Секция «Физико-химическая биология»

Identification of topoisomerase I binding sites and study of it's role in transcriptional regulation in Escherichia coli

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Bacterial topoisomerase I removes excessive negative supercoiling and relaxes DNA molecules in several processes, including transcription and replication [4]. Despite its essentiality for *E. coli*, no data is available about the distribution of topoisomerase binding sites and enzyme's nucleotide motif [3]. It was shown by pull-down experiments that topoisomerase I interacts with RNApol via its C-terminal domain (CTD) [1]. *In vitro* experiments also show that topoisomerase I inhibits R-loop formation by relaxing transcription-induced negative supercoiling [2].

In this work using ChIP-Seq we for the first time identify topoisomerase I binding sites in *Escherichia coli* genome. Additional ChIP-Seq experiments were carried out on *E. coli* culture treated with RNApol inhibitor rifampicin and on *E. coli* cells overexpressing topoisomerase I CTD. We reveal a colocalization of topoisomerase I with RNApol, which supports the existence of a complex between the enzymes *in vivo*. Moreover, we show that topoisomerase performs enzymatic activity both in the form of a complex with RNApol at gene bodies and in an individual form in upstream regions of transcribing genes. In presence of rifampicin topoisomerase leaves gene bodies and enrich promotor zones, which corresponds to RNApol arresting on promotors by rifampicin, leading to abortive transcription. CTD overexpression leads to topoisomerase relocalization from gene bodies to promotor and upstream regions. Finally, we demonstrate that topoisomerase I CTD overexpression, which is likely to partially displace the enzyme from its complex with RNApol, is lethal and results in an accumulation of R-loops *in vivo*.

Taken the data together we conclude that topoisomerase I interacts with RNApol in *E. coli* and this interaction facilitates DNA duplex restoration during transcription.

Источники и литература

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Иллюстрации

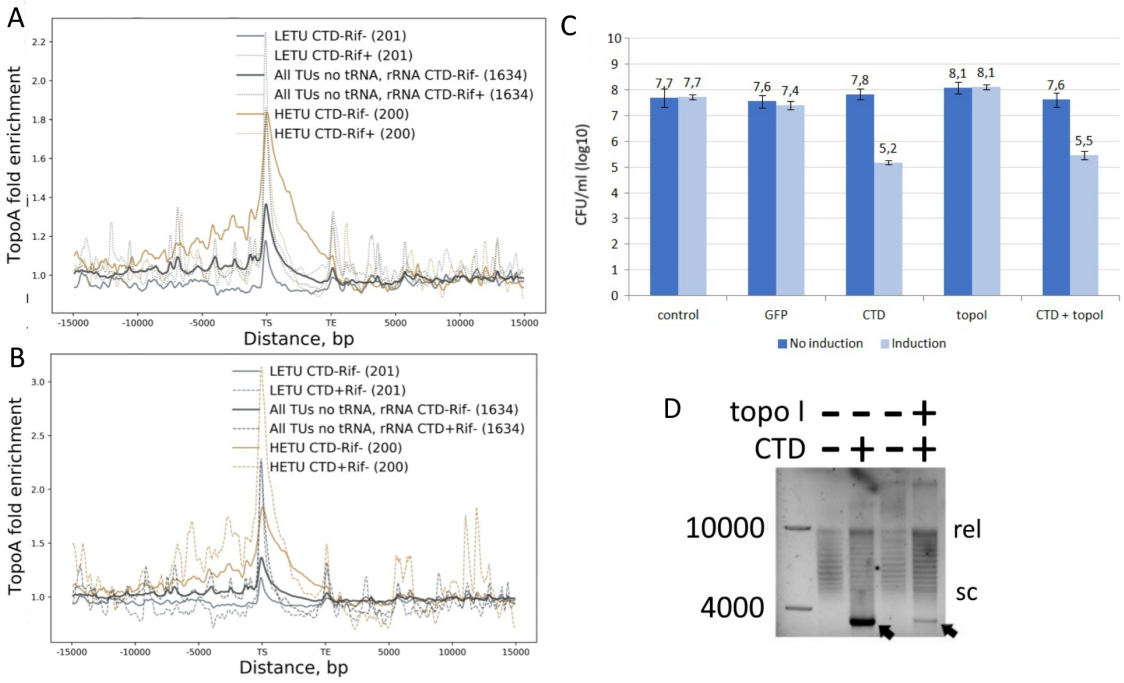

Рис. 1. A. Distribution of topoisomerase I in different groups of transcription units (TU): LETU - lowly expressed transcription units, all transcription units except rRNAs and tRNAs, HETU - highly expressed transcription units; number of genes in each group is stated in brackets. Solid lines represent data for ChIP-Seq experiments without rifampicin and dashed line - experiments with rifampicin. B. Distribution of topoisomerase I in different groups of transcription units (TU): LETU - lowly expressed transcription units, all transcription units except rRNAs and tRNAs, HETU - highly expressed transcription units; number of genes in each group is stated in brackets. Solid lines represent data for ChIP-Seq experiments without CTD overexpression and dashed lines - experiments with CTD overexpression. C. Colony forming units (cfu) per ml (logarifmic scale) of DH5α cells (control), cells with GFP expression (GFP), CTD overexpression (CTD), topoisomerase I expression (topoI) and combined overexpression of topoisomerase I and CTD (CTD + topoI). D. R-loop formation due to CTD overexpression. Electrophoresis of plasmid DNA extracted from cells with and without overexpression of CTD and topoisomerase I (topoI) in the presence of chloroquine at 5 mg/ml. Arrows indicate r-loops, rel - relaxed DNA, sc - supercoiled DNA.