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An in vivo degradation system to study cross-talk between topoisomerases in E. coli

During the E. coli cell cycle, DNA is exposed to coiling variations induced by biological processes such as replication or transcription. Four topoisomerases contribute to the maintenance of DNA homeostasis (TopoI, Gyrase, TopoIII and TopoIV). Among them, TopoI which function appeared to be mostly linked to the relaxation of negative supercoils induced by transcription and TopoIV that promotes replication and segregation by removing positive supercoils and catenanes might be functionally linked. For instance, TopoI mutants are suppressed by duplication of the Topo IV genes. Until now, the study of functional interactions between these topoisomerases has been limited by the difficulty to combine thermosensitive mutants of TopoIV and TopoI mutants that contain different suppressor mutations. To investigate this cross-talk, an inducible degradation system was used that allow rapid depletion of TopoI, TopoIV or both and to monitor cell physiology and DNA topology at low or null TopoI and TopoIV concentration .

Topoisomerase degradation relies on the induction of an exogenous Lon protease that specifically targets the protein(s) of interest fused to a degradation tag. First of all, I validated the degradation system for TopoI and TopoIV; it enabled a drastic reduction of each topoisomerase in 2 hours. Second, I measured consequences of TopoI depletion: although, it rapidly induces over-supercoiling of plasmid DNA, it did not imply growth nor viability defects of the bacteria. Surprisingly, the amount of other topoisomerases (Gyrase and TopoIV) was unchanged and TopoIII was not required for viability. These results suggest that over a short period of time, E. coli can fully tolerate a low amount of TopoI and the associated topological changes. In contrast, degradation of ParE, one of the TopoIV subunits, reduced growth rate and cell viability and induced a well- characterized par phenotype with filamentous and anucleate cells. Surprisingly, when TopoI and ParE were depleted simultaneously, the growth defect associated with ParE degradation is suppressed. These results highlight a novel antagonistic effect between TopoI and TopoIV and further support the utilization of this system to enhance our comprehension of the interaction between topoisomerases.

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Auteur principal: BORDE, Céline (CIRB CDF)

Co-auteurs: BRUNO, Lisa (Collège de France); ESPELI, Olivier (CGM CNRS)

Orateur: BORDE, Céline (CIRB CDF)

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