Three-Dimensional Structure of the 67k N-Terminal Fragment of *E. coli* DNA Topoisomerase I

Research by C.D. Lima, J.C. Wang, and A. Mondragón, Nature ***1994,*** 367, *138*

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CONDENSATION OF THE RESEARCH

PURPOSE OF THE STUDY

To investigate the tertiary structure of a topoisomerase to understand principles of DNA recombination

RESEARCHERS' APPROACH

After overexpression, purification, and crystallization[1] of the 67 kDa N-terminal fragment of *Escherichia coli* topoisomerase, its structure was resolved initially by isomorphous replacement and later by XPLOR stepwise refinement to 2.2 Å using data from a MAR phospor image plate scanner.

OBSERVATIONS

The overall architecture consists of four domains (Fig. 1) forming an elongated toroid around a hole large enough (27.5 Å diameter) to accommodate a piece of B-form DNA. Domain I forms an α/β fold.[2] Domain II consists of three antiparallel β-strands crossing another set of three β-strands connected by a helix and has overall similarity in shape to TATA binding factors.[3] Domain III contains the active site Tyr 319 at an interface made by domains I, III, and IV and next to a large cleft in the main body of the protein. Four helices packing around a central fifth helix stabilize the conserved loop regions in domain III. Tyr 319 interacts with several highly conserved amino acids to form an extensive network of hydrogen bonds, including some water molecules, while a second shell of conserved amino acids surrounds this core. Domain IV consists of four main helices and four smaller helices packing against them as well as two antiparallel strands: There are extensive interfaces between domains I/III and III/IV. The chain trace is considerably interlaced in forming domains II and III, and this suggests that they move as a rigid body away from the rest of the protein providing an open and closed form of the enzyme. Furthermore, the strategic position of Tyr 319 where domains I, III, and IV converge allows for formation of a covalent bond between Tyr 319 and the 5′ phosphoryl end of the

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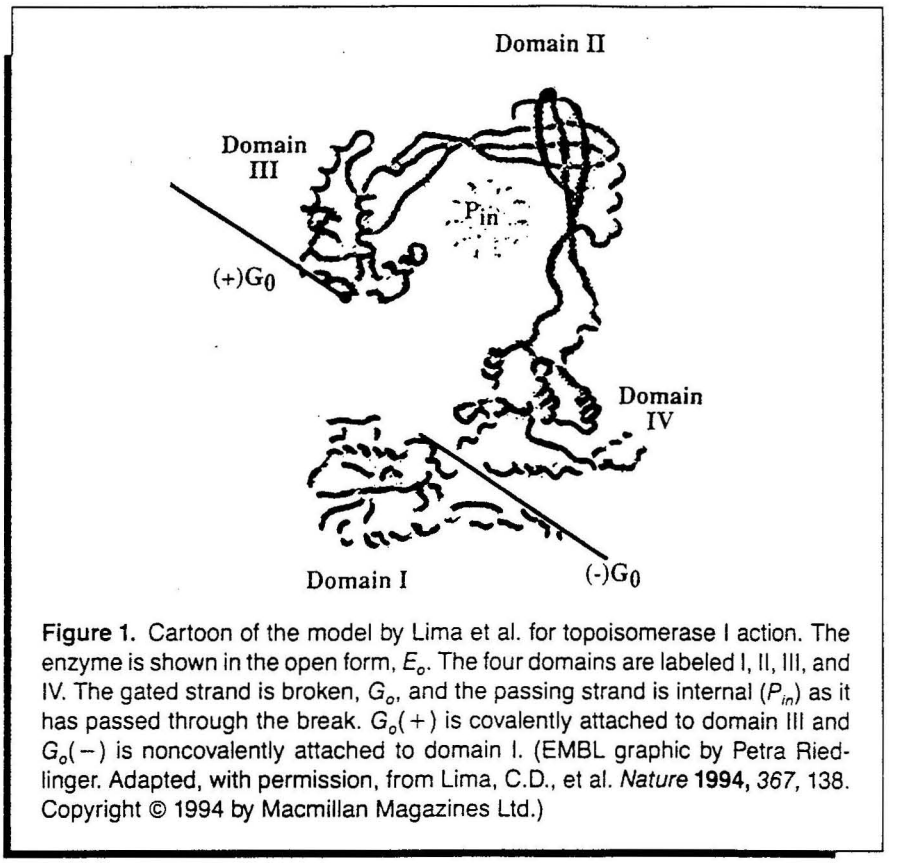

Figure 1. Cartoon of the model by Lima et al. for topoisomerase I action. The enzyme is shown in the open form, E_o. The four domains are labeled I, II, III, and IV. The gated strand is broken, G_o, and the passing strand is internal (P_{in}) as it has passed through the break. $G_o(+)$ is covalently attached to domain III and $G_o(-)$ is noncovalently attached to domain I. (EMBL graphic by Petra Riedlinger. Adapted, with permission, from Lima, C.D., et al. *Nature* **1994,** *367,* 138. Copyright © 1994 by Macmillan Magazines Ltd.)

broken strand coupled to the movement of domains II and III away from the rest of the protein, likely accommodating the passage of either a single- or double-stranded DNA through the break. The domain I topology shows high similarity to nucleotide binding folds and may provide a noncovalent link for the other end of the DNA strand. In the intact *E. coli* enzyme, domain IV extends into the C-terminal domain (missing in the presently studied fragment) that facilitates the strand passage reaction and contains DNA binding cysteine motifs.

COMMENTARY ON THE RESEARCH

E. coli DNA topoisomerase is a 97 kDa metalloprotein and was the first topoisomerase identified.[4] DNA topoisomerases are ubiquitous enzymes used to alter DNA topologies such as in relaxation of supercoiled DNA, interconversion of unknotted and knotted DNA, and catenation. In all these reactions, one or two strands of DNA are transiently broken, a single-stranded or double-stranded DNA is then passed through the break, and finally the break is resealed.[5] In contrast to type II enzymes, DNA topoisomerase I and all other type-I enzymes have no ATP requirement. Mutation experiments on the 67 kDa fragment underline the functional importance of the conserved residues in the active site region.[6] Modelling of DNA through the hole is supported by electrostatic calculations that yield its positively charged nature.[7] DNA polymerase III is another instance for a protein encircling bound DNA. Three acidic residues (Asp 111, Asp 113, and Glu 115) in the vicinity of the active site show spatial similarity with those that coordinate two divalent cations in the exonuclease catalytic site of Klenow fragment.[8]

The structure of *E. coli* topoisomerase I suggests at least two conformations, a closed (E_c) and open form (E_o) where domain III has moved away from the base of the protein to expose the catalytic tyrosine and open the large torus. During the cleavage step, the two ends of the broken strand are separated to form an opening or gate that allows strand passage, such that the enzyme acts as a bridging mechanism[9] to create a gap G_o in the intact DNA strand G_c by mediating covalent and noncovalent interactions with both ends of the strand. Thus the authors postulate minimally three complexes distinguishable in the reaction: unbroken DNA and a closed enzyme (E_cG_c), an opened enzyme with intact DNA (E_oG_c) that allows a second DNA strand from the exterior of the enzyme to pass through the DNA gate to the enzyme interior, and E_oG_o where the second strand may again penetrate but not through the gate. As the reaction proceeds without external energy, all of these steps must be driven by protein–DNA or protein–protein interactions. This fascinating model for type-I DNA topoisomerase function is not only clear and plausible but will also shape, with the aid of the solved structure, many further experimental investigations.

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