Multiscale Structuring of the *E. coli* Chromosome by Nucleoid-Associated and Condensin Proteins

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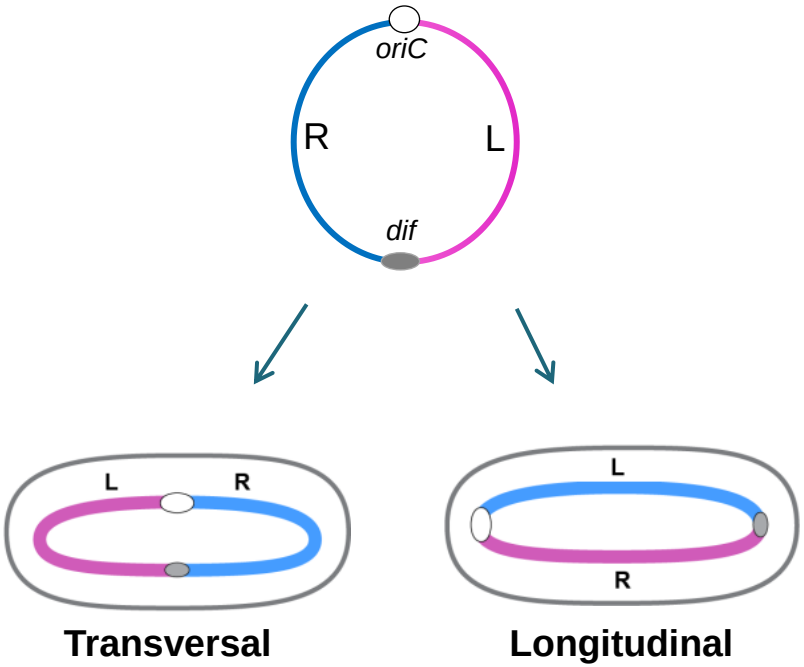
10th April 2018

Chromosome organization in bacteria



- One origin of replication *oriC*
- One *dif* site

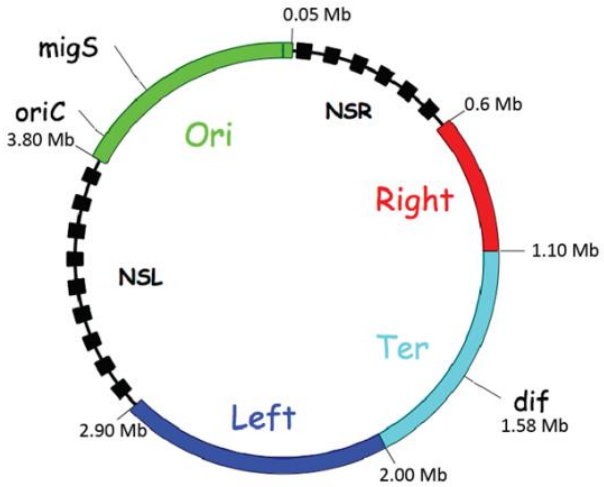
➡ Different chromosome disposition inside the cell

- Segregation and replication occurs concomitantly
- Coordination of cell division with chromosome segregation

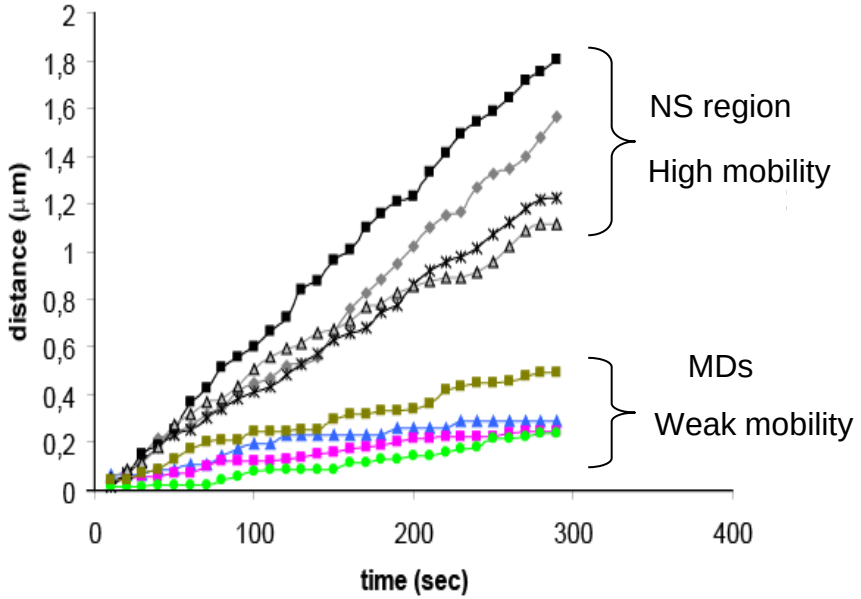
➡ Different chromosome choreography during the cell cycle

