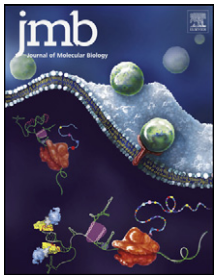

Distinct Mechanism Evolved for Mycobacterial RNA Polymerase and Topoisomerase I Protein–Protein Interaction

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Abstract

We report here a distinct mechanism of interaction between topoisomerase I and RNA polymerase in *Mycobacterium tuberculosis* and *Mycobacterium smegmatis* that has evolved independently from the previously characterized interaction between bacterial topoisomerase I and RNA polymerase. Bacterial DNA topoisomerase I is responsible for preventing the hyper-negative supercoiling of genomic DNA. The association of topoisomerase I with RNA polymerase during transcription elongation could efficiently relieve transcription-driven negative supercoiling. Our results demonstrate a direct physical interaction between the C-terminal domains of topoisomerase I (TopoI-CTDs) and the β′ subunit of RNA polymerase of *M. smegmatis* in the absence of DNA. The TopoI-CTDs in mycobacteria are evolutionarily unrelated in amino acid sequence and three-dimensional structure to the TopoI-CTD found in the majority of bacterial species outside Actinobacteria, including *Escherichia coli*. The functional interaction between topoisomerase I and RNA polymerase has evolved independently in mycobacteria and *E. coli,* with distinctively different structural elements of TopoI-CTD utilized for this protein–protein interaction. Zinc ribbon motifs in *E. coli* TopoI-CTD are involved in the interaction with RNA polymerase. For *M. smegmatis* TopoI-CTD, a 27-amino-acid tail that is rich in basic residues at the C-terminal end is responsible for the interaction with RNA polymerase. Overexpression of recombinant TopoI-CTD in *M. smegmatis* competed with the endogenous topoisomerase I for protein–protein interactions with RNA polymerase. The TopoI-CTD overexpression resulted in decreased survival following treatment with antibiotics and hydrogen peroxide, supporting the importance of the protein–protein interaction between topoisomerase I and RNA polymerase during stress response of mycobacteria.

Introduction

Topoisomerases are essential enzymes that are responsible for controlling DNA topology and facilitating vital cellular processes that include replication, transcription, recombination, and DNA repair [1–5]. The active site for DNA cleavage and rejoining by type IA topoisomerases is evolutionarily conserved at the N-terminal domains (NTDs) of bacterial topoisomerase I and topoisomerase III [6,7]. Bacterial topoisomerase I is responsible for relieving the transcription-driven negative supercoiling, generated behind the RNA polymerase (RNAP) complex during transcription elongation [8–10]. The absence of topoisomerase I activity in *Escherichia coli* has been shown to result in increased R-loop formation via the stable association of the nascent transcript with the unwound DNA [11,12]. This could potentially block transcription elongation [13,14]. A direct association between RNAP and topoisomerase I would facilitate gene expression at highly transcribed loci, including genes induced for survival in stress response.

It was previously reported that the *E. coli* RNAP β′ subunit interacts directly with the zinc ribbon and zinc ribbon-like C-terminal domains (CTDs) of topoisomerase I [15,16]. In *E. coli* and most bacterial species, the CTD of topoisomerase I have multiple zinc ribbon domains, each with a Zn(II) ion coordinated by four cysteines [17–19]. In contrast, the topoisomerase I proteins in Actinobacteria, including mycobacteria,

evolved with topoisomerase I CTDs (TopoI-CTDs) that do not have zinc ribbon or zinc ribbon-like domains [20,21]. Structural determination by X-ray crystallography showed that the *Mycobacterium tuberculosis* topoisomerase I CTD are formed by repeats of a novel protein fold of a four-stranded antiparallel β-sheet, stabilized by a crossing-over α-helix [22]. We speculated that the CTD of mycobacterial topoisomerase I could be involved in protein–protein interactions, among its other functions. The protein–protein interactions of topoisomerase I from *Mycobacterium smegmatis* were studied by co-immunoprecipitation (Co-IP) and pull-down assays coupled to tandem mass spectrometry [23–26]. The assays identified an interaction between DNA-dependent RNAP and topoisomerase I in *M. smegmatis.* This TopoI–RNAP interaction also employed the CTD of topoisomerase I, although the mycobacterial topoisomerase I CTD do not have zinc ribbons. The conservation of this interaction in *M. tuberculosis* with a distinct mode of protein–protein interactions was verified with further studies here.

