ENZYMATIC SYMMETRY BREAKING: HOW DOES TOPOISOMERASE IV DISTINGUISH LEFT FROM RIGHT?

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ABSTRACT
Topoisomerases are essential enzymes that perform the crucial task of maintaining DNA topology and unlinking catenated or knotted DNA. Topoisomerase IV (Topo IV) is a bacterial topoisomerase that transports one segment of DNA through a transient double-strand break in a second segment of DNA. In contrast with topoisomerases from higher organisms, Topo IV demonstrates remarkable selectivity in its strand passage activity. The crossings formed in over-wound DNA (left-handed supercoils) are relaxed faster by Topo IV than those formed in under-wound DNA (right-handed supercoils). How can an enzyme that acts locally distinguish the global topological state of DNA? What mechanisms underlie the asymmetric relaxation of left- versus right-handed supercoils by Topo IV? To address these questions, we developed a magnetic-tweezers based single-DNA crossing unlinking assay. This assay allows us to measure, in real time, individual strand passage events by Topo IV. In conjunction with Monte Carlo simulations of individual DNA crossings, these measurements permit determination of the preferred DNA crossing angle for Topo IV. Surprisingly, the preferred crossing angle is significantly larger than previous estimates and cannot account for the difference in relaxation rates of left- and right-handed supercoiled DNA. Rather, our results demonstrate that symmetry breaking by Topo IV is achieved through an unusual processivity mechanism.