

Micro201
Rudner Lecture 2: plasmid segregation
28 February 2019

Overview:

In this class we will discuss plasmids and their segregation. The background readings include a comprehensive review on this topic (**Ebersbach and Gerdes 2005**) and a relatively recent MicroReview on one of the “motors” that pulls plasmids and chromosomal origins around inside the cell (**Vecchiarelli et al 2012**). The required reading is a beautiful paper in which (Ethan) Garner and colleagues reconstitute R1 plasmid segregation in vitro. The experiments are deceptively “simple”. As with our class on replication, consider carefully how the experiments were actually carried out. In this case, instead of a shaking flask, RHX added, genomic DNA isolated etc., it will be ParM was purified, labeled with a fluorescent dye (how?), and analyzed by TIRF microscopy (can it possibly be this easy?). As always, think about whether the experiments are convincing and what you would do next? And think about what these in vitro experiments are telling us about R1 plasmid segregation inside bacterial cells. Finally, I have included a more recent follow up paper from Jan Lowe’s group (not required reading) that suggests that some of the conclusions in the Garner paper may need some revision. The unraveling of molecular mechanism is dynamic and ever evolving.

If time permits, we will discuss part of paper 2. In this paper, Ringgaard and colleagues visualize the segregation of plasmid pB171 in vivo. The time-lapse imaging is really impressive (and evocative). The mechanism by which this plasmid is partitioned is strikingly different from R1. This segregation system is more relevant to how bacterial chromosomes are segregated. Please be prepared to present Figures 1 and 3.

enjoy.

David

Primary Paper:

Garner, Campbell, Weibel, Mullins Reconstitution of DNA segregation Driven by Assembly of a Prokaryotic Actin Homolog (2007)

Second Paper:

Ringgaard, van Zon, Howard, Gerdes, Movement and equipositioning of plasmids by ParA disassembly (2009)

Background Reading:

Ebersbach and Gerdes, Plasmid Segregation Mechanics (2005)

Vecchiarelli, Mizuuchi, Funnell Surfing biological surfaces: exploiting the nucleoid for partition and transport in bacteria (2012)

Extra Reading:

Gayathri, Fujii, Moller-Jensen, van den Ent, Namba, Lowe A bipolar spindle of antiparallel ParM filaments drives bacterial plasmid segregation