E. coli Topoisomerase IV

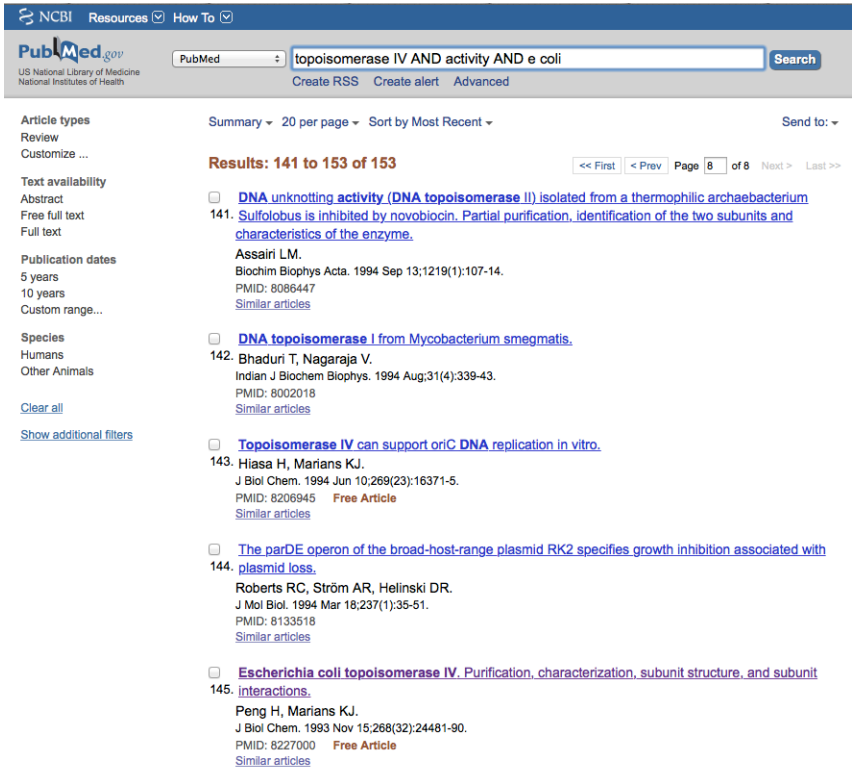
Molecular Function Example

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



“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 H[1], Marians KJ.

