Plasmid Segregation: Is a Total Understanding Within Reach?

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Recent *in vitro* and *in vivo* studies of the proteins responsible for the active partitioning of bacterial plasmids suggest that it may be possible to develop a quantitative, molecular understanding of this form of DNA segregation.

The continual propagation of genetic material from one generation to the next is one of the most basic characteristics of all organisms. In eukaryotes, DNA is segregated into the two daughter cells by a highly dynamic, self-organizing structure called the spindle. While spindle formation and chromosome segregation have been intensely studied for over one hundred years, the ultimate goal of quantitatively explaining how these behaviors arise from the collective interactions of molecules seems far out of reach. Indeed, biologists are still debating basic questions such as the existence of an organizing mechanical scaffold [1], and whether diffusible signals provide a global blueprint that determines spindle morphology [2]. In the last few years tremendous progress has been made in understanding another form of DNA segregation: the partitioning of plasmids in bacteria. Plasmids are non-essential circular pieces of DNA, some of which are actively segregated by cytoskeletal polymers that form dynamic structures analogous to the eukaryotic spindle [3]. A recent live imaging study by Campbell and Mullins [4] indicates that the structure and dynamics of these bacterial spindles can be understood in terms of the *in vitro* behavior of their constituents. This paper, combined with previous work, suggests that it will be feasible to develop a quantitative, biophysically based molecular model of a form of DNA segregation.

The most thoroughly studied plasmid partitioning system is the one responsible for segregating the 100 kilobase multidrug resistant plasmid R1. The active segregation of plasmid R1 uses no host factors and requires just three components: two proteins which the plasmid encodes, ParM and ParR, and a centromere-like DNA sequence called *parC* [3]. About five years ago, immunofluorescence of fixed cells revealed that ParM, an actin homolog, forms filaments [5] with plasmids positioned at the ends [6], suggesting that segregation is caused by ParM polymerization pushing apart plasmids. This view has been further refined through *in vitro* studies which show that while ParM filaments readily nucleate, they are highly unstable, and grow and shrink bidirectionally in an active, fluctuating manner reminiscent of microtubule dynamic instability [7]. ParR binds cooperatively to *parC* and the resulting complex promotes ParM assembly *in vitro* [5].

These results led to a model of segregation in which ParM filaments are continually nucleating and disassembling, searching for ParR–*parC* complexes, and when a ParM filament bridges two plasmids it becomes selectively stabilized and grows, forcing the plasmids apart [7] (Figure 1). Aspects of this model were strikingly confirmed by another *in vitro* study which demonstrated that ParM can push apart ParR–*parC* coated beads in precisely the predicted manner [8]. Thus it seems that the molecules required for the active partitioning of plasmid R1 are known [3], their structures have

been determined [9–11], and their *in vitro* interactions can mimic DNA segregation [8]. But is this all really sufficient to explain what happens *in vivo*?

In the new work, Campbell and Mullins [4] directly studied the behaviors of plasmids and ParM in living *Escherichia coli* cells by using time-lapse fluorescence microscopy. They observed that short, dynamic filaments of ParM seem to grow from the sides of isolated plasmids, implying that ParM filaments are partially stabilized by their interactions with the ParR–*parC* complex *in vivo*, as had been suggested. These structures are reminiscent of the ParM asters formed around isolated ParR–*parC* coated beads *in vitro* [8]. When two plasmids come into close proximity, a ParM bundle polymerizes between them, pushing them apart. The initial encounter between plasmids occurs throughout the cytoplasm and the spindles begin growing at random orientations. The plasmids eventually find their way to opposing poles only because the growing spindle pushes against the cell sides, forcing it to align with the long axis of the cell. Precisely the same process causes *in vitro* spindles — made from two ParR–*parC* coated beads bridged by growing ParM filaments — to orient along the long axis of microchannels [8].

The authors [4] used photobleaching to show that *in vivo* spindles grow symmetrically from both ends, as occurs in the reconstituted system. After elongating for a short while, the ParM filaments suddenly undergo a catastrophic switch to shrinking, indicating that they grow by dynamic instability *in vivo* as they do *in vitro* [7]. These dynamics cause the spindles to continually fall apart and reform independently of the cell cycle, further arguing against any regulation of plasmid segregation by other factors. Amazingly, even the rates of ParM polymerization and depolymerization are similar *in vivo* and *in vitro*, but this may just be a coincidence because these values will depend on various details such as the exact ionic conditions and the concentration of inert proteins.

