Chiral Discrimination by Type II topoisomerases

Keir C. Neuman
Laboratory of Molecular Biophysics
National Heart, Lung, and Blood Institute
National Institutes of Health

Type II topoisomerases are essential ATP-dependent topoisomerase that transport one segment of DNA through a transient double-strand break in a second segment of DNA. In vivo, Type II topoisomerases resolve inappropriate topological states resulting from DNA replication by removing positive supercoils and unlinking catenated daughter strands. In vitro, two type II topoisomerases, *E. coli* Topo IV and hTopo IIα, one of the two isoforms of human Topoisomerase II, preferentially relax positive supercoils. The mechanistic basis of this chiral discrimination remains unclear. We have investigated the mechanisms of DNA supercoil relaxation and chiral discrimination by *E. coli* Topo IV and hTopo IIα with single-molecule magnetic tweezers-based assays. We find that chiral discrimination by Topo IV results from differences in processivity: Topo IV is highly processive on positively supercoiled DNA, but is perfectly distributive on negatively supercoiled DNA. Surprisingly, the processivity of hTopo IIα is nearly equal on positive and negative DNA. For this enzyme, our measurements suggest that chiral discrimination results from differences in the forwards rate of the enzyme turnover rather than in differences in processivity. These data, together with twist dependent relaxation rate measurements, allow a detailed comparison with the mechanism of chiral discrimination by *E. coli* Topo IV, and suggest possible mechanisms of chiral discrimination by type II topoisomerases.