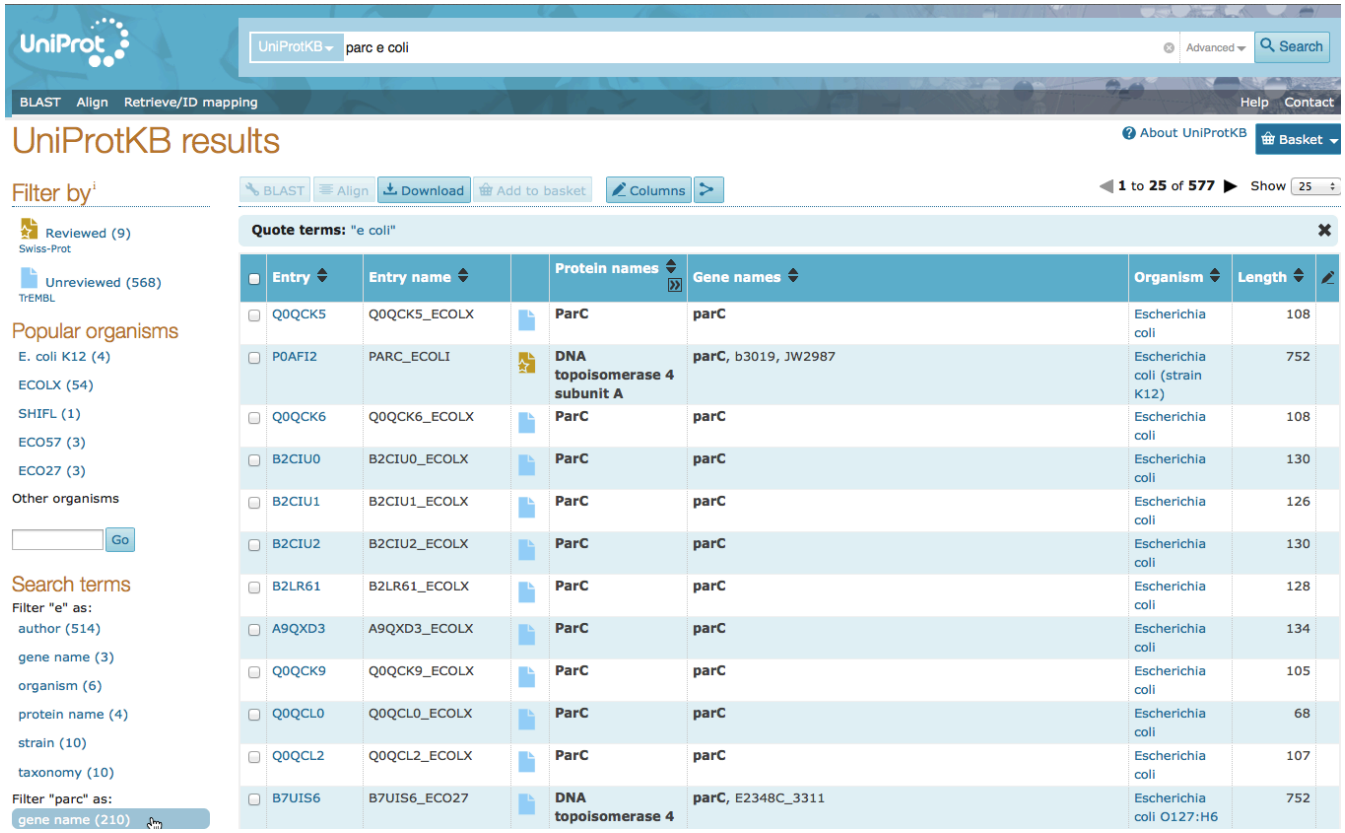
Author information

Abstract

DNA sequence analysis of Escherichia coli parC and parE, encoding the subunits of topoisomerase IV (Topo IV) (Kato J.-I., Suzuki, H., and Ikeda, H. (1992) J. Biol. Chem. 267, 25676-25684), showed that ParC was 22 amino acids longe 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)?

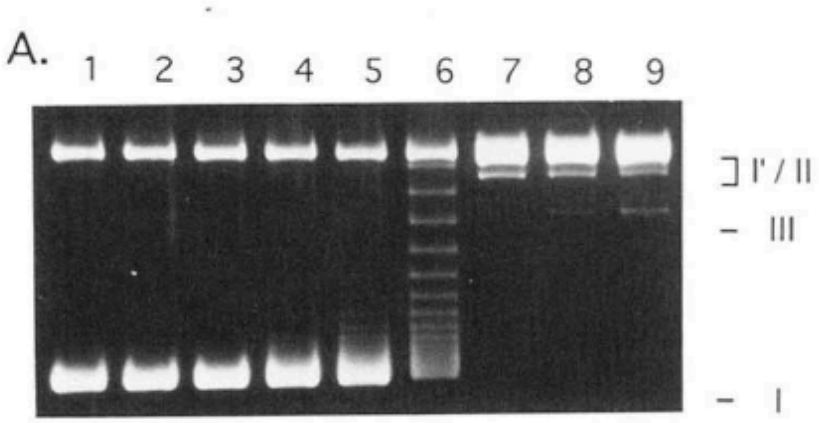
- "Full-length ParC and ParE were required"



Which figures address function?

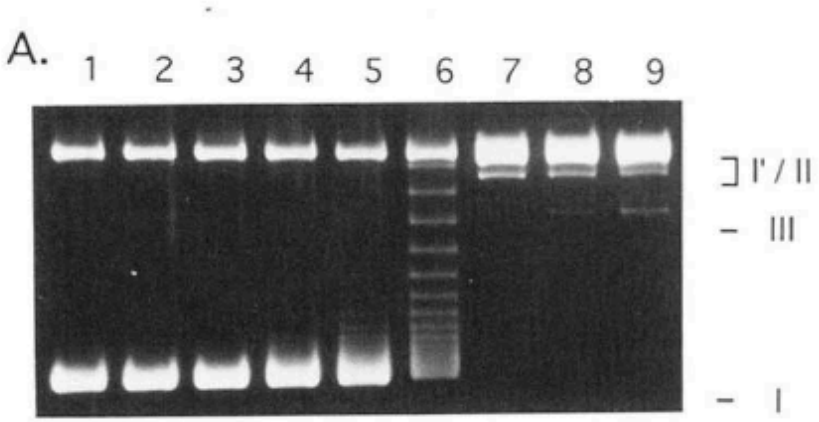
- Do these tell us about the function?
  - Figure 1: sequenced ParC and Part of ParE
  - Figure 2: SDS page of purified proteins
  - Figure 3: Relaxation and decatenation *activities* of TopoIV
  - …