Taken together these results further support the previously developed model of R1 plasmid segregation [7] (Figure 1), and suggest that the entirety of the partitioning process can be understood in-terms of the *in vitro* properties of ParM, ParR and *parC*. Of course there are still many outstanding questions. On a biophysical level, how does ATP hydrolysis give rise to ParM dynamic instability? And how does the interaction with ParR-*parC* stabilize ParM filaments? How can one spindle consist of multiple ParM filaments, as Campbell and Mullins [4] demonstrated, and are the observed 'plasmids' actually clusters of multiple plasmids [12]? Moving up in complexity, it is not obvious that the proposed search-and-capture mechanism can account for all the observed interactions between plasmids. Naively one might expect that the probability of a successful search event would be quite low, particularly if it requires both ParR–*parC* complexes to be in the correct orientation. Furthermore, when separate plasmid foci move in close proximity they transiently diffuse together before forming a spindle, suggesting that plasmids can have some intermediate state of association between being independent and being connected by a growing ParM bundle. Finally, the ultimate question is: can knowledge of the biophysical properties of the R1 plasmid, ParM, and ParR be used to explain the statistics of plasmid partitioning, both the degree to which the *par* locus promotes plasmid stability and the incompatibility of two plasmids which carry the same centromere [13]?

In 1982, Pickett-Heaps, Tippit, and Porter began a review article [14] on eukaryotic spindles by approvingly quoting the classic manuscript, *Mitosis* [15], written 40 years earlier:

"Since about 1870 there has been a succession of periods in which triumph seemed to stand on the threshold as, first, observers of the living cell, then students of the morphology of the fixed cell, and lastly the physiologists, marshaled the evidence furnished by their different attacks.....each of these periods had a corresponding aftermath of disillusion, always accompanied by a new appreciation of the difficulties of the problem."

Now, 25 years later, tools from the molecular revolution have allowed researchers to discover hundreds of proteins involved in chromosome segregation, but while a great deal has been learned, we seem barely closer to understanding the eukaryotic spindle. In contrast, the active segregation of R1 plasmids requires just three components, all of which are well studied. This simplicity — combined with the ingenuity and hard work of many investigators — is allowing researchers to begin to understand the *in vivo* behavior of segregating plasmids in-terms of the *in vitro* properties of the relevant molecules. While much work remains, it seems we may finally be at the threshold of developing a quantitative, molecular understanding of some form of DNA segregation.

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Figure 1. The search-and-capture model of plasmid R1 segregation.
(A) ParM filaments are nucleated throughout the cytoplasm and become stabilized at one end when bound to the ParR–*parC* complex. The free end of the ParM filament searches for another plasmid, but rapidly depolymerizes by dynamic instability if no successful contact is made. (B) When two plasmids come into close proximity the ParM filament can be captured by another ParR–*parC* complex and become stabilized at both ends. (C) The growing ParM bundle pushes the plasmid apart, segregating them to opposite sides of the cell. The ParM filaments eventually depolymerize by dynamic instability, freeing the plasmids to diffuse independently, and the process repeats.

