The bacterial DNA segregation complexes ParB*S* display a twofold phase separation

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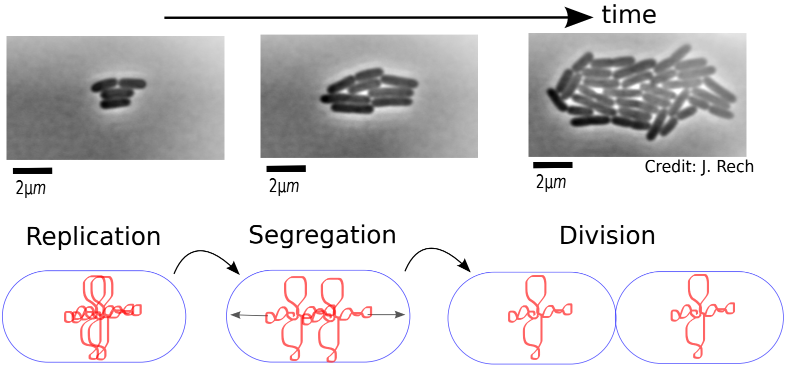
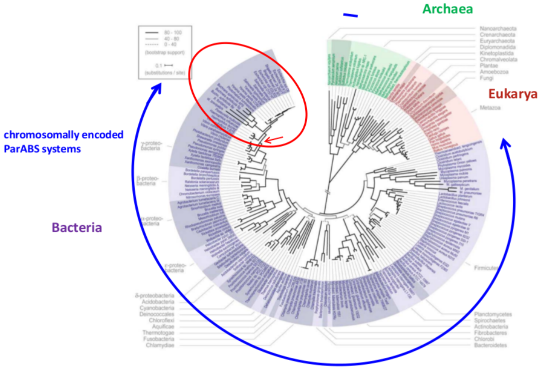
Rencontres scientifiques des Grands Causses V
GDR ADN, Millau
2nd of October 2023

Bacterial DNA segregation



How is the bacterial genome segregated ?

Bacterial DNA segregation: the ParAB*S* system

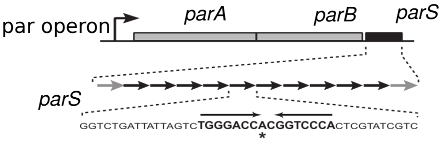


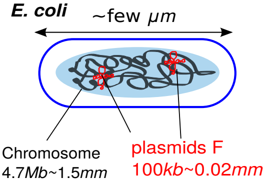
Credit: J-Y. Bouet

→ ParAB*S*: conserved mechanism of liquid-liquid phase separation (**LLPS**)

(Most chromosomes and low-number copy plasmids)

The molecular actors of the ParAB*S* system



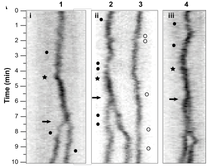


- ParA: “motor” protein (ATPase)
- ParB: binding protein (specific or non-specific binding)
  → promoted recently as a CTPase [Jalal ’20; Osorio-Valeriano ’19; Soh ’19]
- *parS*: specific DNA sequence

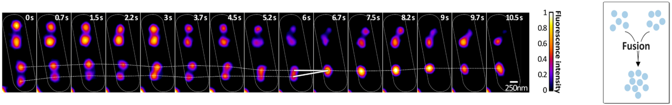
Properties of ParAB*S* complexes

A

B



C

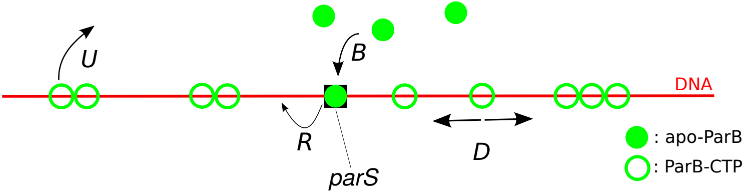


- N_{ParB} ≈200-250 ParB/complex (90% of ParB population)
- exchange/reassembly time $\tau_{\mathrm{ParB}} \sim 1$ min

Guilhas, JCW,...Le Gall, Nollmann **ATP-driven separation of liquid phase condensates in bacteria** *Mol. Cell* '20
JCW... Bouet **Physical modeling of a sliding clamp mechanism for the spreading of ParB...** *iScience* '20