Chromosome organization in *E. coli*

- Genetic analysis: Site-specific recombination to probe DNA collisions



- Chromosome dynamics: FROS analysis

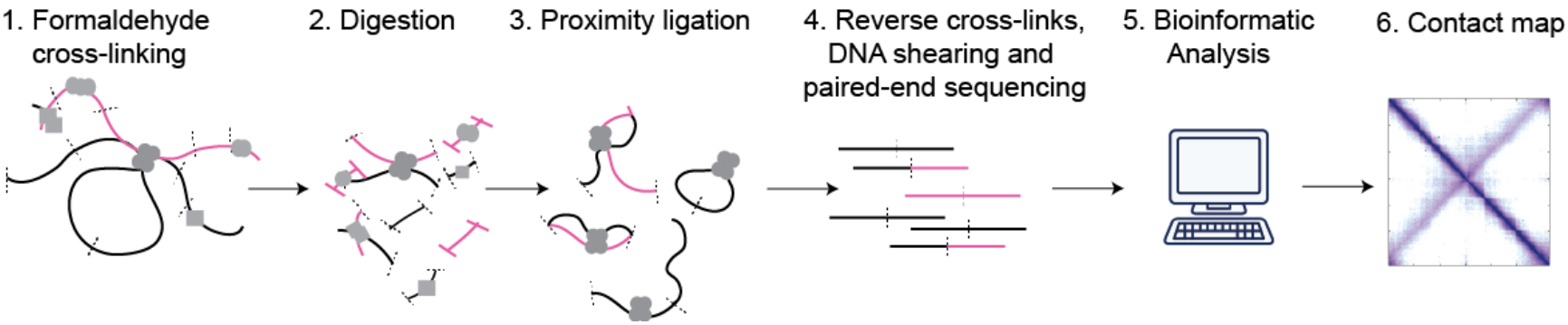


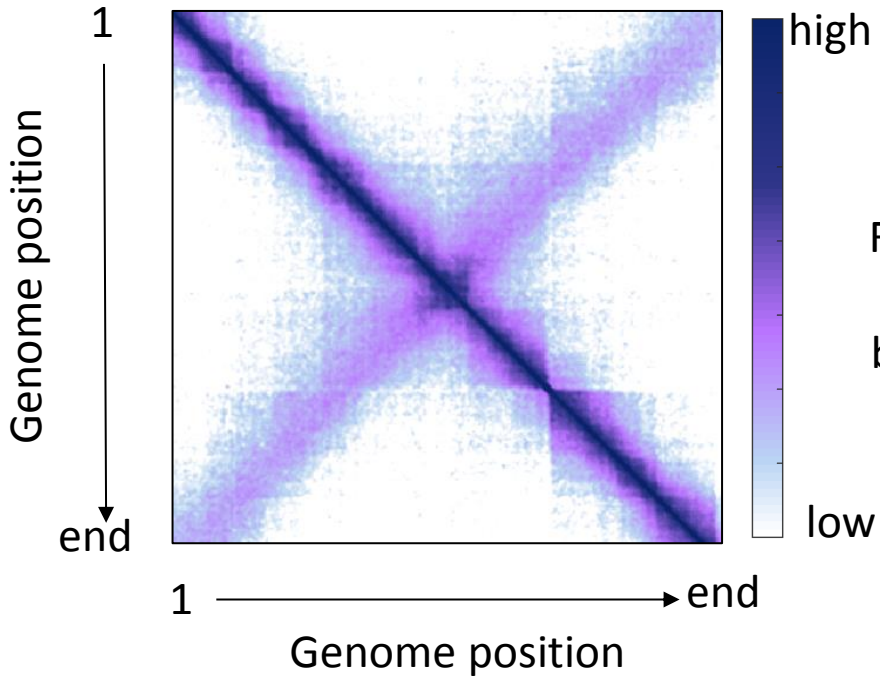
➡ **MD and NSR regions**

➡ **DNA dynamics correlate with MD topography**

Valens et al, 2004
Espéli et al, 2008

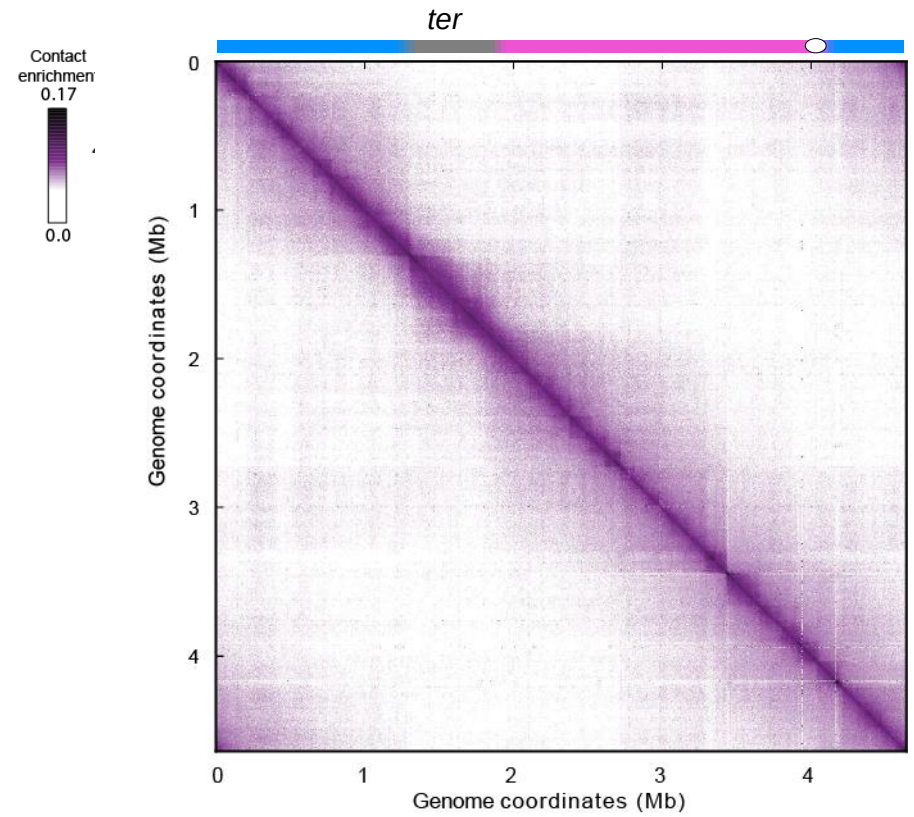
Chromosome conformation capture (3C-seq)





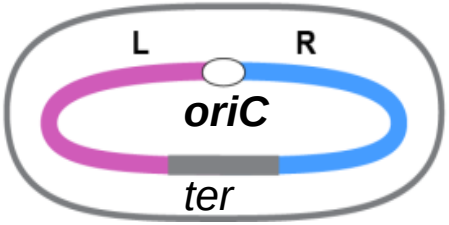
In collaboration with R. Koszul's lab at the Pasteur Institute

A high resolution contact map of the *E. coli* chromosome

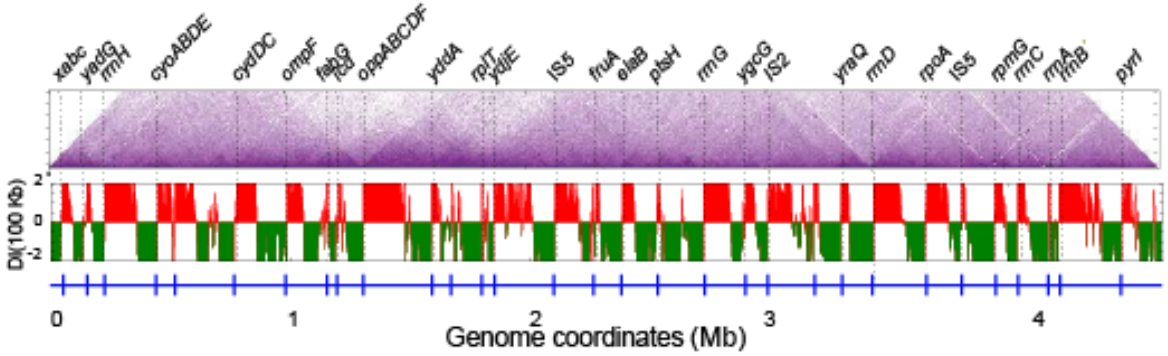


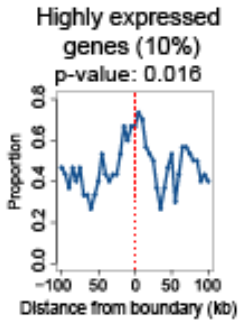
One main diagonal:

