

## **The bacterial DNA segregation complexes ParBS display a twofold phase separation**

The bacterial DNA segregation is prevalently performed through the ParABS system for most chromosome and low-copy number plasmids. It consists in two proteins: (i) ParB a DNA binding protein with a specific binding site at parS. It has been recently found to be a CTP-ase; (ii) ParA, an ATP-ase whose ATP hydrolysis is catalyzed by ParB and (iii) parS, a centromere-like sequence where ParB can bind specifically.

We show that the bacterial DNA segregation consists in a two-fold liquid-liquid phase separation (LLPS) (i) an equilibrium LLPS due to the formation of a droplet of ParB proteins centered on parS. The parS sequence acts as a kinetic catalyzer optimizing both the specificity and the speed of nucleation of the droplet and (ii) an out-of-equilibrium LLPS due to the action of ParA leading to the separation and the positioning of the two replicated droplets of ParB. The energy consumption of ParA is needed to counterbalance the increase of the surface tension.

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