ParB-CTP: Clamping & Sliding (C&S) model

- CTP activity of ParB [Jalal '20; Osorio-Valeriano '19; Soh '19]



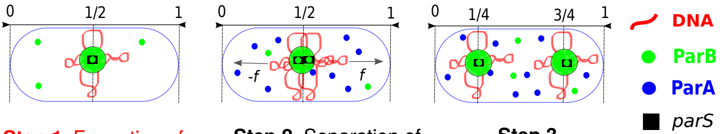
$$N_{\mathrm{ParB}} = R/U \approx 6$$ with $R \approx 0.1s^{-1}$ and $U \approx 0.02s^{-1}$ [Jalal '20]

$$\tau_{\mathrm{ParB}} = 200/R \approx 15 - 30\mathrm{min}$$ to reload ParB after e.g. replication

- → C&S alone cannot explain large scale chromosome architecture

JCW... Bouet **Physical modeling of a sliding clamp mechanism for the spreading of ParB...** *iScience* '20

ParB*S* displays a twofold phase separation



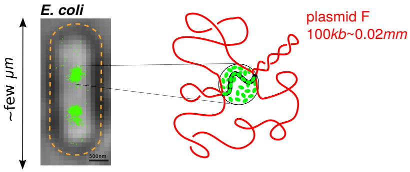
Step 1. Formation of ParB*S*

Step 2. Separation of DNA copies

Step 3. Equipositioning

Equilibrium LLPS

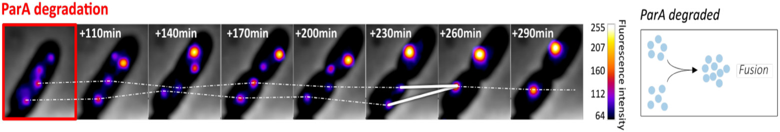
Non-eq. phase separation (ATP)

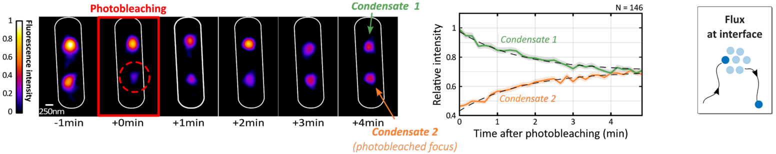


- Membraneless compartments ParB*S* splitted with ATP consumption (ParA)

  → phase separation at- and out of- equilibrium

Liquid-like behaviour of ParB*S* complexes





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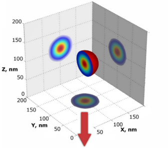
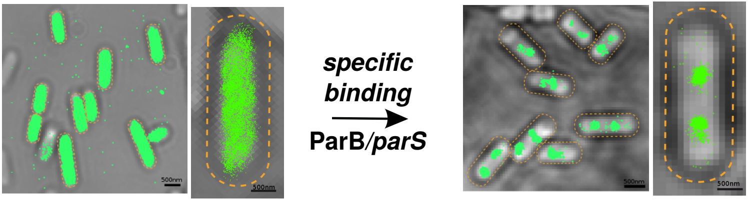
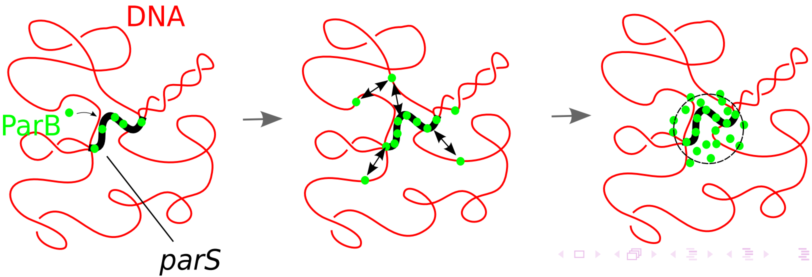


Diameter
37 nm ± 5

Guilhas, JCW,...Le Gall, Nollmann **ATP-driven separation of liquid phase condensates in bacteria** *Mol. Cell* '20

