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Replicon dynamics during the cell cycle in Escherichia coli

During bacterial cell cycle, the replication of the chromosome and plasmids is followed by the segregation of each copy in daughter cells. As replication and segregation must be finely coordinated with cell division many proteins are recruited. Notably the MatP protein, and its DNA binding site matS, which participates in the positioning of the ter regions of sister chromatids at midcell; and the recombinases XerC and XerD which separate dimers by site-specific recombination at the dif site. Nevertheless, the entire mechanism that drive this coordination is not fully understood. It is known that a lot of proteins involved can interact with the Topoisomerase IV (TopoIV), which has a key role in the regulation through its catenane and pre-catenane resolution activity. Catenanes are interlinked DNA molecules, and theses links must be removed to allow their segregation. To better understand the decatenation activity in vivo, I use the model bacterium Escherichia coli with temperature sensitive mutants of TopoIV. The activity is investigated by analyzing catenated plasmids accumulation at non-permissive temperature, as well as their resolution by recovering TopoIV.
Experiments are carried out with a small (2.7kb) and high copy number plasmid, pUC18; its derivative where a dif site has been added, TopoIV activity is strong at this site [4]. To study the effect of subcellular localization on the activity of TopoIV, a matS site has been added on theses plasmids. My results suggest that subcellular position at midcell by matS, and dif sites on the plasmid can improve decatenation, but also that the combination of matS and dif have a synergic effect on TopoIV activity.

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