A

B ParM
   Growth
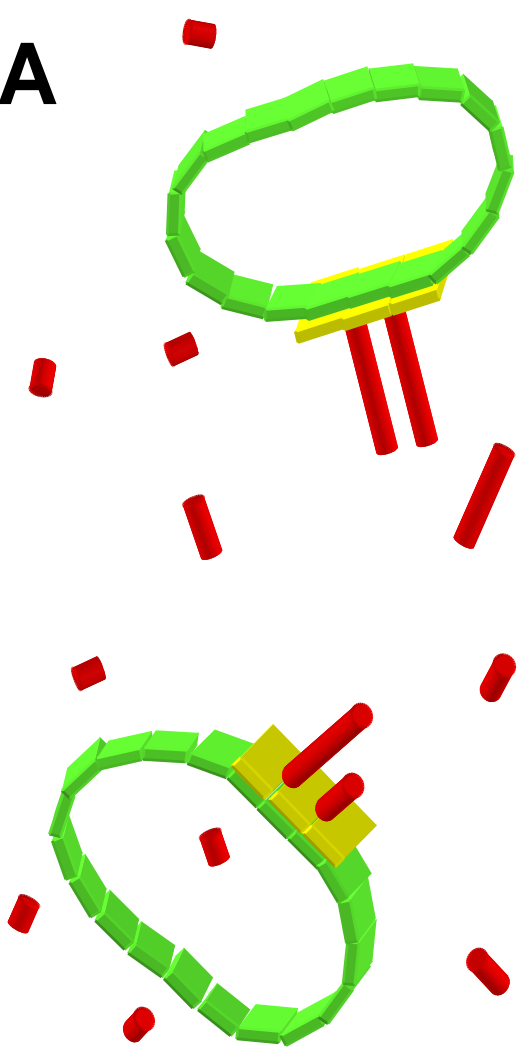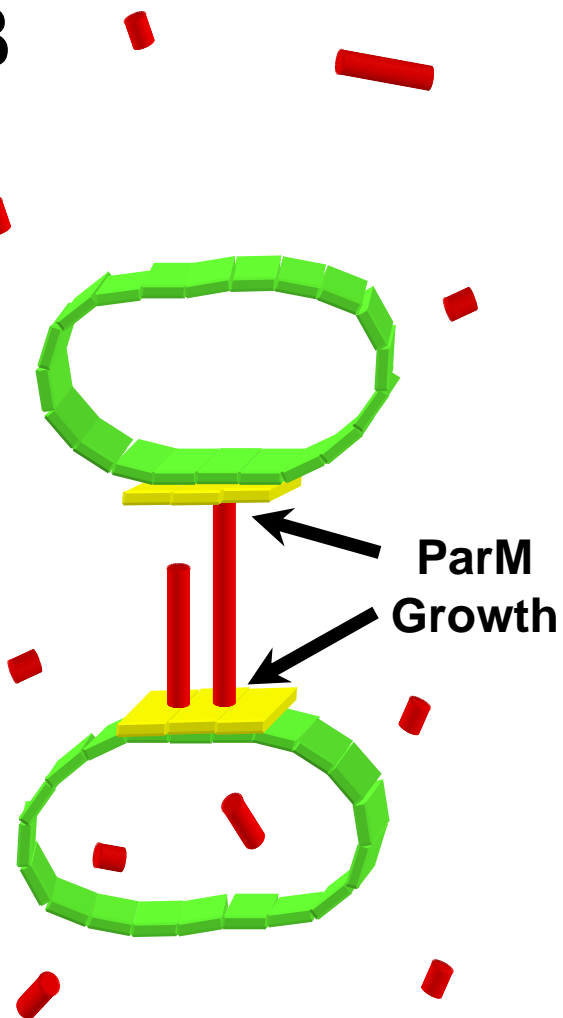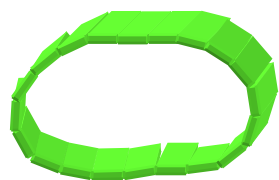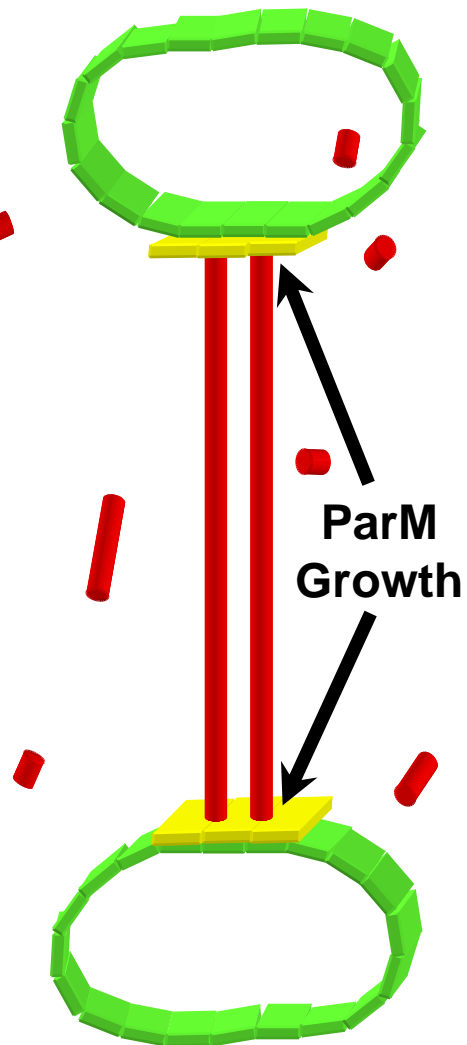
C ParM
   Growth

Plasmid
Containing *parC*   ParM Filament   ParR