Plasmid partition: sisters drifting apart

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Remarkably little is known about how bacterial chromosomes and their plasmids are partitioned to daughter cells prior to cell division, despite extensive experimentation and bioinformatic analysis. An *in vitro* study described in the current issue of *The EMBO Journal* recapitulates many features of plasmid partition *in vivo* and provides insight into how protein patterning on the nucleoid, determined by a diffusion-ratchet mechanism, directs plasmid positioning and partition. Related protein complexes likely use the same mechanism to position and partition newly replicated sister chromosomes and large protein complexes that act in processes other than chromosome segregation.

The fundamentals of chromosome segregation in eukaryotes have been known for decades, with mitosis first being described cytologically at the end of the nineteenth century long before the chemical and genetic nature of chromosomes was known (reviewed in Nasmyth (2001)). A key feature of eukaryotic chromosome segregation is the use of a tubulin-based mitotic spindle to attach to the centromeres of sister chromosomes and to pull them apart during anaphase, a machinery clearly absent in bacteria. Ironically, genetic analyses aimed at revealing the mechanisms of bacterial chromosome, and plasmid segregation have revealed most insight into how plasmids partition, because mutations that lead to severe defects in plasmid partition are readily obtained. Although such studies have shown that some plasmids specify homologues of tubulin or actin to push apart newly replicated sister plasmids, most low copy plasmids encode ParA ATPases featuring a characteristic deviant Walker A motif to ensure their effective partition (reviewed in Howard and Gerdes, 2010; Reyes-Lamothe *et al.*, 2012; Vecchiarelli *et al.*, 2012).

Plasmid-specified ParA ATPases act as part of a three-component system in which ParA-ATP interacts with DNA nonspecifically, whereas multiple dimers of the second protein, ParB, bind plasmid *parS*-sites. ParA-ATP-ParB-*parS* interactions tether plasmid partition complexes to the nucleoid matrix (Figure 1). Related systems can facilitate replication origin positioning as a consequence of adjacent *parS* sites and the consequent segregation of bacterial chromosomes; direct cell division away from the cell poles to midcell; and position large protein complexes regularly over the bacterial nucleoid, or at cell poles (reviewed in Vecchiarelli *et al.*, 2012). Previous *in vivo* and *in vitro*

experiments have led to conflicting views as to whether ParA ATPases function as cytoskeletal filaments whose retraction pulls sister plasmids and chromosomes apart, or whether their action occurs in the absence of filaments or extensive multimerization, with a diffusion-ratchet mechanism underlying their action (Ringgaard *et al.*, 2009; Howard and Gerdes, 2010; Vecchiarelli *et al.*, 2012).

The work of Mizuuchi and colleagues presented in this issue (Hwang *et al.*, 2013), along with a complementary study from the same laboratory (Vecchiarelli *et al.*, 2013), provides convincing evidence for a diffusion-ratchet mechanism. The experiments employed a ‘carpet’ of sonicated nonspecific DNA molecules on the surface of a 25- μ m-deep flow cell to mimic the surface of the nucleoid. Fluorescent proteins and DNA molecules were passed into the flowcell and two-colour total internal reflectance fluorescence microscopy (TIRFM) imaging was used to visualize ParAB-*parS* dynamics in the vicinity of curtain DNA. The results were qualitatively the same irrespective of whether ParAB-*parS* encoded by plasmid P1, or the related F plasmid machinery (SopAB-*sopC*, respectively) was used. Binding and turnover experiments at physiologically relevant concentrations (<1% saturation of carpet DNA, conditions that seem unlikely to favour filament formation), using fluorescence recovery after photobleaching (FRAP), showed that ParA-ATP binds DNA nonspecifically as dimers or small oligomers after a slow conformational change that occurs on ATP binding, and dissociates from DNA slowly in an ATP hydrolysis-independent manner.

