

E. coli Topoisomerase IV

Molecular Function Example

<http://www.ncbi.nlm.nih.gov/pubmed/8227000>

<http://www.jbc.org/content/268/32/24481.full.pdf>

Finding a paper

- I'm interested in Topo IV based on something I heard about it (e.g. drug target)
- Pubmed search: “topoisomerase IV AND activity AND e coli”
- Scan the titles of the older papers

The screenshot shows the PubMed search interface. The search bar contains the query "topoisomerase IV AND activity AND e coli". The results are sorted by "Most Recent" and displayed on page 8 of 8. The search results list several articles, including:

- 141. [DNA unknotting activity \(DNA topoisomerase II\) isolated from a thermophilic archaeobacterium](#). [Sulfolobus is inhibited by novobiocin. Partial purification, identification of the two subunits and characteristics of the enzyme.](#) Assalri LM. *Biochim Biophys Acta.* 1994 Sep 13;1219(1):107-14. PMID: 8086447 [Similar articles](#)
- 142. [DNA topoisomerase I from Mycobacterium smegmatis.](#) Bhaduri T, Nagaraja V. *Indian J Biochem Biophys.* 1994 Aug;31(4):339-43. PMID: 8002018 [Similar articles](#)
- 143. [Topoisomerase IV can support oriC DNA replication in vitro.](#) Hiasa H, Marians KJ. *J Biol Chem.* 1994 Jun 10;269(23):16371-5. PMID: 8206945 [Free Article](#) [Similar articles](#)
- 144. [The parDE operon of the broad-host-range plasmid RK2 specifies growth inhibition associated with plasmid loss.](#) Roberts RC, Ström AR, Helinski DR. *J Mol Biol.* 1994 Mar 18;237(1):35-51. PMID: 8133518 [Similar articles](#)
- 145. [Escherichia coli topoisomerase IV. Purification, characterization, subunit structure, and subunit interactions.](#) Peng H, Marians KJ. *J Biol Chem.* 1993 Nov 15;268(32):24481-90. PMID: 8227000 [Free Article](#) [Similar articles](#)

“Reconstitute activity” looks promising

J Biol Chem. 1993 Nov 15;268(32):24481-90.

Escherichia coli topoisomerase IV. Purification, characterization, subunit structure, and subunit interactions.

Peng H¹, Marians KJ.

+ Author information

Abstract

DNA sequence analysis of *Escherichia coli* parC and parE, encoding the subunits of topoisomerase IV (Topo IV) (Katz J.-I., Suzuki, H., and Ikeda, H. (1992) *J. Biol. Chem.* 267, 25676-25684), showed that ParC was 22 amino acids longer on the N terminus and ParE was 29 amino acids longer on the C terminus than reported previously. *E. coli* strains bearing bacteriophage T7 RNA polymerase-based expression plasmids carrying both intact and truncated parC and parE were used to overproduce the ParC and ParE proteins. Full-length ParC and ParE were required to reconstitute Topo IV activity, whereas the truncated ParC and ParE were inactive. Topo IV activity was supported only by ATP or dATP. The [ATP]_{1/2} for DNA relaxation was 0.45 mM, almost 25-fold higher than the [ATP]_{1/2} for decatenation of kinetoplast DNA. Topo IV activity was inhibited by the quinolone and coumarin antibiotics, although the concentrations required for 50% inhibition of activity were 3-30-fold higher than those required to inhibit DNA gyrase. The norfloxacin-induced DNA cleavage patterns of Topo IV and DNA gyrase were distinct but overlapping. The native forms of ParC and ParE were a dimer and a monomer, respectively; whereas the active form of Topo IV was a heterotetramer, ParC₂ParE₂. The inactivity of the truncated forms of ParC and ParE could be attributed to their failure to form the heterotetramer.

What protein(s)?

- “Full-length ParC and ParE were required”