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

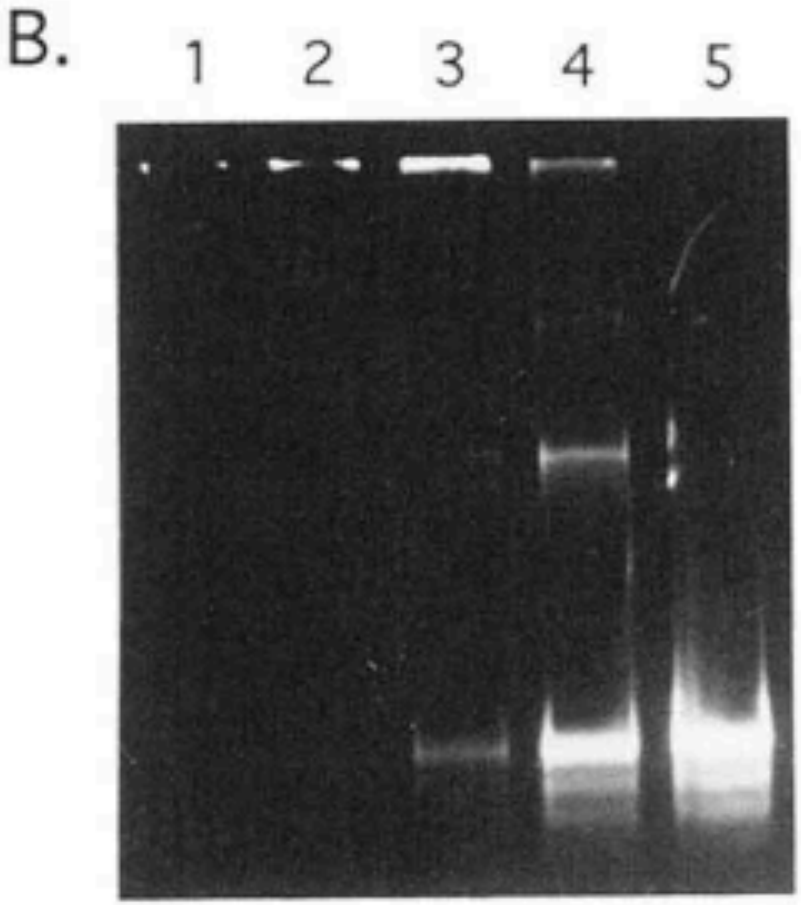
This term has the following 2 child terms, out of 2 total.

- [ ] 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)

