cole, S.T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S.V., Eiglmeier, K., Gas, S., Barry, C.E. 3rd *et al.* (1998) Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature*, **393**, 537–544.
- Bhowmick, T., Ghosh, S., Dixit, K., Ganesan, V., Ramagopal, U.A., Dey, D., Sarma, S.P., Ramakumar, S. and Nagaraja, V. (2014) Targeting *Mycobacterium tuberculosis* nucleoid-associated protein HU with structure-based inhibitors. *Nat. Commun.*, **5**, doi:10.1038/ncomms5124.
- Sasseti, C.M., Boyd, D.H. and Rubin, E.J. (2003) Genes required for mycobacterial growth defined by high density mutagenesis. *Mol. Microbiol.*, **48**, 77–84.
- Manjunatha, U.H., Dalal, M., Chatterji, M., Radha, D.R., Visweswariah, S.S. and Nagaraja, V. (2002) Functional characterisation of mycobacterial DNA gyrase: an efficient decatenase. *Nucleic Acids Res.*, **30**, 2144–2153.
- DiNardo, S., Voelkel, K.A., Sternglanz, R., Reynolds, A.E. and Wright, A. (1982) *Escherichia coli* DNA topoisomerase I mutants have compensatory mutations in DNA gyrase genes. *Cell*, **31**, 43–51.
- Pruss, G.J., Manes, S.H. and Drlica, K. (1982) *Escherichia coli* DNA topoisomerase I mutants: increased supercoiling is corrected by mutations near gyrase genes. *Cell*, **31**, 35–42.
- Bhaduri, T., Bagui, T.K., Sikder, D. and Nagaraja, V. (1998) DNA topoisomerase I from *Mycobacterium smegmatis*. An enzyme with distinct features. *J. Biol. Chem.*, **273**, 13925–13932.
- Godbole, A.A., Leelaram, M.N., Bhat, A.G., Jain, P. and Nagaraja, V. (2012) Characterization of DNA topoisomerase I from *Mycobacterium tuberculosis*: DNA cleavage and religation properties and inhibition of its activity. *Arch. Biochem. Biophys.*, **528**, 197–203.
- Jain, P. and Nagaraja, V. (2006) Indispensable, functionally complementing N and C-terminal domains constitute site-specific topoisomerase I. *J. Mol. Biol.*, **357**, 1409–1421.
- Kumar, R., Riley, J.E., Parry, D., Bates, A.D. and Nagaraja, V. (2012) Binding of two DNA molecules by type II topoisomerases for decatenation. *Nucleic Acids Res.*, **40**, 10904–10915.
- Sikder, D. and Nagaraja, V. (2000) Determination of the recognition sequence of *Mycobacterium smegmatis* topoisomerase I on mycobacterial genomic sequences. *Nucleic Acids Res.*, **28**, 1830–1837.
- Komolov, K.E. and Koch, K.W. (2010) Application of surface plasmon resonance spectroscopy to study G-protein coupled receptor signalling. *Methods Mol. Biol.*, **627**, 249–260.
- Bhaduri, T., Sikder, D. and Nagaraja, V. (1998) Sequence specific interaction of *Mycobacterium smegmatis* topoisomerase I with duplex DNA. *Nucleic Acids Res.*, **26**, 1668–1674.
- Ahmed, W., Bhat, A.G., Leelaram, M.N., Menon, S. and Nagaraja, V. (2013) Carboxyl terminal domain basic amino acids of mycobacterial topoisomerase I bind DNA to promote strand passage. *Nucleic Acids Res.*, **41**, 7462–7471.
- Leelaram, M.N., Bhat, A.G., Godbole, A.A., Bhat, R.S., Manjunath, R. and Nagaraja, V. (2013) Type IA topoisomerase inhibition by clamp closure. *FASEB J.*, **27**, 3030–3038.
- Bugreev, D.V. and Nevinsky, G.A. (2009) Structure and mechanism of action of type IA DNA topoisomerases. *Biochemistry*, **74**, 1467–1481.
- Macvanin, M. and Adhya, S. (2012) Architectural organization in *E. coli* nucleoid. *Biochim. Biophys. Acta*, **1819**, 830–835.
- Ahmed, W., Menon, S., Godbole, A.A., Karthik, P.V. and Nagaraja, V. (2014) Conditional silencing of topoisomerase I gene of *Mycobacterium tuberculosis* validates its essentiality for cell survival. *FEMS Microbiol. Lett.*, **353**, 116–123.
- Macvanin, M., Edgar, R., Cui, F., Trostel, A., Zhurkin, V. and Adhya, S. (2012) Noncoding RNAs binding to the nucleoid protein HU in *Escherichia coli*. *J. Bacteriol.*, **194**, 6046–6055.
