Molecular characterization and modeling of a bacterial DNA segregation apparatus

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75% – 25%

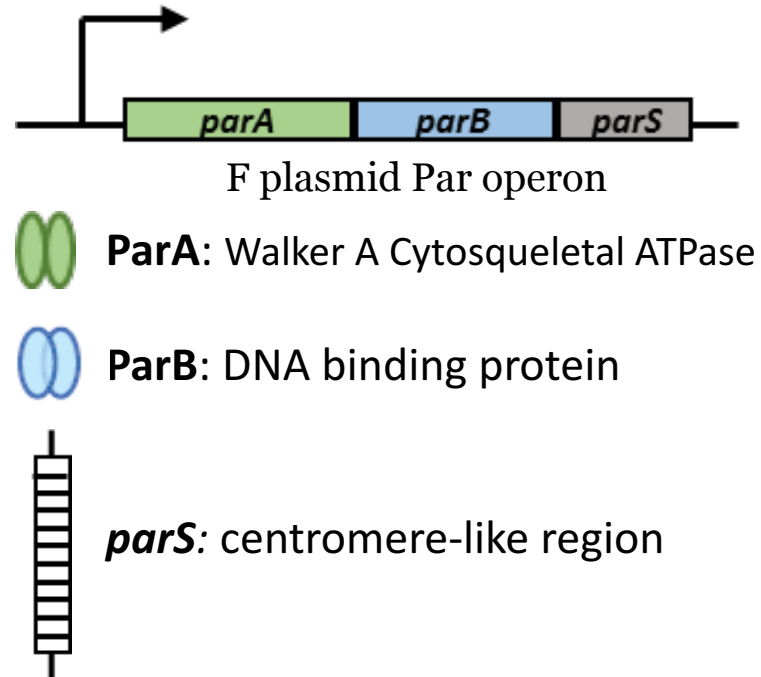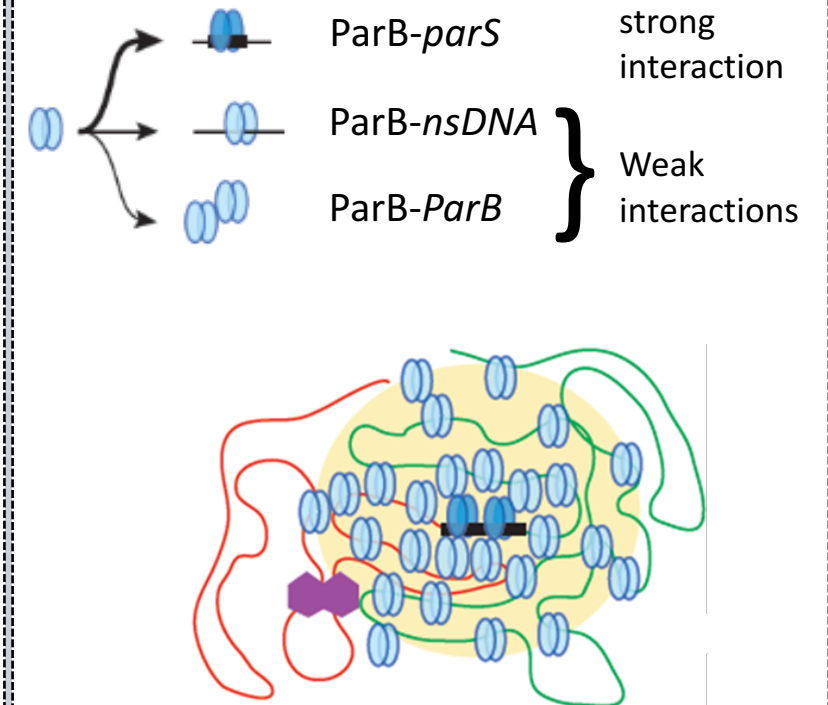# Objectifs scientifiques – exposé du sujet

Bacterial DNA

Partition

- Minimalistic system, Type I ParABS
- Widespread on bacterial chromosomes and plasmids
- Recently discovered in Archaea