- ✓ TRANSVERSAL Disposition

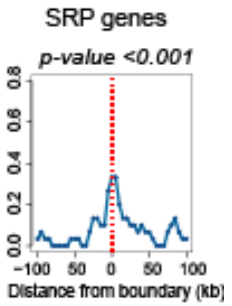


- ✓ 31 CIDs (size: from 40 to ~300 kb)



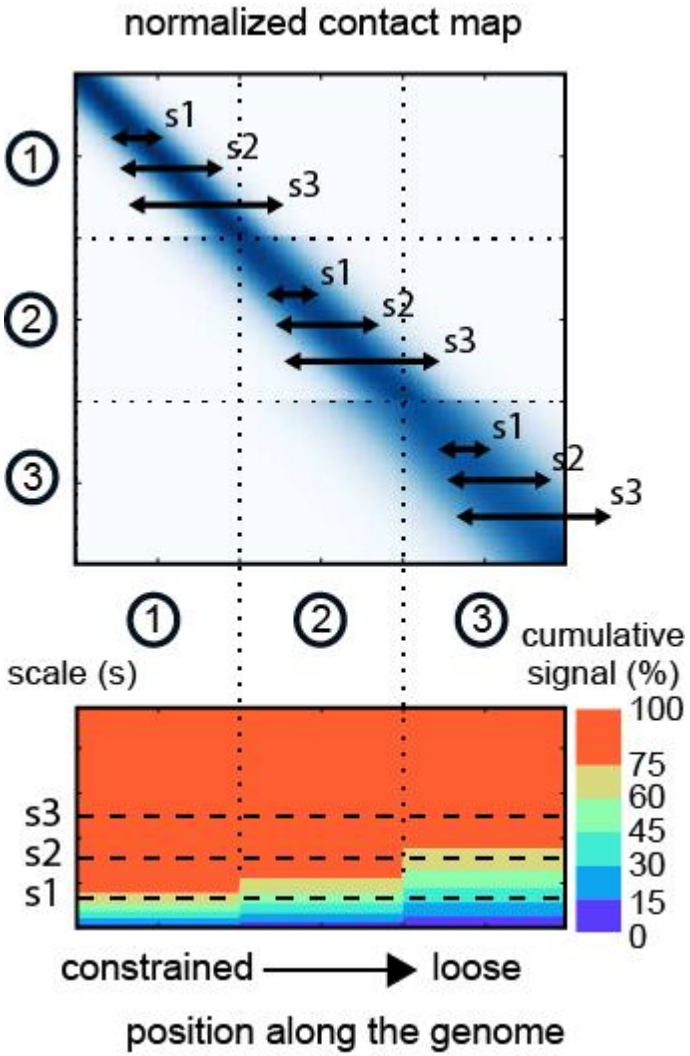


(22/31)



(9/31)

Scalogram: a new tool to analyze 3C contact frequencies

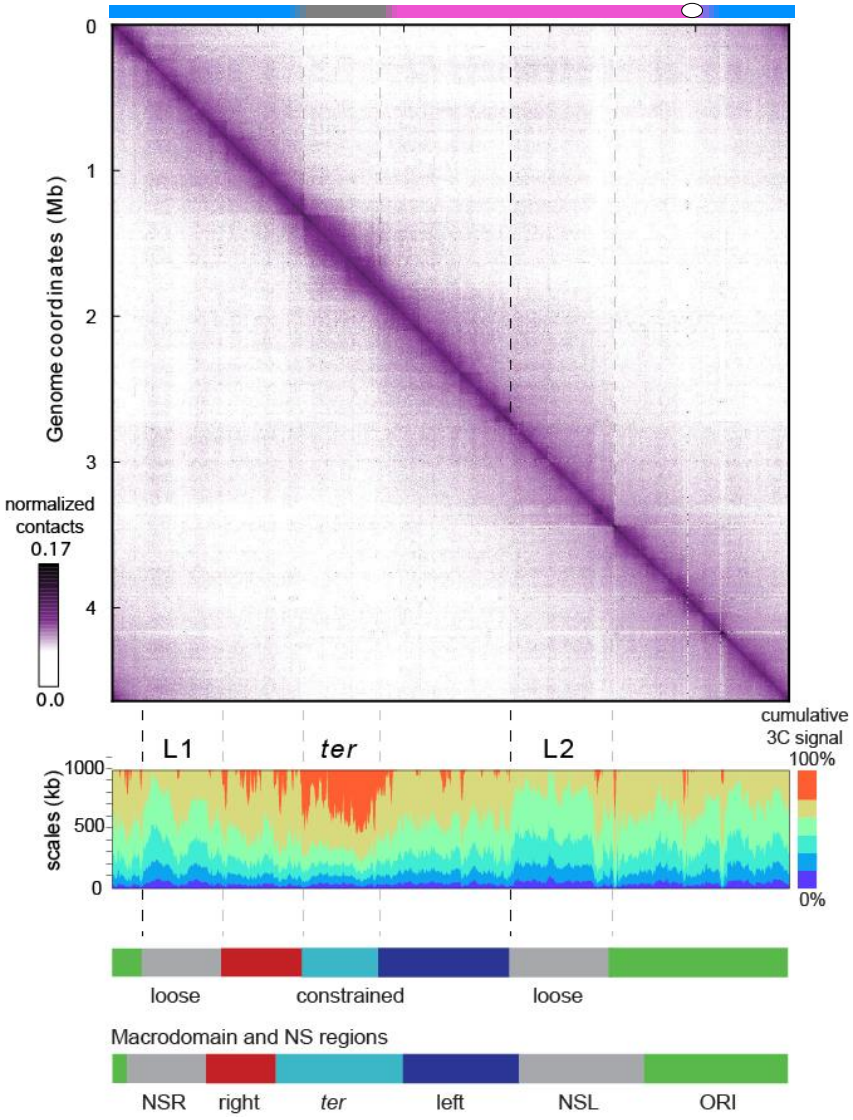


- Using windows of various sizes, the normalized contacts are sum
- This sum represents the fraction of total contacts made by a bin within a window size

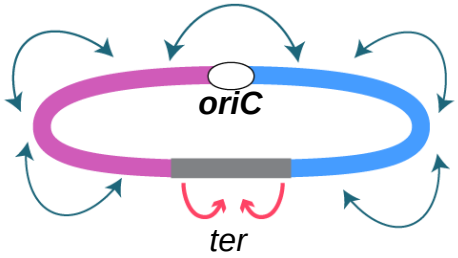
Reveals to which extent a genomic region can "see" their flanking sequences