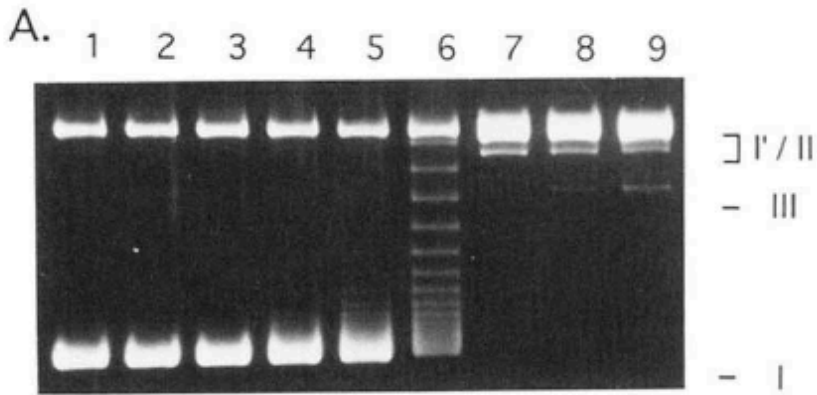
The screenshot shows the UniProtKB search results page for the query 'parc e coli'. The search bar at the top contains 'UniProtKB parc e coli' and a search button. Below the search bar, there are navigation links for 'BLAST', 'Align', 'Retrieve/ID mapping', 'Help', and 'Contact'. The main heading is 'UniProtKB results'. On the left side, there are filters for 'Reviewed (9) Swiss-Prot' and 'Unreviewed (568) TrEMBL'. Below these are 'Popular organisms' including 'E. coli K12 (4)', 'ECOLX (54)', 'SHIFL (1)', 'ECO57 (3)', and 'ECO27 (3)'. There are also 'Other organisms' and a 'Go' button. The 'Search terms' section shows filters for 'e' (author: 514, gene name: 3, organism: 6, protein name: 4, strain: 10, taxonomy: 10) and 'parc' (gene name: 210). The main table displays the search results with columns for 'Entry', 'Entry name', 'Protein names', 'Gene names', 'Organism', and 'Length'. The table contains 13 rows of results, including entries for ParC and DNA topoisomerase 4 subunit A in Escherichia coli.

Entry	Entry name	Protein names	Gene names	Organism	Length
Q0QCK5	Q0QCK5_ECOLX	ParC	parC	Escherichia coli	108
P0AFI2	PARC_ECOLI	DNA topoisomerase 4 subunit A	parC, b3019, JW2987	Escherichia coli (strain K12)	752
Q0QCK6	Q0QCK6_ECOLX	ParC	parC	Escherichia coli	108
B2CIU0	B2CIU0_ECOLX	ParC	parC	Escherichia coli	130
B2CIU1	B2CIU1_ECOLX	ParC	parC	Escherichia coli	126
B2CIU2	B2CIU2_ECOLX	ParC	parC	Escherichia coli	130
B2LR61	B2LR61_ECOLX	ParC	parC	Escherichia coli	128
A9QXD3	A9QXD3_ECOLX	ParC	parC	Escherichia coli	134
Q0QCK9	Q0QCK9_ECOLX	ParC	parC	Escherichia coli	105
Q0QCL0	Q0QCL0_ECOLX	ParC	parC	Escherichia coli	68
Q0QCL2	Q0QCL2_ECOLX	ParC	parC	Escherichia coli	107
B7UIS6	B7UIS6_ECO27	DNA topoisomerase 4	parC, E2348C_3311	Escherichia coli O127:H6	752

Which figures address function?

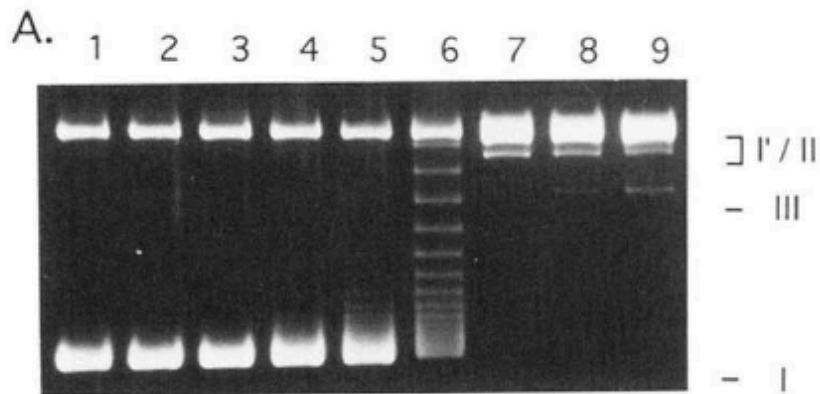
- Do these tell us about the function?
 - Figure 1: sequenced ParC and Part of ParE
 - Figure 2: SDS page of purified proteins
 - Figure 3: Relaxation and decatenation *activities* of TopoIV
 - ...

Figure 3



- Panel A: relaxation assay
 - Supercoiled DNA runs faster on gels
 - When they added increasing amounts of both proteins, DNA converted to relaxed

Figure 3



- Panel A shows GO:0003916 ! DNA topoisomerase activity but can we get more specific?