The Partition System

F plasmid Par operon

ParA: Walker A Cytosqueletal ATPase

ParB: DNA binding protein

parS: centromere-like region

The Model

ParB-*parS* strong interaction

ParB-*nsDNA* } Weak interactions

ParB-*ParB*

High Resolution Chromatin Immunoprecipitation: ChIP-sequencing

Roxanne E. Diaz[1], Aurore Sanchez[1], Véronique Anton Le Berre[2] and Jean-Yves Bouet[1,*]

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[2]Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés, Université de Toulouse, UPS, INSA, INP, CNRS, F-31077 Toulouse, France.
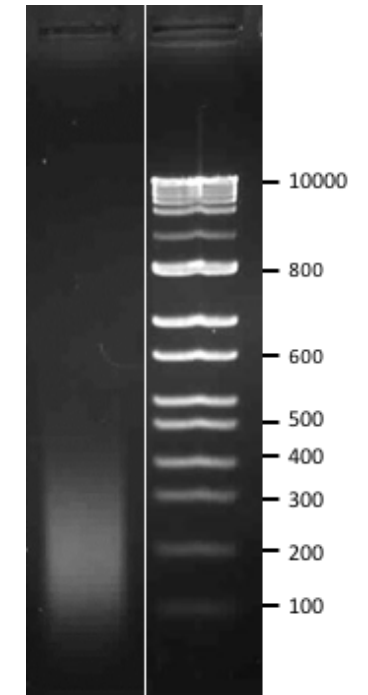
In press

DNA profile of ChIP sample
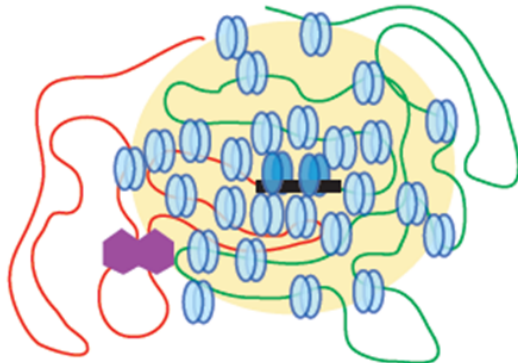
- To study ParB-DNA interactions used High resolution ChIP-sequencing
- Average DNA fragment size ~200bps

Imaging centromere-based incompatibilities: Insights into the mechanism of incompatibility mediated by low-copy number plasmids

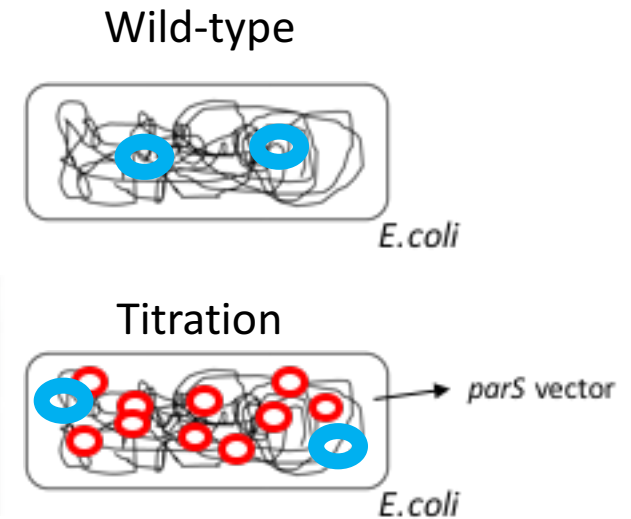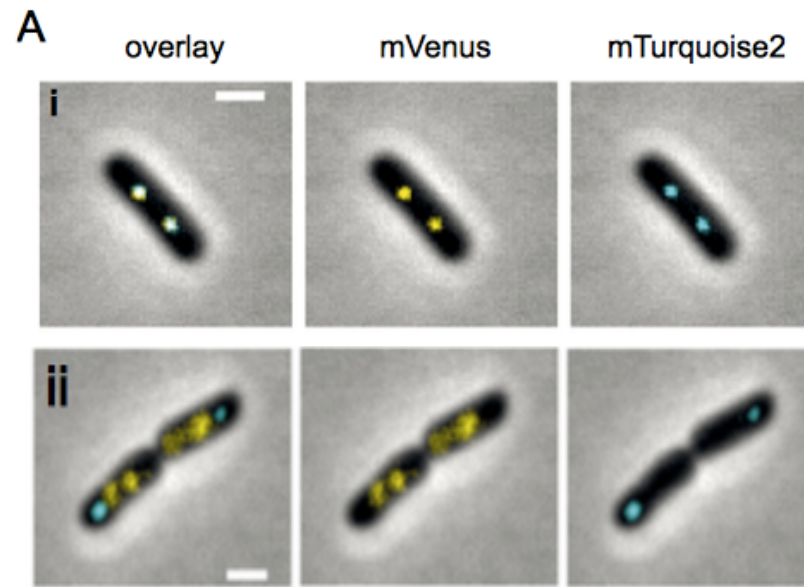Roxanne Diaz [a,b], Jérôme Rech [a,b], Jean-Yves Bouet [a,b,*]