Lioy and Cournac et al, 2018

A high resolution contact map of the *E. coli* chromosome



- ✓ *Scalogram* reveals 2 modes of DNA contacts:
  - in the Mb range outside ***ter*** / constrained contacts around ***ter***

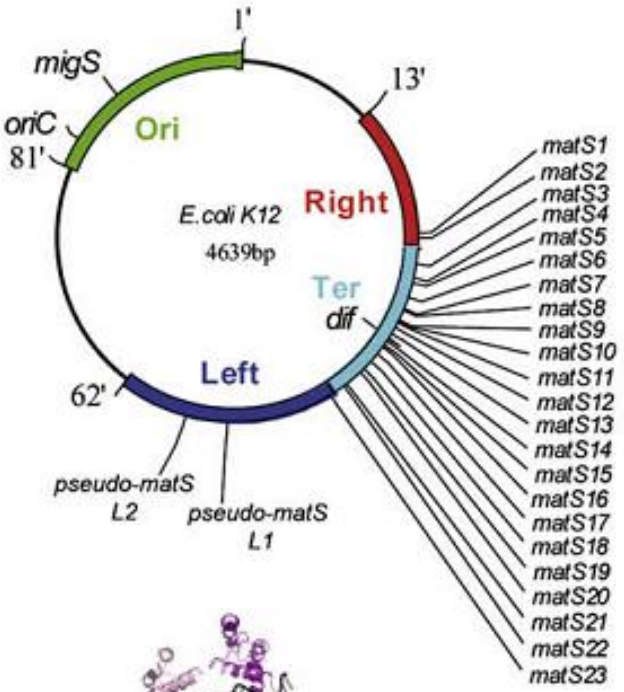


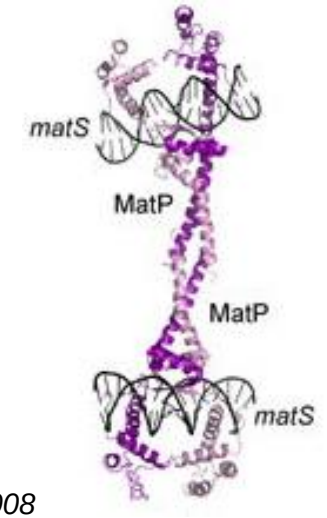
- ✓ Good correlation with MD and NSR regions

Lioy and Cournac et al, 2018

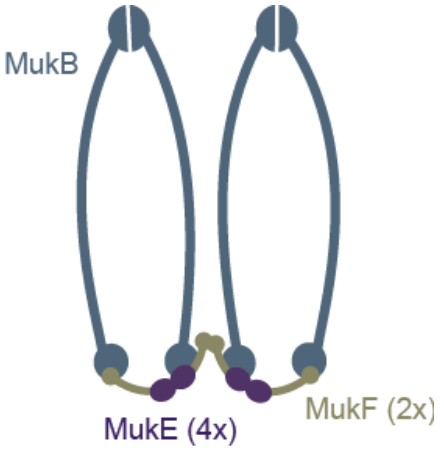
Factors involved in chromosome organization in *E. coli*