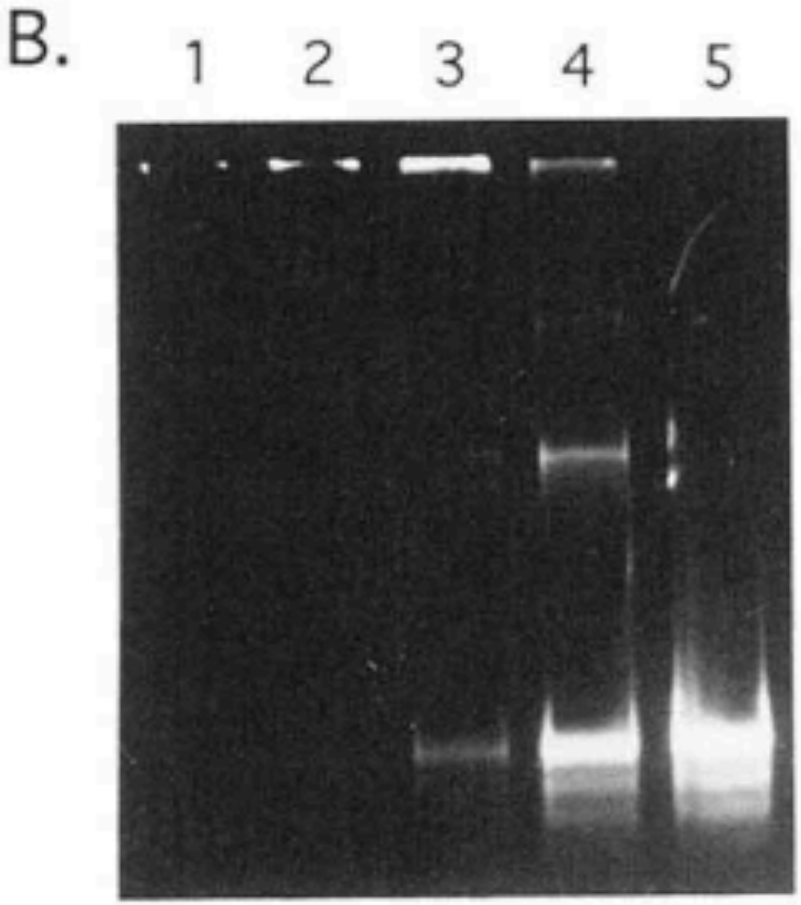
- Type I:
  - Catalysis of a DNA topological transformation by transiently cleaving one DNA strand at a time to allow passage of another strand; changes the linking number by +1 per catalytic cycle." [PMID:8811192]
- Type II:
  - Catalysis of a DNA topological transformation by transiently cleaving a 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



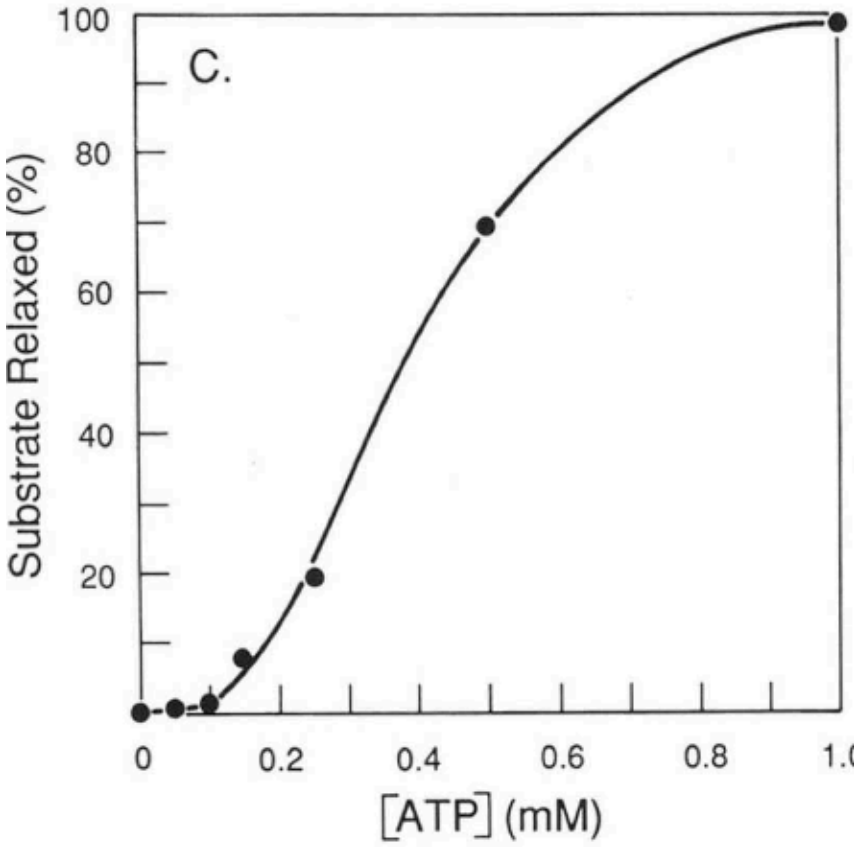
- Catenated DNA is rings linked together like chain mail. Doesn't enter the gel
  - Topo II can take these apart. Individual rings run to the bottom of the gel
  - Topo I can't

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 Paper: GO annotation for *E. coli* ParC