[a] Laboratoire de Microbiologie et Génétique Moléculaires, Centre National pour la Recherche Scientifique, F-31000 Toulouse, France
[b] Laboratoire de Microbiologie et Génétique Moléculaires, Université de Toulouse, UPS, F-31000 Toulouse, France

- Probing partition complex formation
  - Increase/decrease ParB levels
- Dual-labeled F plasmid
- Validation of titration hypothesis
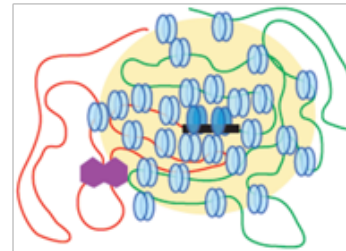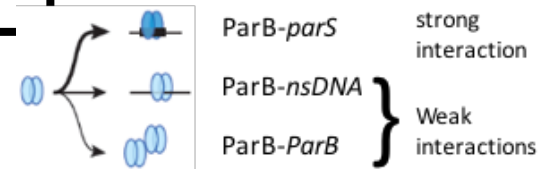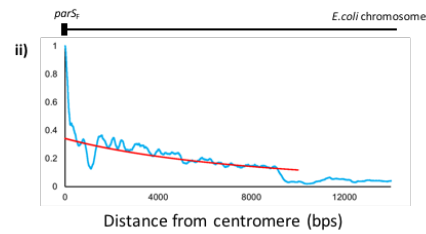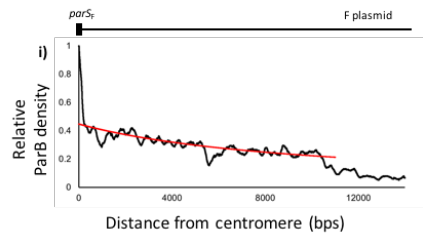 - Titration of ParB using high-copy number vectors containing *parS*
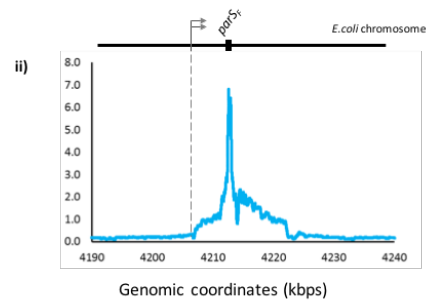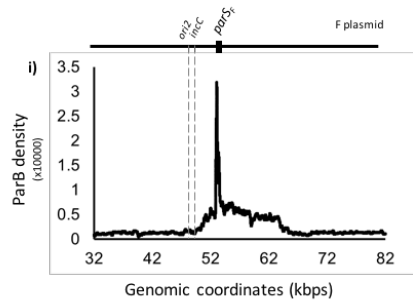
The stochastic self-assembly mechanism of ParB-*parS* partition complexes is conserved and robust on bacterial chromosome and plasmids