- Specific organizers (**MatP/matS**) / Ter MD

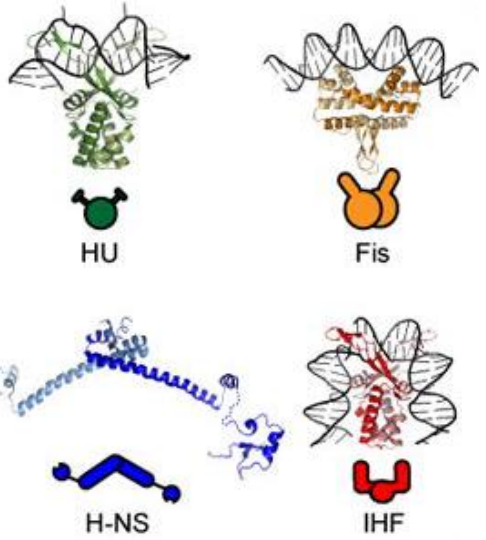




- SMC complex: **MukBEF**

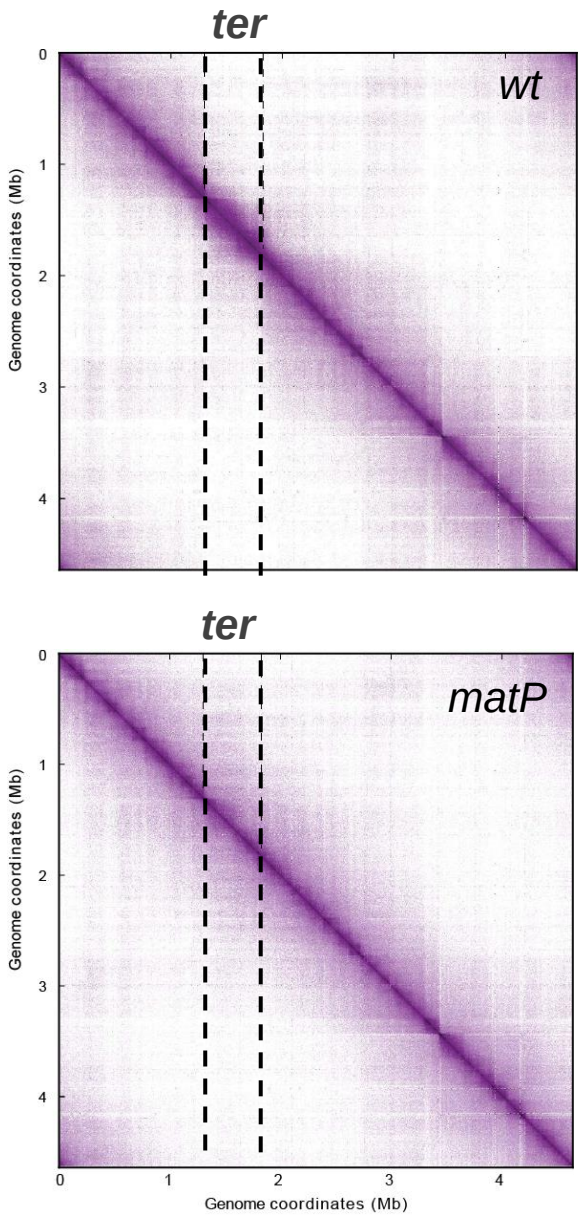


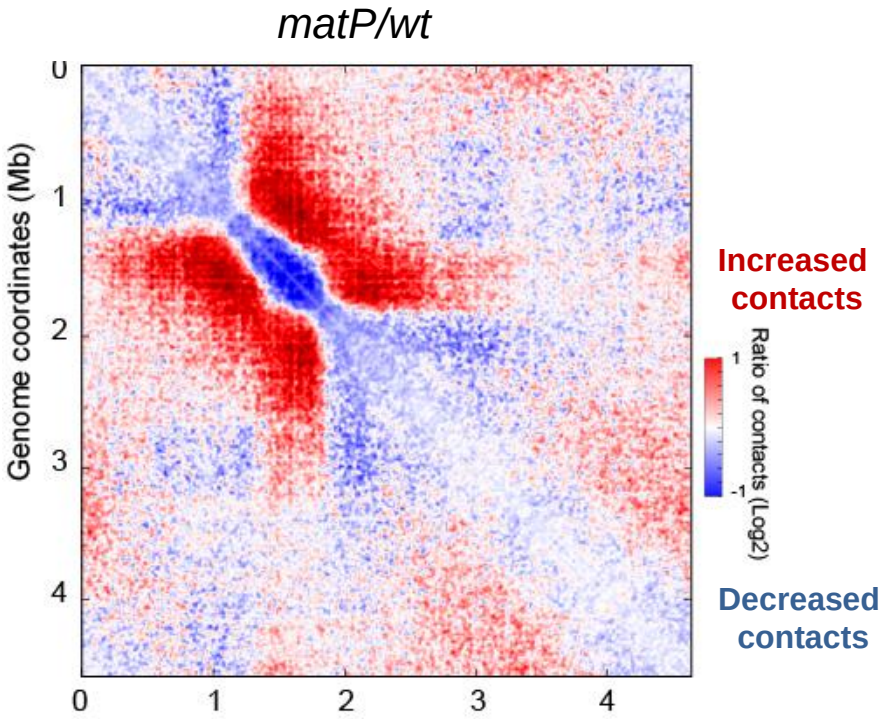
- Nucleoid associated proteins (NAPs)



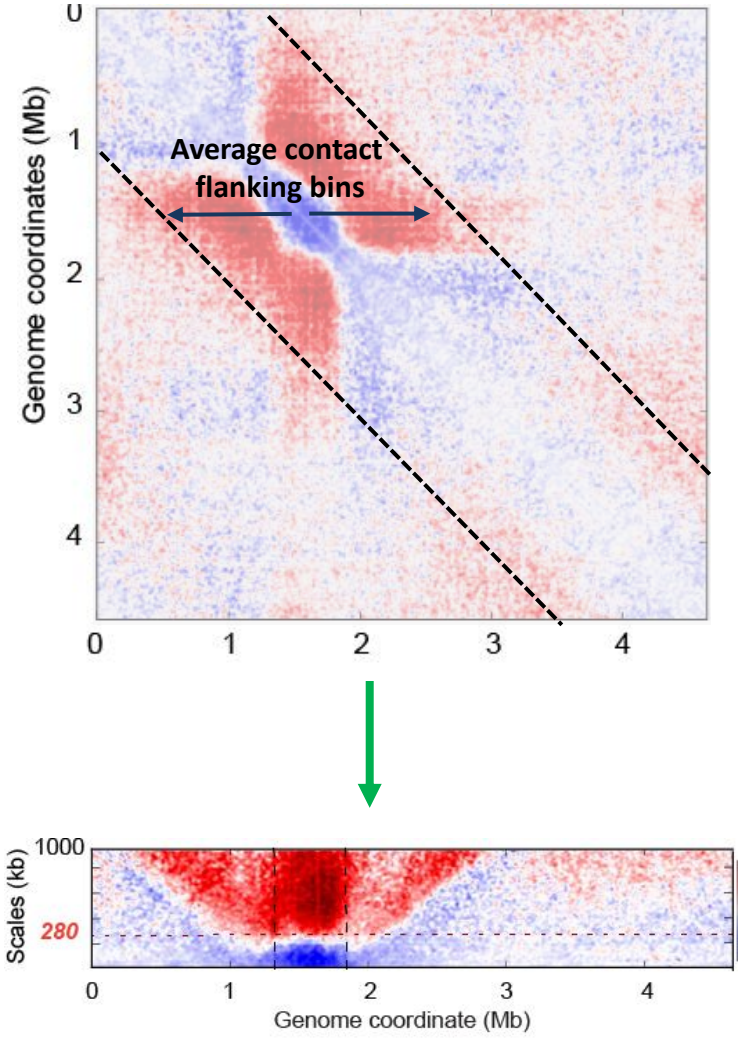
Mercier et al, 2008
Dupaigne et al, 2012
Badrinarayanan et al, 2015

Organization of the *ter* region in the absence of MatP





Ratio-plot: a new tool to compare contact maps

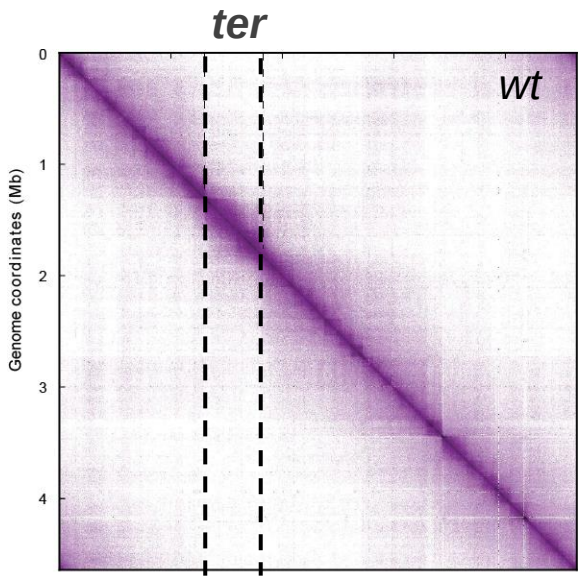


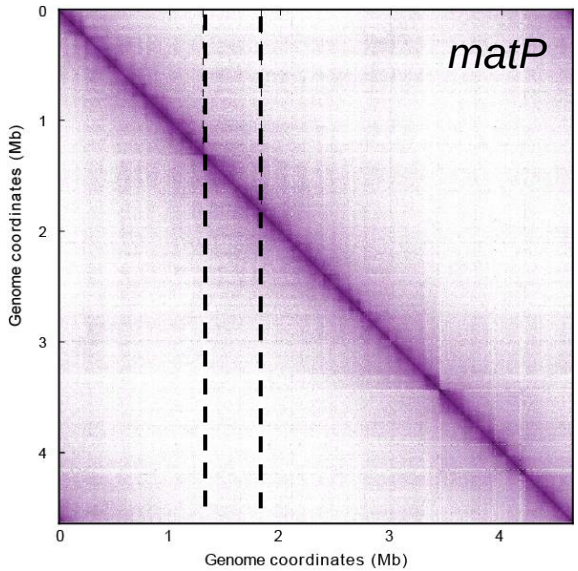
- Ratio-plots represent the **average contacts** made by a bin along the genome with its flanking bins at increasing distances (from 5kb to 1000kb)

