Mini review

DYNAMIC INSTABILITY - A COMMON DENOMINATOR IN PROKARYOTIC AND EUKARYOTIC DNA SEGREGATION AND CELL DIVISION

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Abstract: Dynamic instability is an essential phenomenon in eukaryotic nuclear division and prokaryotic plasmid R1 segregation. Although the molecular machines used in both systems differ greatly in composition, strong similarities and requisite nuances in dynamics and segregation mechanisms are observed. This brief examination of the current literature provides a functional comparison between prokaryotic and eukaryotic dynamically unstable filaments, specifically ParM and microtubules. Additionally, this mini-review should support the notion that any dynamically unstable filament could serve as the molecular machine driving DNA segregation, but these machines possess auxiliary features to adapt to temporal and spatial disparities in either system.

Key words: Dynamic instability, Microtubules, ParM filaments, R1 plasmid, Mitosis, Mitotic spindle, Brownian ratchet, Cytoskeleton evolution, Catastrophe/recovery

Microtubule-mediated sister chromatid segregation during anaphase is a fundamental process in the nuclear division of most eukaryotic cells [1, 2]. In a related prokaryotic process, proper segregation of duplicated R1 antibiotic resistance plasmid is achieved using the ParM/ParR/*par*C actin-like motility system encoded in the *par* operon of R1 [3, 4]. While these systems differ in overall macromolecular structure and composition, notable similarities are evident in these seemingly distantly related processes [5, 6]. Importantly and most

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pertinent to this examination, plasmid or chromosome segregation is initiated and driven by dynamic instability, the switching between slow polymerization (recovery) and rapid depolymerization (catastrophe) of ParM filaments and microtubules in prokaryotes and eukaryotes, respectively [1, 3].

Microtubules (MTs) have well-documented roles in eukaryotic cells ranging from organelle positioning and intracellular organization to vesicular trafficking and mitotic chromatid segregation [1, 2]. To properly align and separate sister chromatids, the cell must correctly orient the mitotic spindle both spatially and temporally [1]. Spatial regulation is achieved through bipolar centrosome localization, physical "tug-of-war" of spindle fibers on either side of the metaphase plate and cyclin/CDK complex activity [7, 8]. Temporal regulation of mitotic events is driven by cyclin/CDK complex activity and in the metaphase/anaphase transition, by anaphase-promoting complex (APC) [9]. Once the chromosomes are properly aligned along the metaphase plate, the MTs of the mitotic spindle undergo catastrophe effected by the GTPase activity of α-tubulin [1]. This pulling force is then coupled to chromatid segregation by microtubule and kinetochore-binding protein complexes, such as the Dam1 ring and Ska1 complexes in budding yeast and humans, respectively [10, 11]. These complexes translate the force generated from MT depolymerization into chromatid movement toward either centrosome [10, 11] (Fig. 1A).

ParM is an *E. coli* actin homologue used to segregate duplicated R1 plasmid DNA along the long axis of a dividing cell to either pole. The machinery required for R1 segregation is self-encoded within the *par* operon: ParR binds to *parC* nucleotide repeats that can be thought of as a centromere within R1 (Fig. 1B). This binary complex can then bind dynamically unstable ParM filaments. After binding of ParM to the ParR/parC complex, the ParM filaments are protected against depolymerization [3]. It is thought that ParM filaments bind both R1 plasmids using a "search and capture" method in which unconnected ParM filaments come into close enough proximity to form a stabilized bundle of filaments which can then polymerize and separate both R1 plasmids [3, 12]. Additionally, recent electron cryomicroscopy data have suggested that ParR-bound ParM filaments form bundles that appear to initiate R1 segregation near the nucleoid region in *E. coli* [13].

The utility of ParM filament polymerization in R1 segregation may make ParM seem an attractive mechanism for segregation of *E. coli* chromosomes. However, one major problem with this notion is that *E. coli* use multi-fork replication, where one *ori* will fire more than once per cell cycle to compensate for having slower genomic DNA replication than cell division. The ParM filament system would need to have a regulatory mechanism to distinguish between segregation of two separated chromosomes and the multi-fork chromosome. Therefore, it has been proposed that *E. coli* chromosomes bearing *parC* elements will be defective in segregation [14].
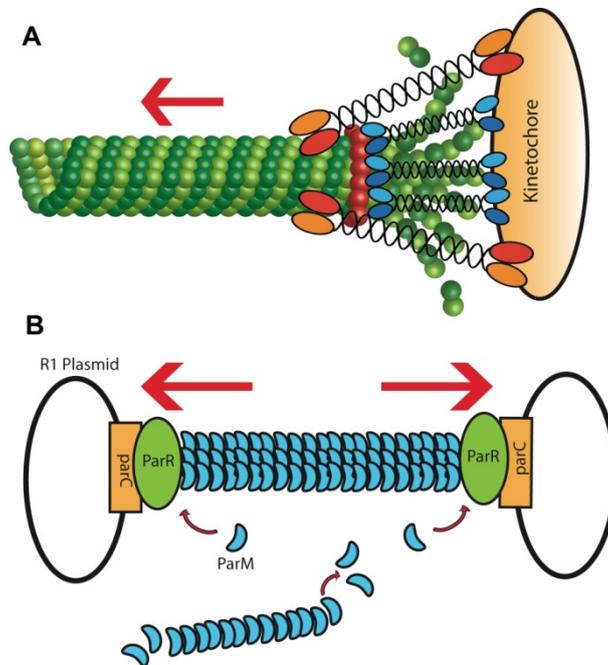
Fig. 1. DNA segregation mechanisms in eukaryotic cells and *E. coli*. A – illustrates MT-kinetochore attachment and chromosome pulling in a eukaryotic cell. Destabilization and catastrophe of the MT promotes pulling of the chromosomes to their respective poles. Dam1 ring complex is portrayed as red spheres. B – illustrates ParM-mediated plasmid R1 segregation. Dynamic instability of unbound ParM filaments supplies free ParM monomers that can be incorporated into ParM filaments actively segregating R1 using a pushing mechanism. Large red arrows indicate direction of DNA movement in both (A) and (B).