Roxanne Diaz[1], Jean-Charles Walter[2], Jérôme Rech[1], Véronique Anton Leberre[3], Andrea Parmeggiani[2] and Jean-Yves Bouet[1,#]

ParB$_F$ propagates similarly on chromosome and plasmid DNA

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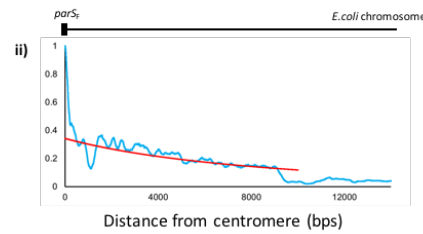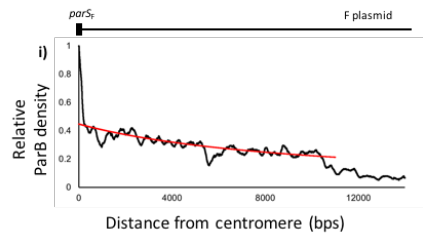**ParB$_F$ propagates similarly on chromosome and plasmid DNA**

ParB-*parS* — strong interaction

ParB-*nsDNA* — Weak interactions

ParB-*ParB* — Weak interactions

ParB$_F$ stochastic propagation on DNA is robust in a wide range of ParB concentration

B)
parS$_F$ *E.coli* chromosome

- 0.5-fold ParB$_F$
- 8-fold ParB$_F$
- 14-fold ParB$_F$
- 40-fold *parS*$_F$ excess
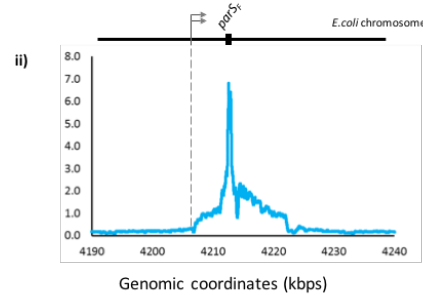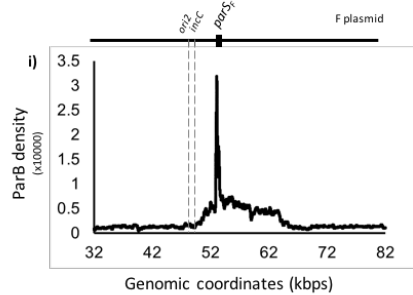- 100-fold *parS*$_F$ excess

The stochastic self-assembly mechanism of ParB-*parS* partition complexes is conserved and robust on bacterial chromosome and plasmids

Roxanne Diaz[1], Jean-Charles Walter[2], Jérôme Rech[1], Véronique Anton Leberre[3], Andrea Parmeggiani[2] and Jean-Yves Bouet[1,#]

ParB_F propagates similarly on chromosome and plasmid DNA

ParB-*parS* — strong interaction
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ParB-*ParB* — Weak interactions

ParB_F stochastic propagation on DNA is robust in a wide range of ParB concentration

- 0.5-fold ParB_F
- 8-fold ParB_F
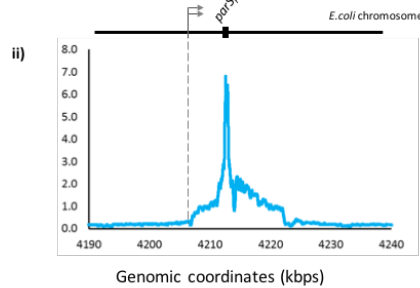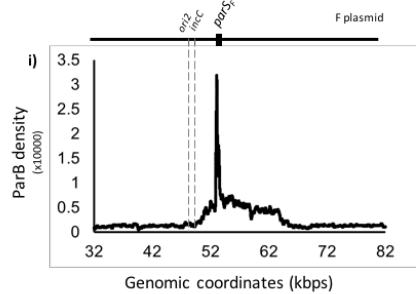- 14-fold ParB_F
- 40-fold *parS*_F excess
- 100-fold *parS*_F excess