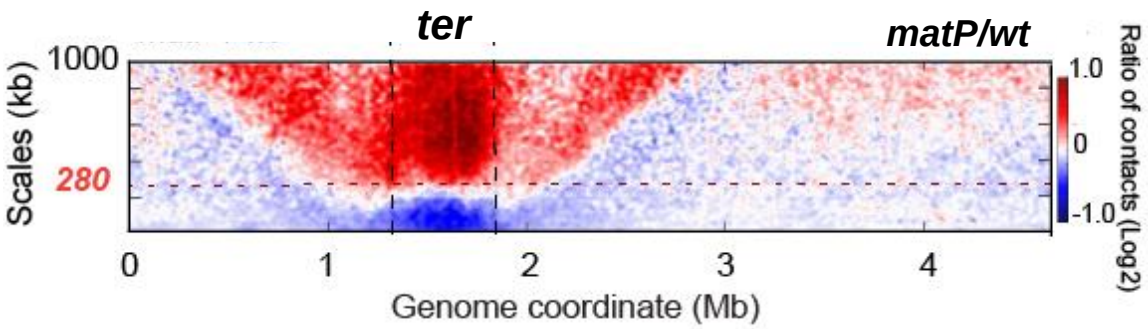
2D → 1D

Shows the ability of a loci to interact with its flanking sequences along the entire genome

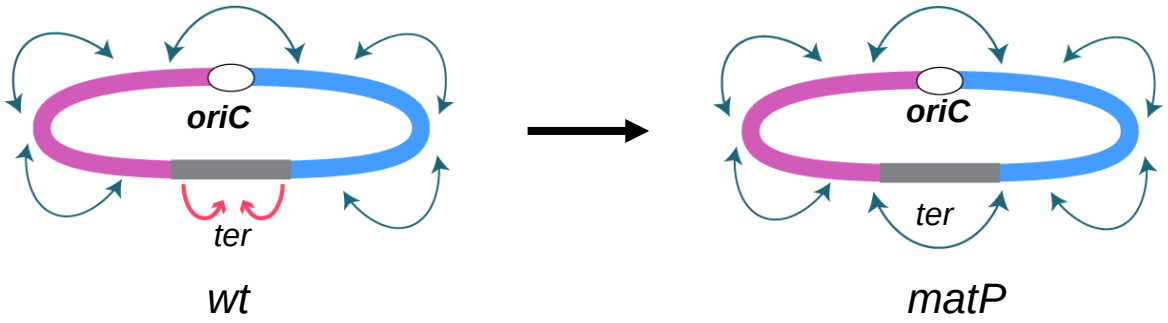
Organization of *ter* and a role for MatP in *ter* insulation





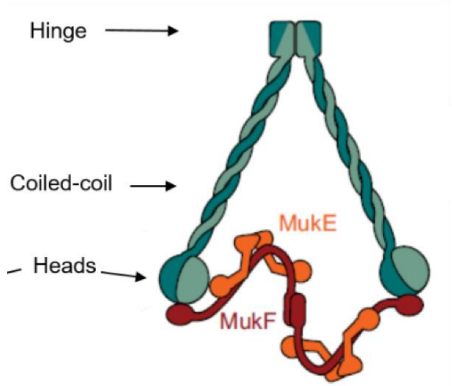


- ✓ enrichment of long-range contacts within ***ter*** and its flanking domains in the absence of MatP (>~280 kb).
  - ➢ Confirmed by Genetics
- ✓ ***ter*** now appears similar to the rest of the genome

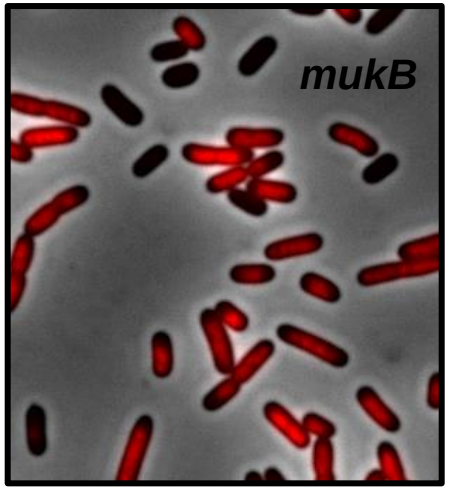


MukBEF complex

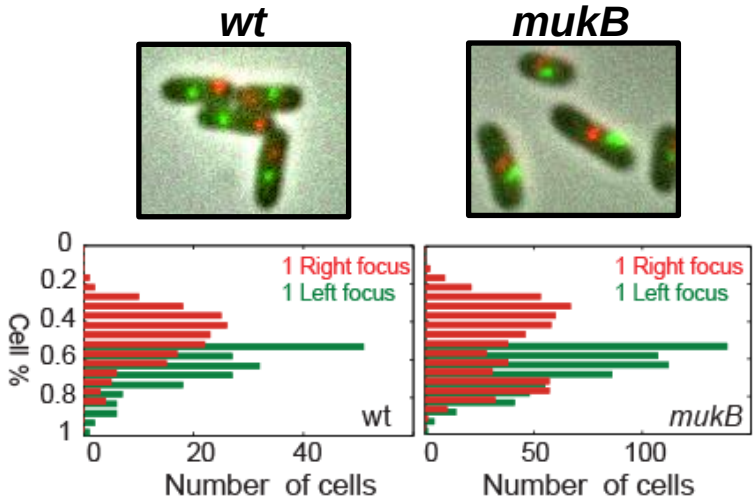
- **MukBEF :**
  - Distantly related in sequence to SMC-ScpAB
  - Role in chromosome segregation
  - Role in chromosome positioning
  - Interplay with TopoIV and **MatP**