- Ni Bhriain, N. and Dorman, C.J. (1993) Isolation and characterization of a topA mutant of *Shigella flexneri*. *Mol. Microbiol.*, **7**, 351–358.
- Dame, R.T. and Goosen, N. (2002) HU: promoting or counteracting DNA compaction? *FEBS Lett.*, **529**, 151–156.
- Guo, F. and Adhya, S. (2007) Spiral structure of *Escherichia coli* HU alpha beta provides foundation for DNA supercoiling. *Proc. Natl. Acad. Sci. U.S.A.*, **104**, 4309–4314.
- Sikder, D., Unniraman, S., Bhaduri, T. and Nagaraja, V. (2001) Functional cooperation between topoisomerase I and single strand DNA-binding protein. *J. Mol. Biol.*, **306**, 669–679.
- Bensaid, A., Almeida, A., Drlica, K. and Rouviere-Yaniv, J. (1996) Cross-talk between topoisomerase I and HU in *Escherichia coli*. *J. Mol. Biol.*, **256**, 292–300.
- Marians, K.J. (1987) DNA gyrase-catalyzed decatenation of multiply linked DNA dimers. *J. Biol. Chem.*, **262**, 10362–10368.
- Perkins, J.R., Diboun, I., Dessailly, B.H., Lees, J.G. and Orengo, C. (2010) Transient protein-protein interactions: structural, functional, and network properties. *Structure*, **18**, 1233–1243.

49. Malik, M., Bensaid, A., Rouviere-Yaniv, J. and Drlica, K. (1996) Histone-like protein HU and bacterial DNA topology: suppression of an HU deficiency by gyrase mutations. *J. Mol. Biol.*, **256**, 66–76.
50. Rouviere-Yaniv, J., Yaniv, M. and Germond, J.E. (1979) *E. coli* DNA binding protein HU forms nucleosome like structure with circular double-stranded DNA. *Cell*, **17**, 265–274.
51. Li, Y., Stewart, N.K., Berger, A.J., Vos, S., Schoeffler, A.J., Berger, J.M., Chait, B.T. and Oakley, M.G. (2010) *Escherichia coli* condensin MukB stimulates topoisomerase IV activity by a direct physical interaction. *Proc. Natl. Acad. Sci. U.S.A.*, **107**, 18832–18837.
52. Sengupta, S., Shah, M. and Nagaraja, V. (2006) Glutamate racemase from *Mycobacterium tuberculosis* inhibits DNA gyrase by affecting its DNA-binding. *Nucleic Acids Res.*, **34**, 5567–5576.
53. Chatterji, M. and Nagaraja, V. (2002) GyrI: a counter-defensive strategy against proteinaceous inhibitors of DNA gyrase. *EMBO Rep.*, **3**, 261–267.
54. Espeli, O., Lee, C. and Mariani, K.J. (2003) A physical and functional interaction between *Escherichia coli* FtsK and topoisomerase IV. *J. Biol. Chem.*, **278**, 44639–44644.
55. Hayama, R. and Mariani, K.J. (2010) Physical and functional interaction between the condensin MukB and the decatenase topoisomerase IV in *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.*, **107**, 18826–18831.
56. Vos, S.M., Stewart, N.K., Oakley, M.G. and Berger, J.M. (2013) Structural basis for the MukB-topoisomerase IV interaction and its functional implications in vivo. *EMBO J.*, **32**, 2950–2962.
57. 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.
58. Huang, F. and He, Z.G. (2010) Characterization of an interplay between a *Mycobacterium tuberculosis* MazF homolog, Rv1495 and its sole DNA topoisomerase I. *Nucleic Acids Res.*, **38**, 8219–8230.
59. Yang, Q., Huang, F., Hu, L. and He, Z.G. (2012) Physical and functional interactions between 3-methyladenine DNA glycosylase and topoisomerase I in mycobacteria. *Biochemistry*, **77**, 378–387.
60. Yang, Q., Liu, Y., Huang, F. and He, Z.G. (2011) Physical and functional interaction between D-ribokinase and topoisomerase I has opposite effects on their respective activity in *Mycobacterium smegmatis* and *Mycobacterium tuberculosis*. *Arch. Biochem. Biophys.*, **512**, 135–142.
61. Vos, S.M., Lyubimov, A.Y., Hershey, D.M., Schoeffler, A.J., Sengupta, S., Nagaraja, V. and Berger, J.M. (2014) Direct control of type IIA topoisomerase activity by a chromosomally encoded regulatory protein. *Genes Dev.*, **28**, 1485–1497.