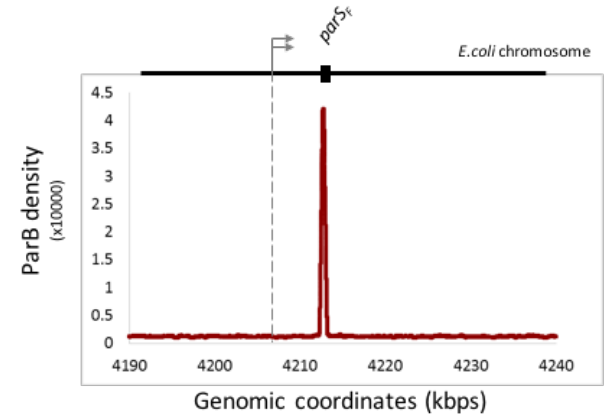**The multimerization interface of ParB is required for its caging**

The stochastic self-assembly mechanism of ParB-*parS* partition complexes is conserved and robust on bacterial chromosome and plasmids

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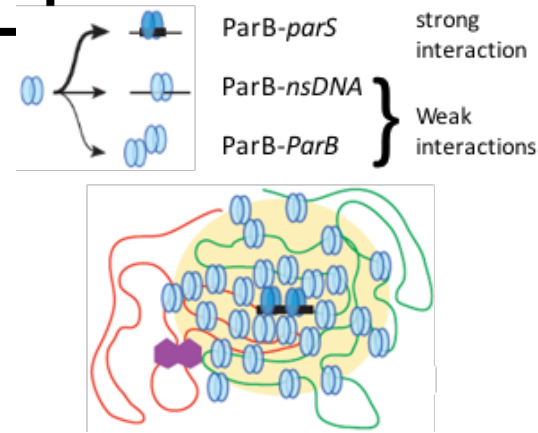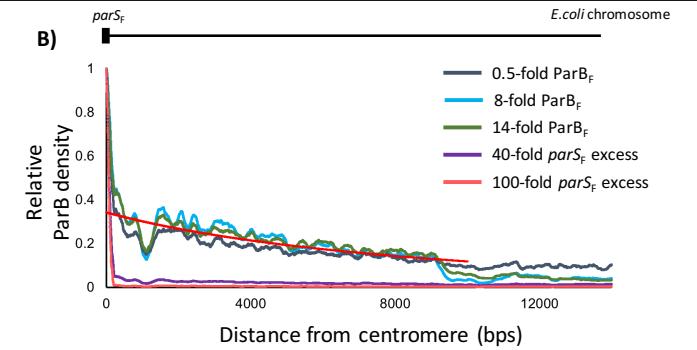
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B)
- 0.5-fold ParB_F
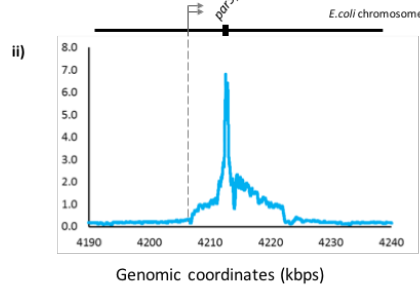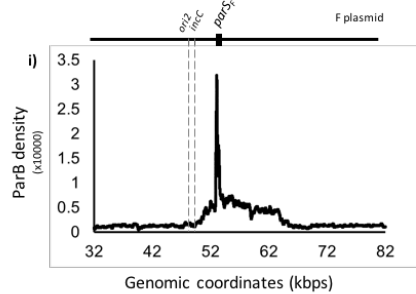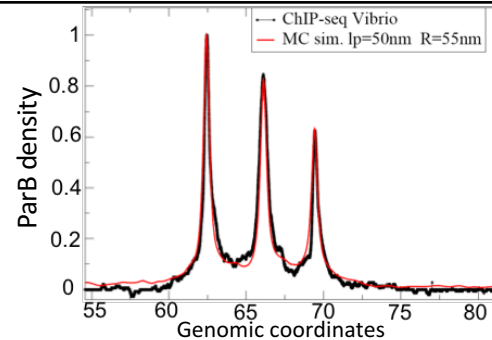- 8-fold ParB_F
- 14-fold ParB_F
- 40-fold *parS*_F excess
- 100-fold *parS*_F excess