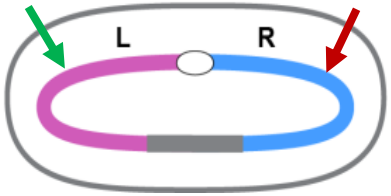


~20 % anuclated cells



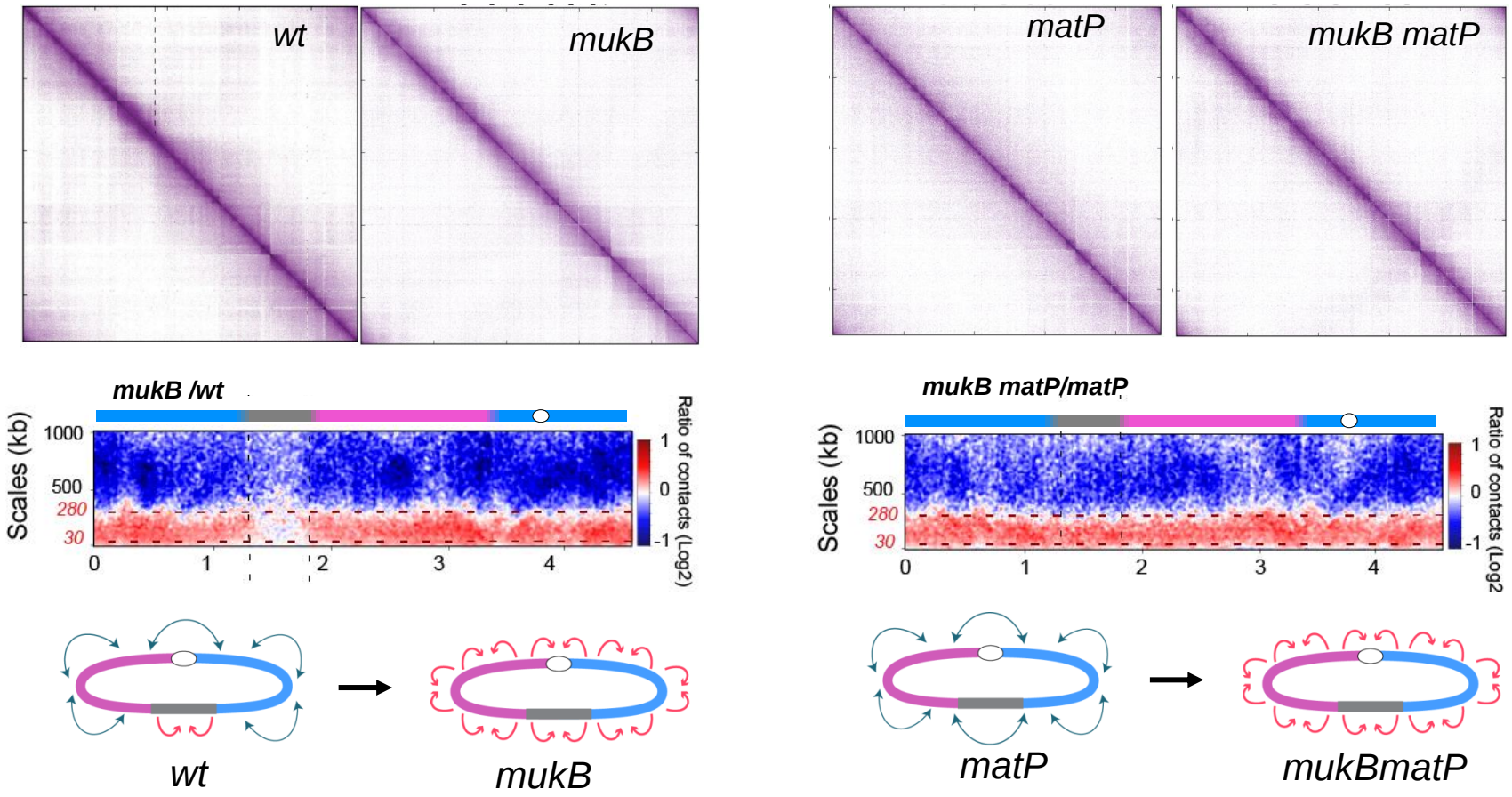
Increased Right locus mispositioning (11% vs 22 %)





Nolivos et al, 2016
Lioy and Cournac et al, 2018

MukBEF is essential to promote long range communications

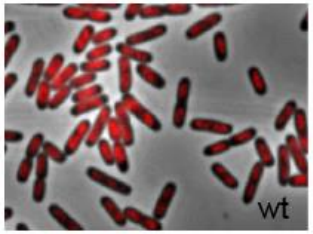


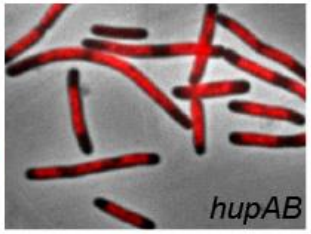
- ✓ **MukBEF** promotes **intra-arm** DNA contacts in the Mb range
- ✓ **MatP** restricts DNA contacts and prevents **MukBEF** activity in ***ter***

3 major NAPs in *E. coli*

HU:

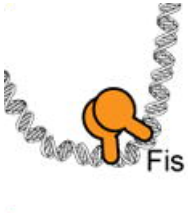
- DNA bending
- Transcriptional factor - low sequence specificity
- Hi-C *C. crescentus*: promote short-range contacts

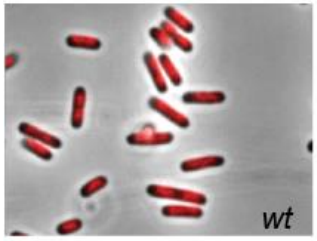


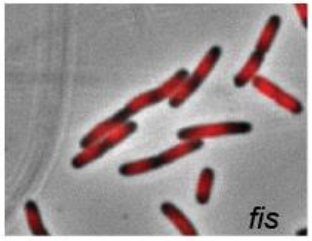


Fis:

- DNA bending
- The most abundant NAP in exponential phase
- Transcriptional factor: binds to AT rich regions

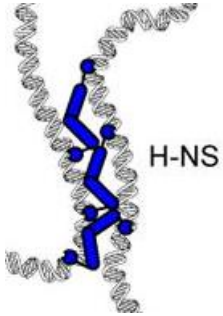


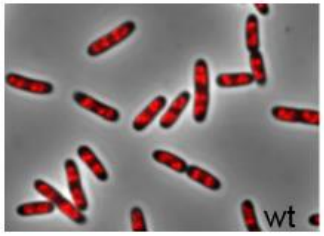


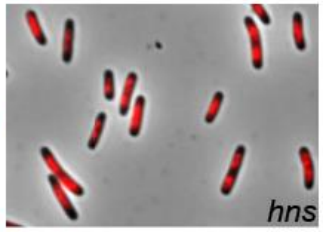


H-NS:

- DNA bridging.
- Oligomerizes along DNA
- Xenogeneic silencer (AT rich regions)