Reaction of appropriate concentrations of ParA, ParB, *parS*-containing plasmid and ATP led to the formation of DNA carpet-bound plasmid partition complexes with 5–20 plasmid copies associated with 500–1500 ParA monomers and 2–3-fold fewer dimers of ParB. Stimulation of ParA-ATP hydrolysis by the high local concentration of *parS*-bound ParB led to release of ParA and the associated plasmid partition complexes from the carpet. In order to facilitate observation and analysis, complexes containing many more plasmid molecules were formed by preincubating plasmid with ParAB. These larger complexes behaved similarly to the smaller complexes, but now partition complex ‘jumping’ and repositioning could be observed directly. The ParA concentration associated with plasmid clusters determined the extent of anchoring of the complexes to the carpet; tightly anchored complexes had many ParA-ParB-*parS* tethers,

Figure 1 Schematic of diffusion-ratchet model for ParAB-*parS*-mediated partition in the confined *in vivo* situation. ParA-ATP undergoes a slow conformational change after ATP binding (filled blue circles) and then associates nonspecifically with the nucleoid and plasmid DNA. Interaction of multiple ParB dimers (red circles) bound to *parS* sites with multiple adjacent ParA-ATP molecules (pale blue circles) tethers plasmid partition complexes to the carpet. ParB-stimulated hydrolysis of bound ParA-ATP molecules releases ParA-ADP (pale blue stars) and consequent stepwise loss of tethering. This leads to the formation of a ParA-ATP-depletion zone on the nucleoid. Lateral transfer of the plasmid because of confinement leads to its continuing association with the ParA-ATP gradient at the edge of the depletion zone (panels A and B). After plasmid replication and decatenation, the sisters are free to diffuse independently, when they associate independently with the ParA-ATP gradients at distal edges of the depletion zone (panels C–E). The equilibrium state is when two sisters are at the quarter positions of the nucleoid. The slow reformation from ParA-ADP of a ParA-ATP form that can associate with the nucleoid provides the dynamic instability that underlies the diffusion ratchet.

whereas as the ParA concentration associated with a complex decreased as ATP hydrolysis was stimulated by ParB, tethered Brownian ‘wiggling’ was observed just prior to eventual release of a complex from the carpet. At this point, both the plasmid complex and the surrounding carpet region were ParA-depleted. Once the plasmid complex was detached, the depletion zone recovered to its steady-state ParA-ATP-bound state over a few minutes. The ‘rolling’ of some plasmid complexes along the edge of the ParA-depletion zone and occasional bidirectional splitting of complexes were also observed, consistent with the nucleoid-bound ParA-ATP gradient at the edges of the depletion zones directing the transport of partition complexes (Figure 1). These observations recapitulate strikingly the patterns that have been observed *in vivo*, both for *E. coli* and Gm⁺ bacterial systems (Ringgaard *et al.*, 2009; Hatano and Niki, 2010; Soberón *et al.*, 2011).

In this correspondent’s opinion, the controversy of whether ParA ATPases function through dynamic instability of a diffusion-ratchet mechanism, or through retraction of a ParA filament bound to plasmid *parS* sites, is now resolved in favour of the former model, which is strongly supported by the *in vitro* dynamics data presented in this issue, and in the complementary paper (Hwang *et al.*, 2013; Vecchiarelli *et al.*, 2013). Furthermore, ParA filaments only form *in vitro* at non-physiological high protein concentrations, whereas the retracting filamentous ParA-derived structures described *in vivo* are likely artefacts of the imaging protocols used (see Vecchiarelli *et al.* (2012), for a critical discussion).

Despite the present study recapitulating many aspects of *in vivo* plasmid partition, a limitation is that the 25 µm of solvent-accessible space in the flowcell allowed the diffusion away of complexes from the carpet, so that they could not be followed by TIRFM. The confined space between the *in vivo* nucleoid and the inner cell membrane, which is likely to be no more than 100 nm, and of the order of the effective cross-section of plasmid molecules undergoing partition, will lead to released partition complexes moving laterally through ongoing interactions with the carpet. Hence, the partition complexes will survey the changing ParA-ATP gradient, leading to complex partition and multiple complexes being positioned equidistantly on the nucleoid, as observed *in vivo*. Furthermore, the size of the plasmids undergoing partition means that they will be excluded from the bulk nucleoid, and their positioning and partition will be restricted to the nucleoid surface.

Future work should try to more closely address the *in vivo* situation, by using a DNA carpet below a shallow flowcell, thereby mimicking the confined *in vivo* situation. Combined with better sensitivity and spatial and temporal resolution *in vitro*, and by higher resolution *in vivo* experiments, a more complete mechanistic understanding of the positioning and partition reactions should emerge. The work adds to the body of evidence showing that the dynamic instability intrinsic to diffusion-ratchet mechanisms plays disparate yet important roles in biology, as proposed originally by Turing (1952); for example, in addition to playing roles in protein patterning as described by Hwang and colleagues, diffusion-ratchet mechanisms determine digit number in mammals and the stripe patterning on a zebra (Sheth *et al.*, 2012).

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Conflict of interest

The author declares that he has no conflict of interest.

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