Child Terms

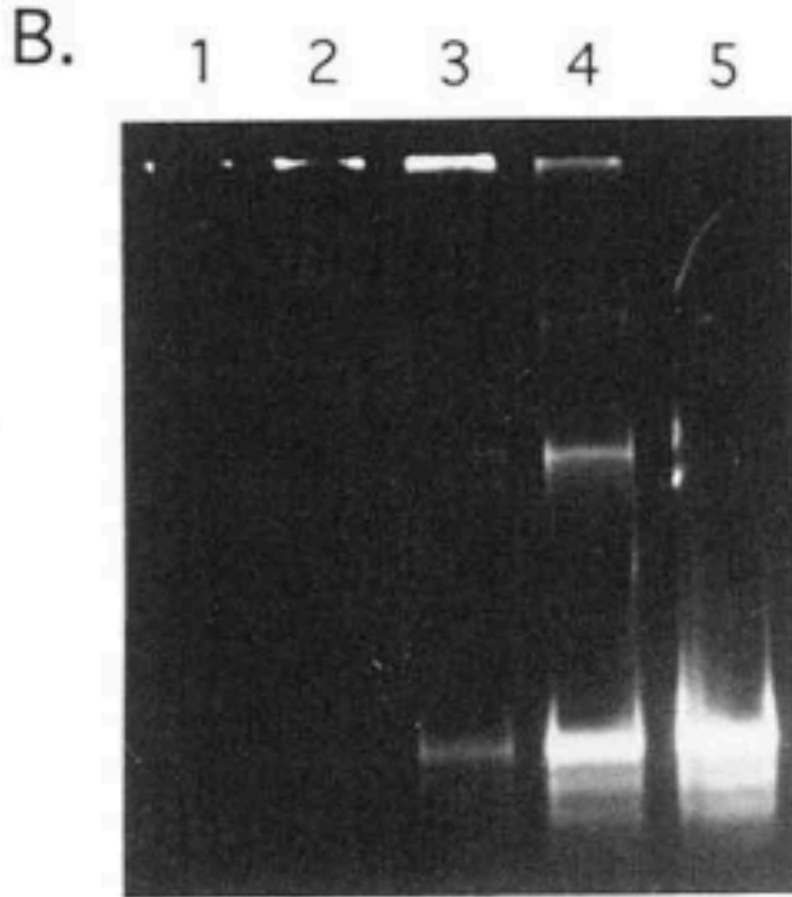
This term has the following 2 child terms, out of 2 total.

- [] GO:0003917 ! DNA topoisomerase type I activity
- [-] GO:0061505 ! DNA topoisomerase II activity (2)
 - [] GO:0003918 ! DNA topoisomerase type II (ATP-hydrolyzing) activity
 - [] GO:0061506 ! DNA topoisomerase type II (ATP-independent) activity

Type I vs Type II topoisomerases – check term definitions (or wikipedia)

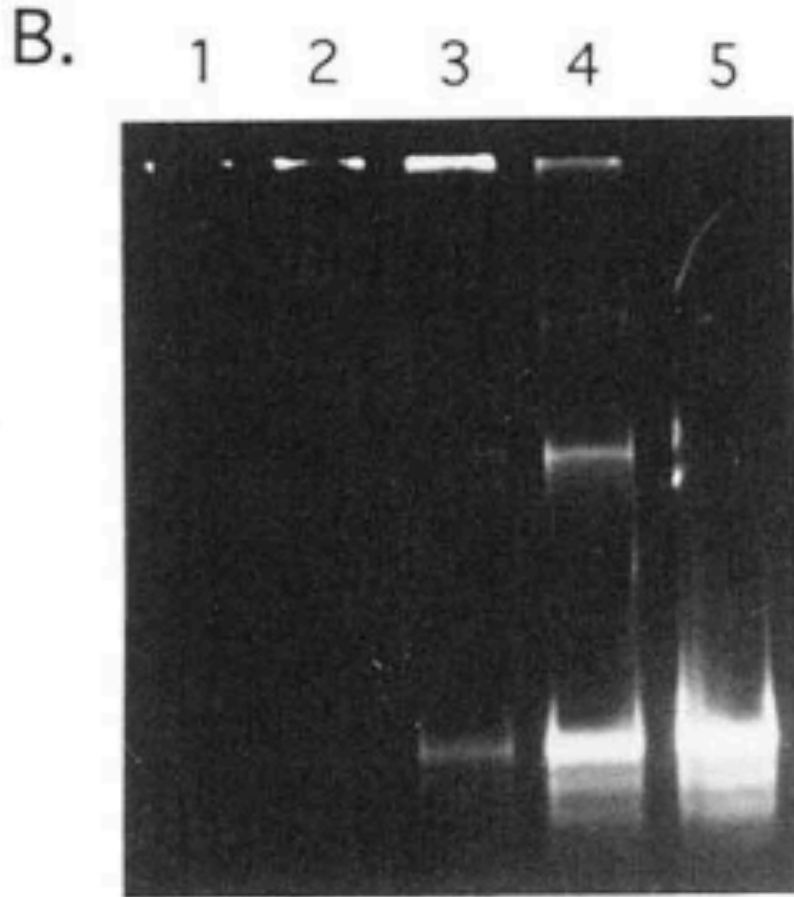
- Type I:
 - Catalysis of a DNA topological transformation by transiently **cleaving one DNA strand** at a time to allow passage of another strand; changes the linking number by +1 per catalytic cycle." [PMID:8811192]
- Type II:
 - Catalysis of a DNA topological transformation by transiently **cleaving a pair** of complementary DNA strands to form a gate through which a second double-stranded DNA segment is passed, after which the severed strands in the first DNA segment are rejoined; changes the linking number in multiples of 2." [GOC:dph]