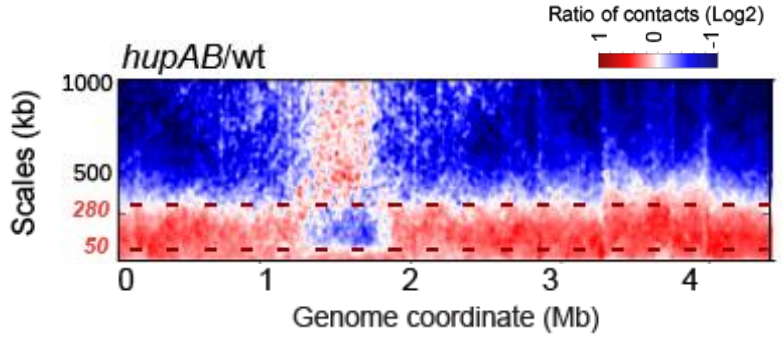




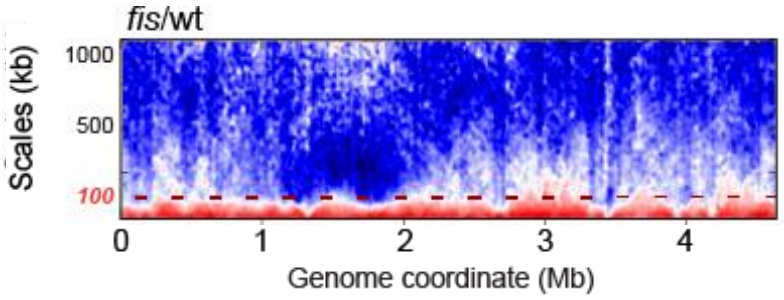
➡ **Exact role in chromosome organization is unkown**

Le T et al, 2013
Badrinarayanan et al, 2015

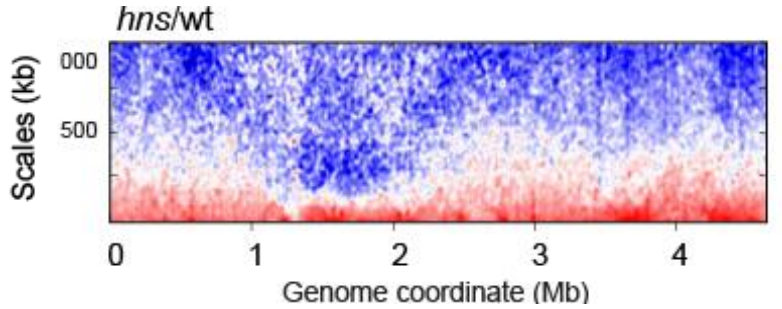
Different activities of 3 major NAPs



- ✓ **HU** promotes **intra-arm** DNA contacts in the Mb range (similar to **MukBEF**)
- ✓ **HU** promotes optimal DNA contacts in ***ter*** (~300 kb)



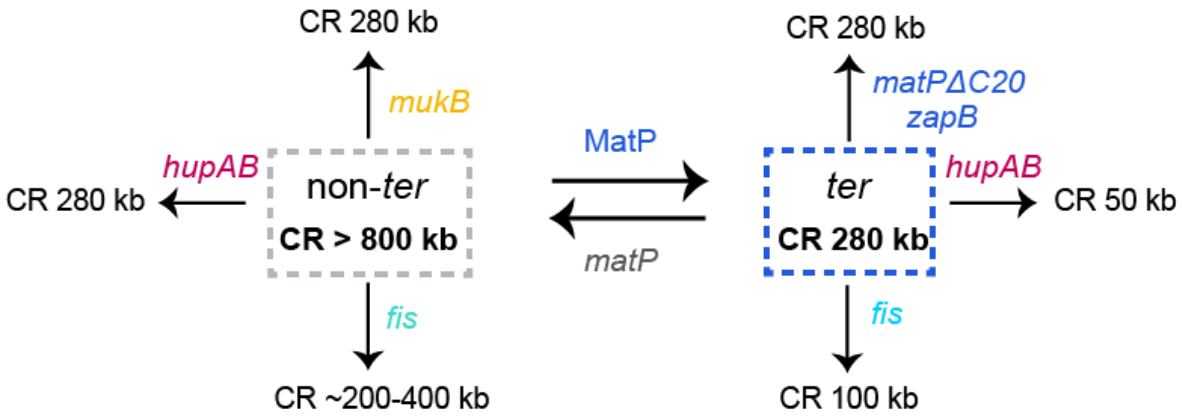
- ✓ **Fis** is required for long-range interactions
  - ➢ Confirmed by Genetics
- ✓ No correlation between DNA binding/increased DNA contacts
- ✓ Variable effect along the genome (ter and non ter)

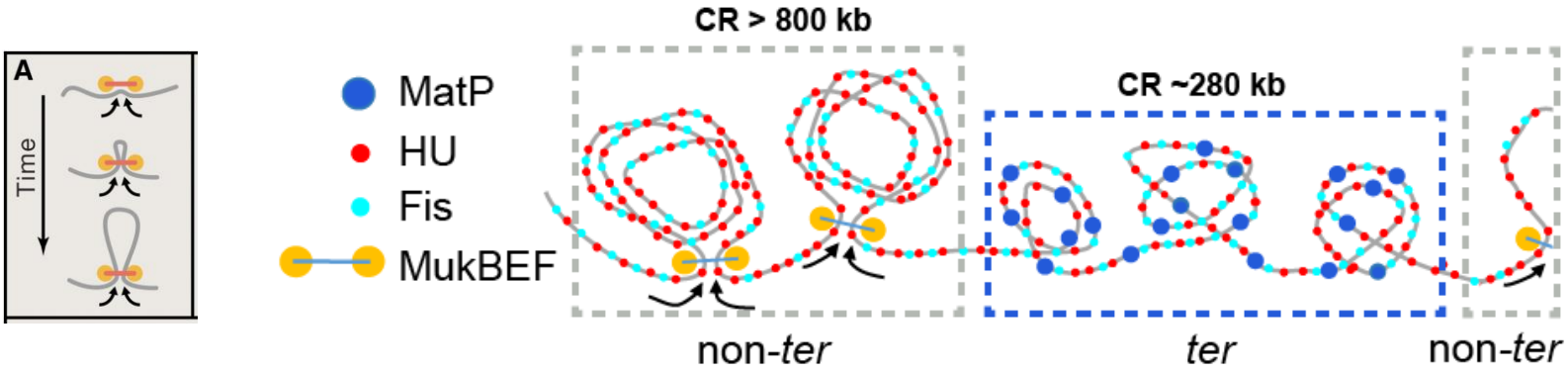


- ✓ **H-NS** slightly effect on long-range contacts
- ✓ Increased short-range contacts in *hns* cells.
- ✓ Correlation with DNA binding sites (~70%)

HU and **Fis**: Long range effect
H-NS : Restricts short range contacts (Gene repressor)

Multiscale organization of the chromosome





Acknowledgments

I2BC

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