The multimerization interface of ParB is required for its caging

Chromosomal ParB also propagates stochastically from *parS* sites

ChIP-seq Vibrio
MC sim. lp=50nm R=55nm
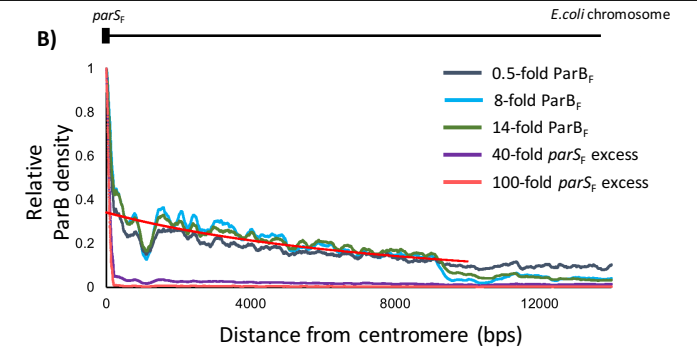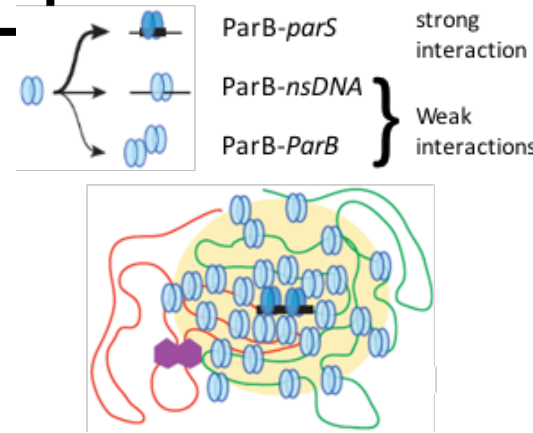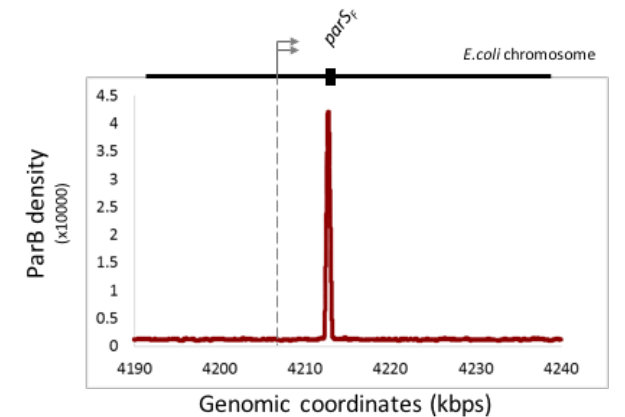
a) Intérêt du caractère interdisciplinaire b) difficultés rencontrées

A)

- Interactions outside of primary lab
  - Able to see how other labs function

- Close contact with bioinformatician
  - Treatment of raw ChIP-seq data
  - Genome reference creation

- Direct interaction with sequencing platform
  - Understanding of library preparation and sequencing process

B)

- Difficulty learning bioinformatic needs for ChIP-seq data
  - In end learned new skills!

Perspectives : a) pour le doctorant   b) pour le projet

A)

- Defending in December 2017
- Looking for a post-doc!

B)

- Use ChIA-PET to determine partition complex interactions with nucleoid
- Determine role of DNA superhelicity on partition complex formation

Retour d'expérience des deux encadrants

- APR2014 = 2nd collaborative project between the two teams
- Allows the initiation and realization of this project, which without it would not have been on track
- A monthly meeting between the PhD student, the 2 directors and people implicated in the project
- Objectives were reached and even developed beyond
- The APR program is not only highly valuable for the two teams but also essential for the development of projects