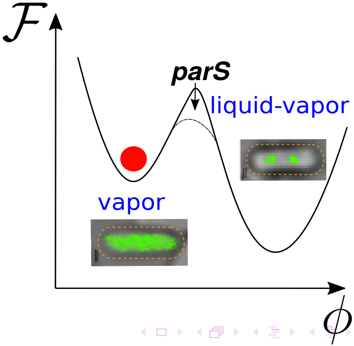
The phase separation of ParB is catalyzed by *parS*



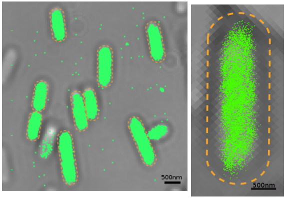


The phase separation of ParB is catalyzed by *parS*

$$\mathcal{F} = U - TS$$



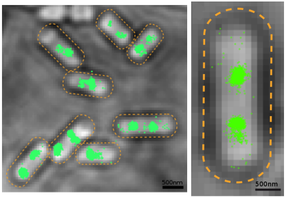
Liquid-like behaviour of ParB*S* complexes

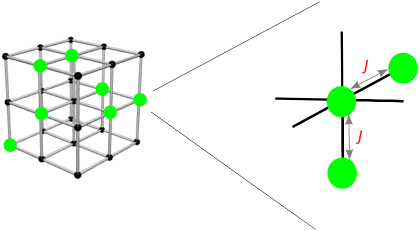


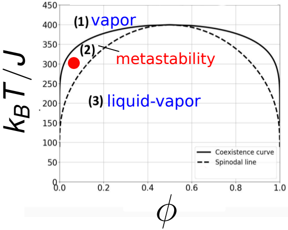
specific binding

→

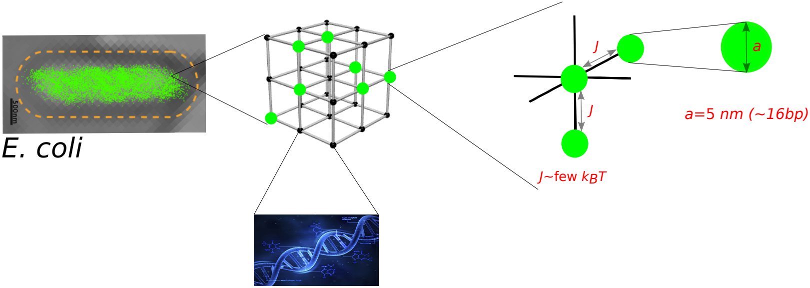
ParB/*parS*







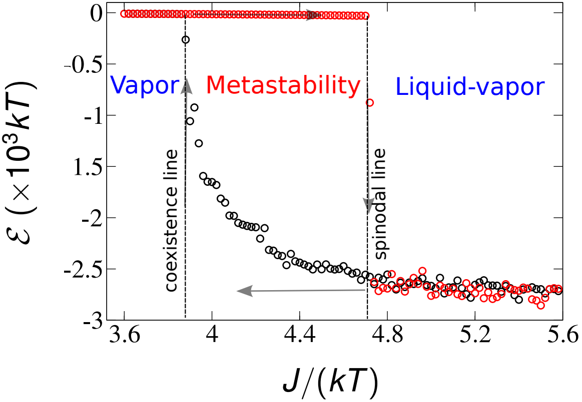
ParB on the nucleoid modeled by a Lattice Gas



$$\mathcal{E} = -J \sum_{\langle i,j \rangle} \phi_i \phi_j - \varepsilon_{parS} \sum_i \phi_i$$

- Diffusion $D \sim 1 \mu m^2.s^{-1}$ & Interaction energy J

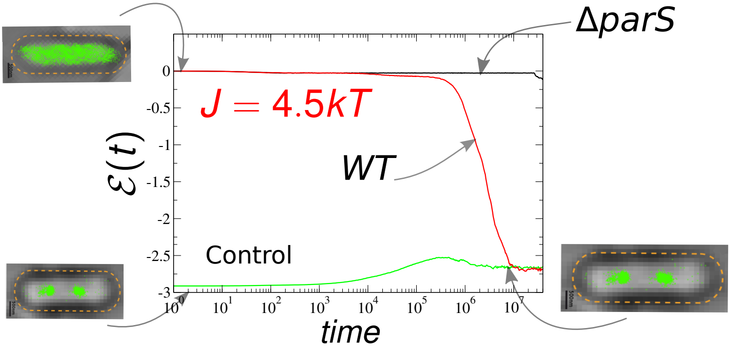
Phase diagram of the ParB*S* system



1. 1st order phase transition (jump in the energy & hysteresis)
2. Metastable region for $J \sim$ few $k_B T$

