Introduction

Now I work on two projects:

- The first one is aimed to study genomic distribution of TopoI and TopoIV in *E.coli*. Further it will be referred to as **Topo-Seq**.
- The second project is going to be called **SMC-ParABS**.

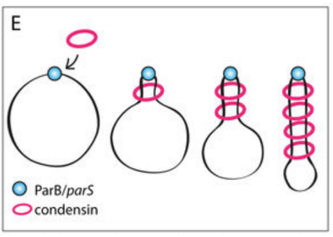
SMC-ParABS

Here are a few words if someone asks me what I do in Skoltech with bacteria:

> *The way you pass things on from generation to generation whether you are a bacterium, an oak tree, or a human cell is continuity of cell dividing and producing two new cells. It is a complete mystery how the sister DNAs are disjoined from each other. How do you ensure that the one goes to the left, one goes to the right, then cell divides down the middle. There is a huge mess of DNA mass inside a cell, that replicates, then separates, and still is doing well. The major organizers of the process are topoisomerases and SMC proteins. SMC complexes are ring-shaped molecules that entrap the DNA inside. SMC is thought to assemble the sisters into distinct structures by moving across the DNA via loop extrusion. This feature of SMC dynamics is shared by many organisms including bacteria. Due to their simplicity, bacteria give us a great opportunity to get towards the understanding of how things work.*

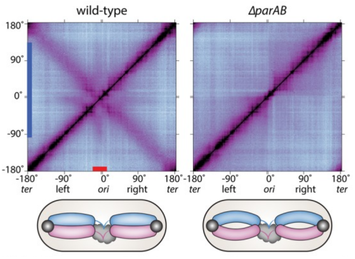
Loop extrusion by SMC loaded on ParB-*parS* sites

In *Bacillus subtilis* and *Caulbacter crescentus*, SMC is loaded by ParB proteins at parS sites, where it encircles DNA and starts to form a loop. This process is called **loop extrusion**.

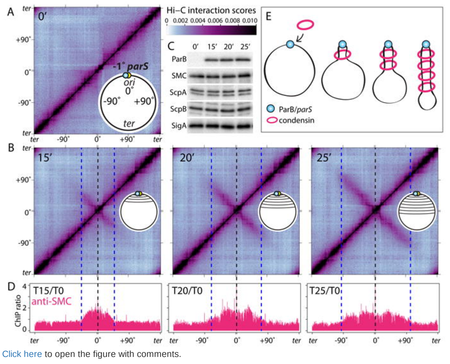


SMC complex in mediates the interaction between chromosome arms by loop extrusion

Loop formation draws contiguous DNA in on itself and away from noncontiguous DNA. This feature is called **arm alignment** or **replichore juxtaposition**. Arm alignment is directly visible on the following Hi-C contact map as a distinctive secondary diagonal (perpendicular to the main diagonal).



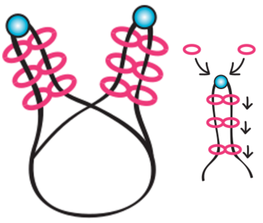
Inducing ParB synthesis in both *B. subtilis* and *C. crescentus* leads to progressive arm alignment from *ori* to distal parts. This wonderful experiment [look at the figure below] is the first-ever experimental proof of the loop extrusion in action. And yes, bacterial people were the pioneers of this fundamental mechanism [at least in the context of Hi-C]. The following figure should be included in classical molbio textbooks.



Click here to open the figure with comments.

Linking loop extrusion to segregation

Loaded by ParB, SMC translocates from parS to distal parts of the chromosome [how far is unclear]. This action is thought to individualize the origins of replicated chromosomes, thereby helping to segregate replicated chromosomes.



This is how the sisters segregate.
SMC is drawn as a handcuff, although nobody knows whether it's true or not

ParABS and SMC. Evolution. *E. coli* phenomenon

ParB works closely with and likely coevolves with SMC to ensure chromosome organization and chromosome segregation in bacteria. In *B. subtilis*, SMC complexes are required to segregated newly replicated origins. In contrast, *Caulobacter* SMC is not required for survival. Conversely, ParABS (ParA-ParB-parS) is essential in *Caulobacter*, but not in *B. subtilis*.

With the exception of *Escherichia coli*, all other bacteria for which we have detailed information about how their genomes are organized, contain the ParABS system. This is accompanied by the fact that the cross-diagonal, observed *B. subtilis* and *C. crescentus* is absent in *E. coli*. There is a distant SMC homolog, called the MukBEF, in *E. coli*. Notably, ParABS systems do not exist in this bacterium, leaving open the question of how MukBEF complex loads on the chromosome anf how the segregation process works.

What to do

We want to introduce one by one ParA-ParB-parS and the canonical SMC in *E. coli* and see if the cross diagonal appears on Hi-C map. It sounds like fun. Actually it is. However, we may address the issues of segregation physiology by knocking out the MukBEF. Then see whether there were the differences between ParB-parS and ParA-ParB-parS strain. Evolution of ParABS-SMC in β^3-proteobacteria [and in Archaea] is of interest too.

Topo-Seq

What is done

- We have derived an *imp*-mutant DY330 strain of *E. coli*. *Imp* encodes a component of bacterial cell wall. Deletion of 23 aminoacid at 330-352 position of imp protein is required for increased membrane permeability of anzaic acid, a TopoI poison.
- We have extracted TopoI protein for *in vitro* supercoling experiments with the anzaic acid.

Week 0

What had been done during the last week

- A literature review of SMC-ParABS system [and now is on progress]
- I've found candidate ParA and ParB for introduction into *E. coi* by BLASTp search. Both ParA and ParB belong to *Vibrio cholerae*. This bacterium, just like as *B. subtilis* and *C. crescentus*, has got a pronounced cross-diagonal on Hi-C contact map.
- We are going to use ciprofloxacin for TopoIV-Seq experiments. However it also binds GyrA subunit of gyrase. Crosslinking of gyrase to DNA can serve as a sourse of bias in TopoIV-Seq. Single amino acid substitution (S83L) in the quinolone-resistance determining region (QRDR) of *gyrA* gene makes gyrase resistant for ciprofloxacin-mediated DNA crosslinking. For this, we applied oligonucleotide recombeneering approach. However, colonies has grown after transformation have resembled *Streptococci*. Ironically, the PCR results were positive [with *E. coli* primers]. Next week we send them for sequencing.