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RNAP:EcTopoI interaction is essential to prevent R-loops formation in Escherichia coli

Научный руководитель – Северинов Константин Викторович

Галивонджян А.Х.[1], *Сутормин Д.А.*[2]

1 - Московский государственный университет имени М.В.Ломоносова, Факультет биоинженерии и биоинформатики, Москва, Россия, *E-mail: a.galivondzhyan@yandex.ru*; 2 - Сколковский институт науки и технологий, Москва, Россия, *E-mail: sutormin94@gmail.com*

During transcription negative and positive supercoils are formed downstream and upstream of elongating RNAP [6]. Excessive negative supercoiling is thought to prevent DNA duplex restoration which can lead to annealing of nascent RNA to DNA template strand thus forming a heteroduplex structure called R-loop [4]. These RNA-DNA heterodimers can lead to transcription stalling and RNA degradation [1]. Moreover, in bacteria R-loops can be used as primers for constitutive stable DNA replication (cSDR) - a type of ectopic replication that is known to be responsible for genomic instability [5]. It was extensively demonstrated *in vivo* and *in vitro* that type I topoisomerases of *E. coli*, particularly TopoI, prevent deleterious R-loops formation by relaxation of excessive negative supercoiling [7, 2]. TopoI interacts with RNAP by C-terminal and Zn-binding domains (CTD and ZBD correspondingly) which was demonstrated by pull-down experiments [3] and further validated by our ChIP-Seq data. We hypothesized that interaction between TopoI and RNAP is essential for *in situ* relaxation of transcription-induced negative supercoiling providing the mechanism for effective inhibition of R-loops formation.

To check this hypothesis we examined the effect of TopoI:RNAP complex disruption on *E. coli* physiology. To disrupt the complex, we overexpressed TopoI competitors: CTD and catalytically inactive topoisomerase mutant TopoI Y319F. We showed that plasmids purified from cells with competitors' overproduction have an altered topological state that corresponds to hypernegatively supercoiled form (Fig. 1A). A similar topological phenomenon was previously demonstrated for *topA*-null mutants and was linked to R-loops accumulation [7]. Then, using DRIP-Seq method we mapped R-loops genome-wide. We observed that disruption of TopoI:RNAP complex by overexpression of CTD leads to drastic accumulation of R-loops in active transcription units (Fig. 1B). Respectively, with dot-blot analysis using S9.6 antibodies specific for R-loops we showed that TopoI Y319F overproduction leads to significant accumulation of R-loops (Fig. 1C). We conclude that TopoI-RNAP complex is essential to prevent R-loops accumulation during transcription in *Escherichia coli*.

References

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Illustrations

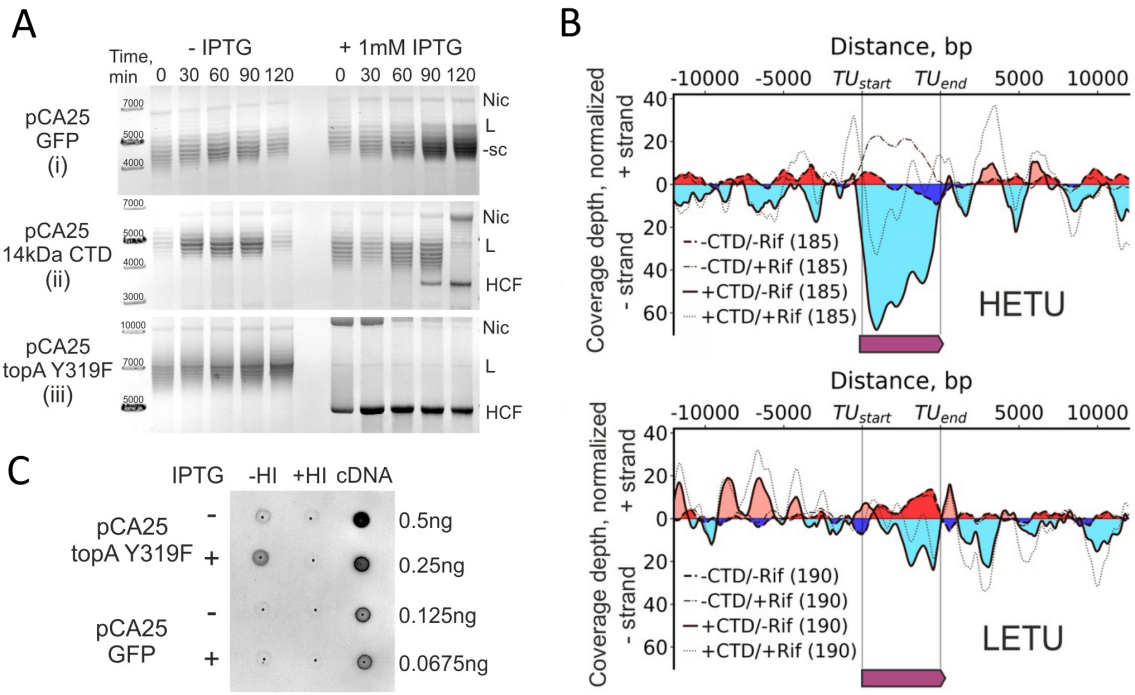

Рис. 1. (A) Supercoiling of pCA25 GFP (i), pCA25 14kDa CTD (ii) or pCA25 topA_strep Y319F (iii) plasmids extracted from exponentially growing E. coli DY330 topA-SPA culture in LB supplemented with 0.5% glucose. At OD600∼0.2 cultures were bisected and one half was induced with 1 mM IPTG (+ 1 mM IPTG), the second half served as a non-induced control (- IPTG). The cultures were sampled every 30 min. Nic – nicked plasmid, L – linear plasmid, -sc – negatively supercoiled plasmid, HCF – hyper-compacted form of a plasmid. Separation performed in 1% agarose gel in TAE buffer supplemented with 5 mkg/mL chloroquine. For DNA visualization gel was stained with EtBr after separation. (B) Metageneanalysis of normalized strand-specific read coverage depth obtained in DRIP-Seq experiments with exponentially growing E. coli DY330 topA-SPA cultures. Area under curve for -CTD/-Rif experiment (dashed line) shown in dark-blue for "-" strand and in dark-red for "+" strand. Area under curve for +CTD/-Rif experiment (solid line) shown in light-blue for "-" strand and in light-red for "+" strand. Left plot represents coverage for a set of highly-transcribed transcription units (HETU), right – for low-transcribedunits (LETU). Number of transcription units involved in metagene analysis are indicatedin parenthesis. Schematic transcription unit shown as a purple oriented rectangle below. (C) Dot-blot with S9.6 antibodies against RNA:DNA hybrids of total nucleic acids extracted from E. coli DY330 topA-SPA harboring pCA25 topA_strep Y319F or pCA25 GFP plasmids. Cultures were grown in LB supplemented with 0.5% glucose until OD600∼0.2 and thenbisected. One half wasinduced with 1 mM IPTG (samples marked with +), the second half served as a non-induced control (marked with -). Nucleic acids were extracted in 1h after induction and were treated with RNAse III to remove double-stranded RNA. As a control, samples were additionally treated with RNAse HI (+HI) or left untreated (-HI). cDNA synthesized with Maxima H Minus Reverse Transcriptase was usedas a standard (on the right). Quantification of dot-blot signals is shown on the right.