Guilhas, JCW,...Le Gall, Nollmann **ATP-driven separation of liquid phase condensates in bacteria** *Mol. Cell* '20

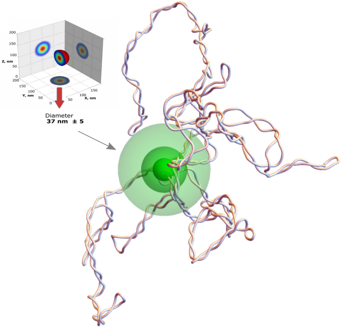
Kinetic of ParB*S* nucleation



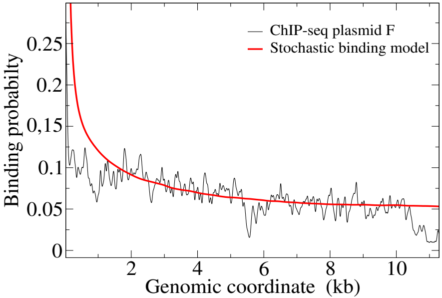
- *parS* is needed to catalyze LLPS *in vivo* (but it is not enough):
  → speed of nucleation and specificity boosted with ParB-CTP ?

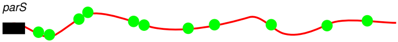
Guilhas, JCW,...Le Gall, Nollmann **ATP-driven separation of liquid phase condensates in bacteria** *Mol. Cell* '20

DNA conformation: The stochastic binding model



$\sigma = -0.06$

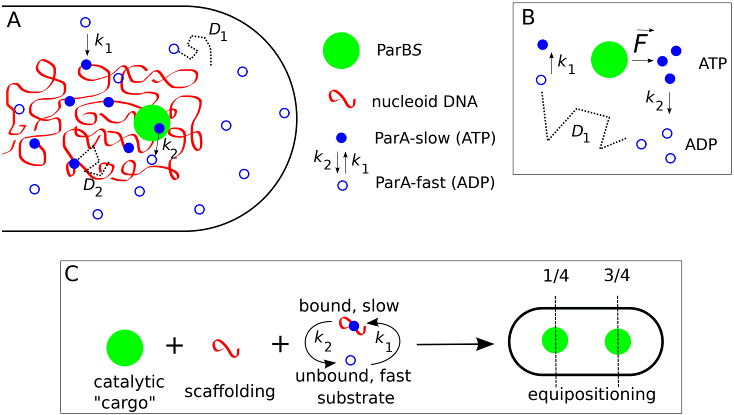




- DNA organization accessible through ParB ChIP-seq profile

JCW, ..., Bouet, Junier, **Supercoiled DNA and non-eq. formation of protein complexes**, *PLOS Comp. Biol.* '21

ParA gradient is mediating equipositioning of ParB*S*



JCW *et al* **Surfing on proteins waves: proteophoresis as a mechanism for bact. gen. part.** *Phys Rev Lett* '17

Reaction-Diffusion equations

ParA-slow (ATP): $$\frac{\partial v}{\partial t} = D_2 \, \Delta v + k_1 \, u(\mathbf{r}, t) - k_2 \, v(\mathbf{r}, t) \sum_i S(\mathbf{r} - \mathbf{r}_i(t))$$

