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Single-nucleotide resolution detection of Topo IV cleavage activity in the E. coli genome with Topo-Seq

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During replication of circular chromosomes, positive DNA supercoiling formed by DNA polymerase can prevent chromosomes segregation by formation of tangles and catenanes. Topoison are a class of proteins that resolve such topological challenges and support proper DNA replication and segregation processes. In *Escherichia coli,* topoisomerase IV (Topo IV) is considered to be the main decatenase [1].

Topo IV is a classical type-IIA topoisomerase (Figure A). *In vitro* and *in vivo,* Topo IV efficiently decatenates circular DNA molecules and resolves precatenanes. Topo IV can also relax positive supercoils but is unable to introduce negative supercoiling below the equilibrium, which distinguishes it from DNA gyrase, a homologous type-IIA topoisomerase. *E. coli* Topo IV has several known protein partners that modulate its activity and thus might influence its genomic distribution. *In vivo*, the interaction between the condensing complex MukBEF and Topo IV is necessary for the timely segregation of newly replicated regions containing the origin of replication [2]. In the Ter macrodomain of *E. coli,* MatP protein displaces MukBEF-TopoIV complex. At the same time, in the middle of the Ter macrodomain, in the vicinity of a *dif*-site, a Topo IV cleavage hot-spot is located. At this site, resolution of chromosome dimers and catenanes presumably occurs, and Topo IV activity is mediated by XerC/XerD recombinase [3, 4].

Using Topo-Seq [5], a method that employs the stabilization properties of topoisomerase poisons and allows mapping cleavage sites strand specifically and with a single-nucleotide resolution, we identified several thousands of Topo IV cleavage sites in the *E. coli* genome and derived a Topo IV cleavage motif. The motif contains several determinants that were not reported previously and lacks the signs of DNA wrapping observed in the DNA gyrase cleavage motif [5] (Figure C). On a whole-genome level, Topo IV cleavage sites were significantly depleted in the Ter macrodomain and highly enriched in a 50-60 kb region containing the origin of replication *oriC*. We found two strong non-canonical Topo IV cleavage signals near the *dif*-site (Figure B). Finally, we found that Topo IV enrichment was increased downstream of active transcription units, where positive supercoiling is accumulated (Figure D). These results significantly expand the knowledge about Topo IV activity in the *E. coli* genome.

References

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Illustrations

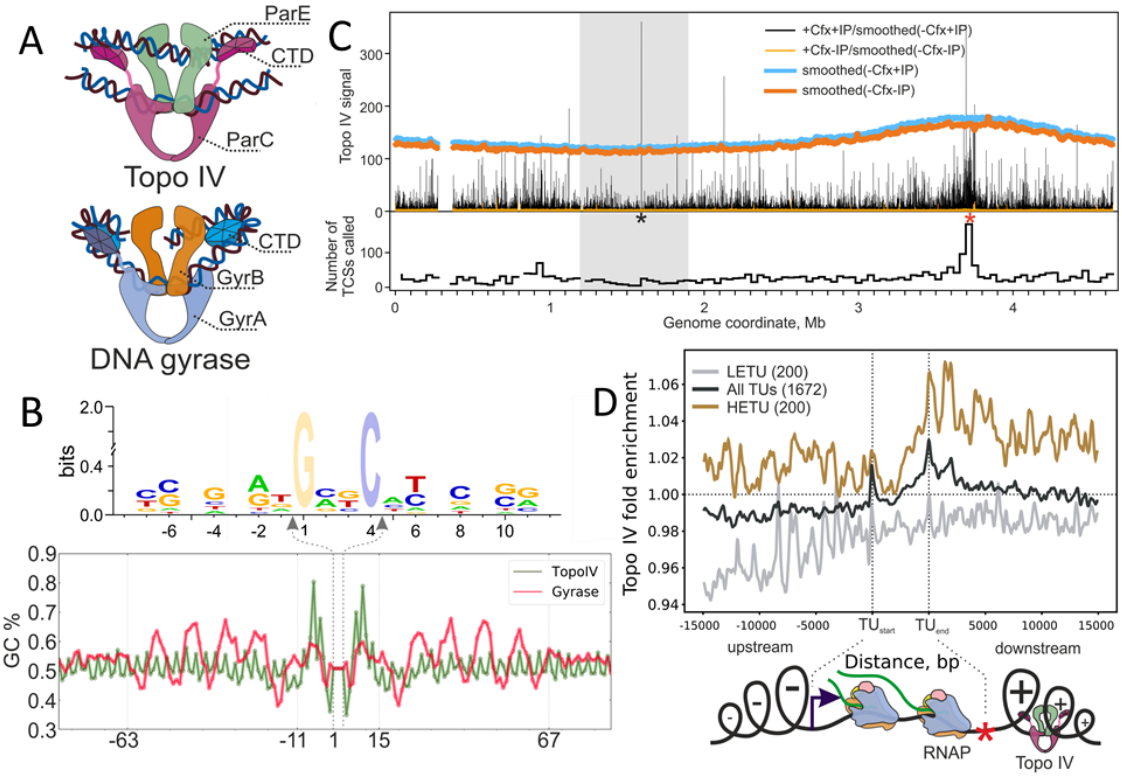

Рис. (A) Cartoon representations of Topo IV and DNA gyrase complexes with DNAs. (B) A logo representation of Topo IV cleavage motif determined with Topo-Seq. The Topo IV motif (green) and the DNA gyrase 'combined' motif (red) are shown as plots of GC content below. Vertically dashed arrows indicate the positions of cleavage sites. (C) Topo IV enrichment over the E. coli genome. A black asterisk marks the position of the dif-site; a red asterisk marks the position of oriC; a gray rectangle indicates the E. coli chromosome Ter macrodomain. A plot representing the number of topoisomerase cleavage sites in 40 kb-wide genomic bins is shown below. (D) Metagene plot showing Topo IV enrichment in TUs, their upstream, and downstream regions. Enrichment is shown for all transcription units (TUs, black curve), highly-expressed (HETU, orange curve), and least-expressed (LETU, grey curve) sets. The number of TUs in each group is indicated in parentheses. Bottom, graphical representation of the Liu & Wang twin-domain model showing localization of RNA polymerase and Topo IV.