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A kinetic clutch governs uncoiling by type IB topoisomerases

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Type IB topoisomerases (Top1B) are essential enzymes that relax excessive DNA supercoiling associated with replication and transcription and are important drug targets for cancer chemotherapy. The natural compound camptothecin (CPT) and the cancer chemotherapeutics derived from it, irinotecan and topotecan, are highly specific inhibitors of human nuclear Type IB topoisomerase (nTop1). We employed a magnetic-tweezers based single-molecule DNA supercoil relaxation assay to measure the torque dependence of human nuclear Top1 relaxation (nTop1) and inhibition by CPT. For comparison, we examined the human mitochondrial (Top1mt) topoisomerase and an N-terminal deletion mutant of nTop1 (Top68). Despite substantial sequence homology in their core domains, nTop1 and Top1mt exhibit dramatic differences in sensitivity to torque and CPT, with Top68 betraying intermediate characteristics. In particular, nTop1 displays nearly torque-independent religation probability, distinguishing it from other Top1B enzymes studied to date. Kinetic modeling reveals a hitherto unobserved torque-independent transition linking the DNA rotation and religation phases of the enzymatic cycle. The parameters of this transition determine the torque sensitivity of religation, and the efficiency of CPT binding. This "kinetic clutch" mechanism explains the molecular basis of CPT sensitivity and more generally provides a framework with which to interpret Top1B activity and inhibition.