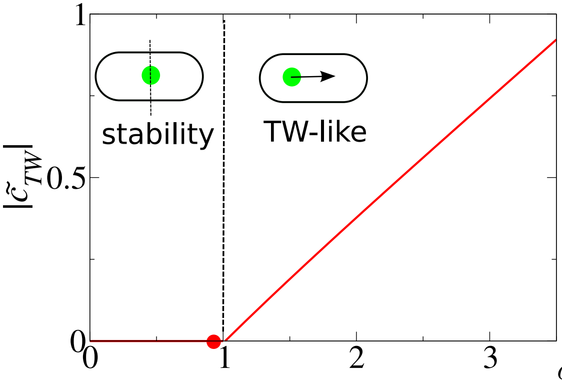
ParA-fast (ADP): $$\frac{\partial u}{\partial t} = D_1 \, \Delta u - k_1 \, u(\mathbf{r}, t) + k_2 \, v(\mathbf{r}, t) \sum_i S(\mathbf{r} - \mathbf{r}_i(t))$$

ParB*S*: $$m\gamma \frac{d\mathbf{r}_i}{dt}(t) = \varepsilon \int_V \nabla v(\mathbf{r}', t) \, S(\mathbf{r}' - \mathbf{r}_i(t)) \, d^3\mathbf{r}'$$

- Feedback between the partition complexes and ParA densities
  $\rightarrow$ Non-linear system with dynamical instability

JCW *et al* **Surfing on proteins waves: proteophoresis as a mechanism for bact. gen. part.** *Phys Rev Lett* '17

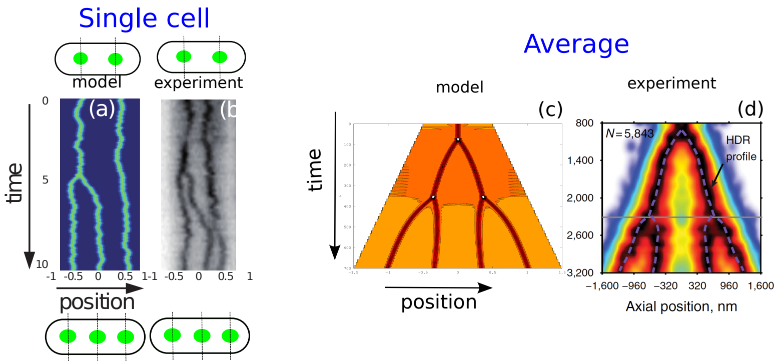
Dynamical instability



$$\frac{\alpha}{\alpha_c} = \frac{\varepsilon N_p}{m\gamma D_2}$$

JCW *et al* **Surfing on proteins waves: proteophoresis as a mechanism for bact. gen. part.** *Phys Rev Lett* '17

ParA gradient is mediating equipositioning of ParB*S*



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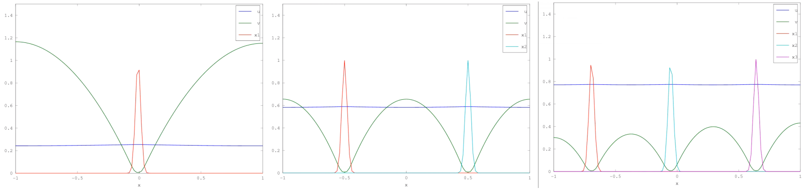

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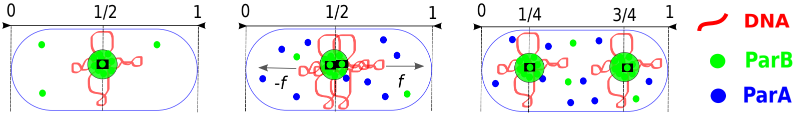

Step 1. Formation of ParB*S*

Equilibrium LLPS

Step 2. Separation of DNA copies

Step 3. Equipositioning

Non-eq. phase separation (ATP)

- ParB-CTP: increased specifity and speed of LLPS ?

Collaborators and Institutions

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Biophysical modeling

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Super resolution microscopy

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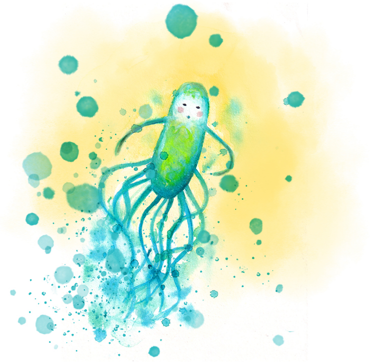
Université Montpellier

cnrs dépasser les frontières

LabEx NUMEV

Thank you! Any question ?



Mon Amie Alphée, Eds. Encre de Siagne. Drawing A. Jeuillard