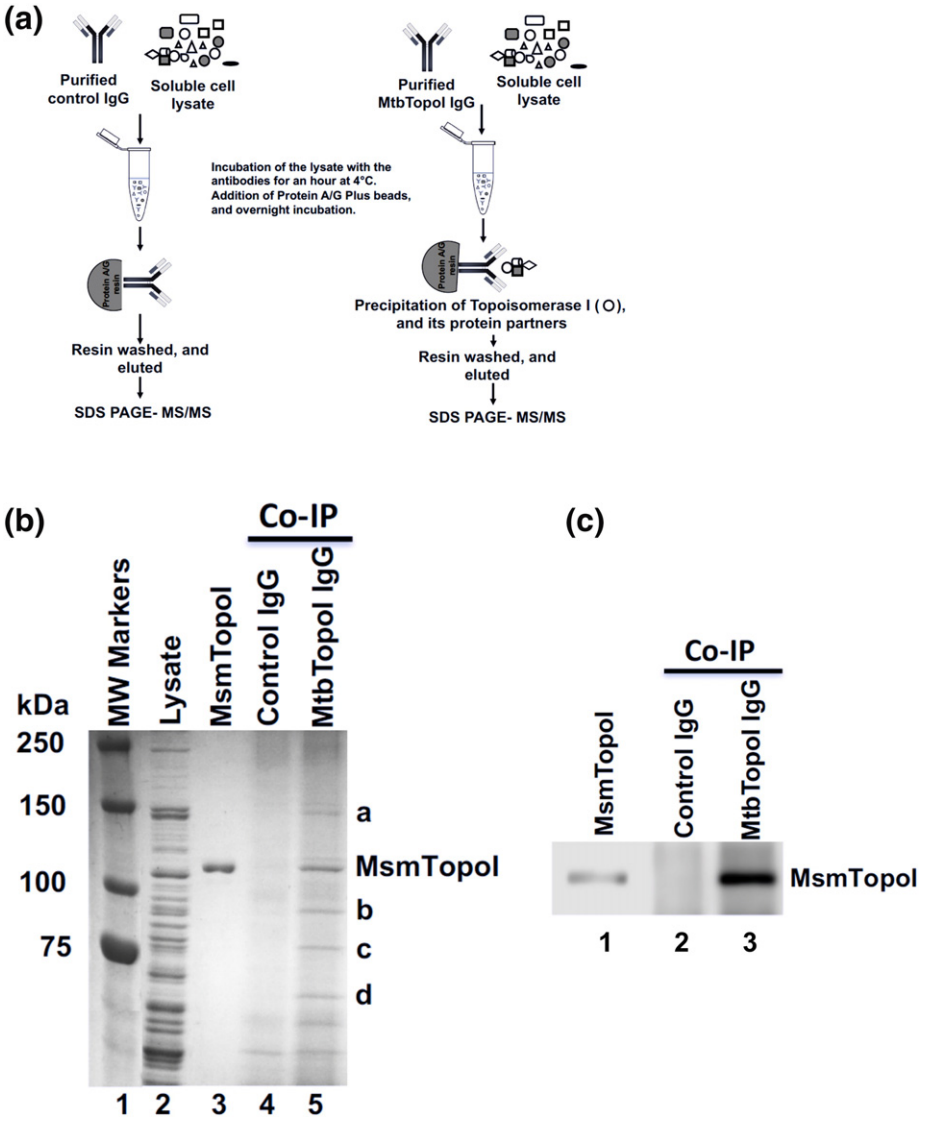

Fig. 1. Co-IP of topoisomerase I and interacting proteins from *M. smegmatis* lysate. (a) Schematic of the Co-IP. (b) Proteins were stained with Coomassie blue following SDS-PAGE. Lane 1: MW standards; lane 2: 15 μg of total *M. smegmatis* proteins in soluble lysate; lane 3: purified recombinant MsmTopoI. Proteins were immunoprecipitated from *M. smegmatis* lysate (500 μg total proteins) by pre-immune rabbit antibodies (lane 4) or antibodies raised against MtbTopoI (lane 5). Bands a–d were selected for LC MS/MS analysis. (c) A fraction of the eluates from Co-IP reactions were electrophoresed on a SDS-PAGE and immunoblotted with TopoI antibodies. Lane 1: 25 ng of purified MsmTopoI. The efficiency of the Co-IP assay was verified by analyzing a small fraction of eluates from the reaction of *M. smegmatis* lysate with pre-immune rabbit antibodies (lane 2), or antibodies raised against MtbTopoI (lane 3).