Figure 3B



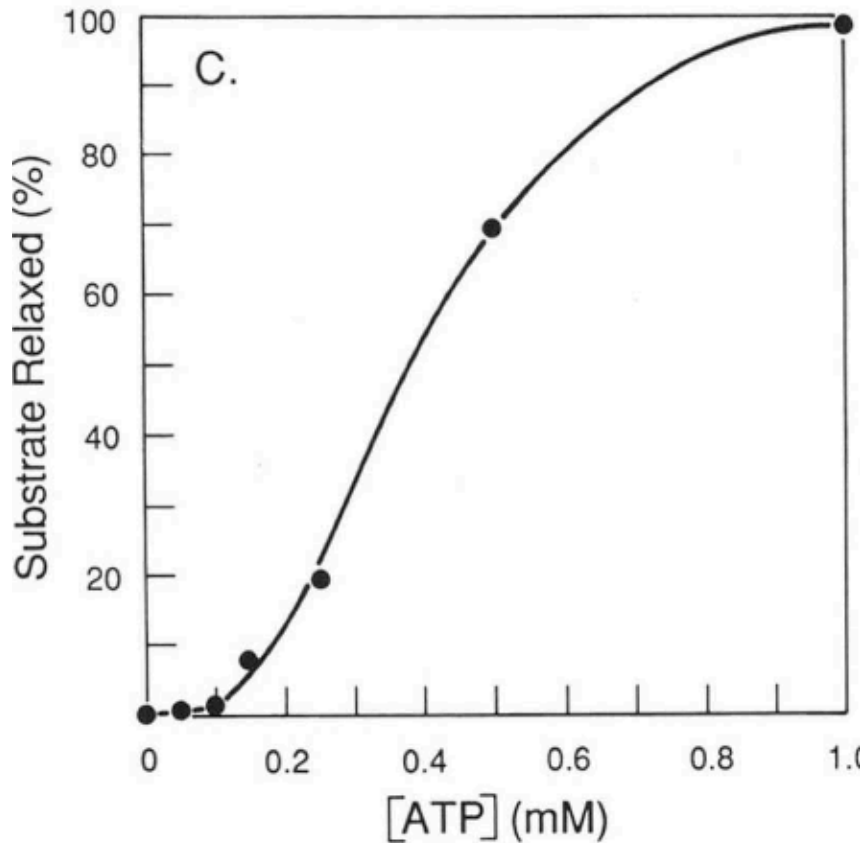
- Catenated DNA is rings linked together like chain mail. Doesn't enter the gel
 - Topo II can take these apart. Individual rings run to the bottom of the gel
 - Topo I can't

Figure 3B



- 3B shows GO:
0061505 ! DNA
topoisomerase II
activity
 - But is it ATP-dependent
or independent?
 - Standard reaction mixtures
(20 pl) containing ... 1 mM
ATP (unless indicated
otherwise), ...

Figure 4



- Shows ATP dependence: GO:0003918 ! DNA topoisomerase type II (ATP-hydrolyzing) activity

Example Pader: GO annotation for *E. coli* ParC

TableEdit

ECOLI:PARC

Qualifier	<input type="text" value=""/>
GO ID	<input type="text" value="GO:0003918"/>
GO term name	DNA topoisomerase type II (ATP-hydrolyzing) activity
Reference	PMID: <input type="text" value="8227000"/>
Evidence Code	<input type="text" value="IDA: Inferred from Direct Assay"/>
with/from	
Aspect	F
Notes	<input type="text" value="Topoisomerase assay in Fig 3. ATP dependent decatenation means it is a Type II from Fig 4"/>
Status	complete
<input type="button" value="Public"/> <input type="button" value="Refresh"/> <input type="button" value="Save Row"/> <input type="button" value="Cancel"/>	