The ParM/ParR/*par*C system must be both temporally and spatially regulated to effect duplicated R1 segregation to either pole of a rod-shaped bacterium and ensure equal distribution of this low-copy plasmid [3]. Interestingly, this is achieved without homologues of the aforementioned eukaryotic regulatory mechanisms. Duplicated R1 plasmid DNA can be transported to both poles of an *E. coli* cell within seconds. This occurs multiple times in one cell cycle, suggesting disconnect between septum formation and cell division and R1 segregation, unlike in mammalian nuclear and cell division. After the plasmid DNAs have reached both poles, ParM filaments dissociate rapidly leaving the DNAs at either pole. ParM filaments maintaining R1 at either pole throughout the cell cycle will ensure that each daughter cell will be allocated equal numbers of plasmid DNAs [15].

It has been proposed that ParM monomers are able to add into the pre-existing ParM filament in an iterated fashion with steric constraints in the ParR/ParC complex, enabling addition of one monomer at a time [13, 15]. Additionally, it is thought that ParM filaments are stabilized by ATP cap formation similar to eukaryotic MTs (GTP cap), and that ParM filaments require ParR binding the

parC element of R1 for stabilization and plasmid segregation; however, precise mechanisms which spatially enable the efficient bipolar transportation of R1 remain elusive [16]. An interesting model reminiscent of eukaryotic MT dynamics describes a solution for allowing the parental and nascent R1 plasmid to be effectively divided to each pole of the cell.

The Brownian ratchet model suggests that thermal fluctuations effect the movement of a target filament-binding molecule to allow sporadic additions of component monomers, thereby increasing the overall length of the filament [6]. This phenomenon is used to the advantage of rod-shaped bacteria as it solves the crucial problem of orientation and finding the long axis of the cell. While eukaryotes use centrosomes as nucleation sites for mitotic spindle formation and markers at either pole, the bacterium might use the ParM filament to "feel out" either pole. When R1-bound ParM filaments extend and hit improper axes of the cell, the filaments reorient and repeat the process until the long axis of the cell is found (Fig. 2). The addition of new ParM monomers to the ParM filament acts as a pushing force, allocating each of the two ParM-bound R1 plasmids to either pole.
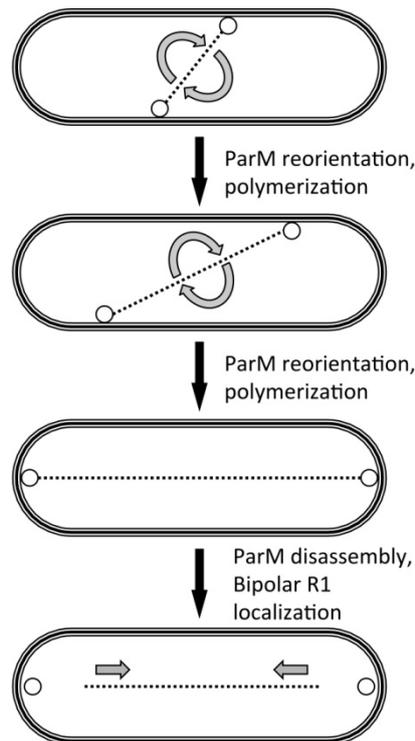

Fig. 2. Model for Brownian ratchet-mediated reorientation of ParM filaments in a rod-shaped bacterium. Dashed lines represent ParM filaments, circles represent R1 plasmids and the grey arrows symbolize reorientation of the ParM filament.

The model that ParM filaments are able to find the long axis of the cell via a Brownian ratchet mechanism was supported *in vitro*, as ParM filaments were capable of maneuvering *parC*-conjugated beads through a microfabricated channel with a length close to that of the long axis of a bacterium [3].

Interestingly, although ParM filaments are structurally asymmetric, the rate of monomer addition is equal on both ends [15]. Moreover, they exhibit a rapid dissociation driven by nucleotide hydrolysis to segregate R1. Unlike in eukaryotes, where nucleotide hydrolysis-driven dynamic instability is used as a direct effector of chromatid segregation, *E. coli* uses a rather indirect mechanism. Unbound ParM filaments display prominent dynamic instability; catastrophe is achieved through ATP hydrolysis, resulting in subsequent transient depolymerization of the unbound ParM structures. The resulting monomeric ParM molecules are then potentially "fed" into the elongating ParM filaments bound to the ParR/*parC* complex [3].

The ParM and MT systems examined here also illustrate and help answer the question of why pushing or pulling mechanisms are used in different cell types. Given that the compression force required to buckle a filament is inversely proportional to its length [6], smaller prokaryotic cells would utilize a pushing mechanism to generate force for plasmid segregation whereas larger eukaryotic cells would use a pulling mechanism to move chromatids [2]. This works to the advantage of the prokaryote, because the non-polar ParM filament polymerizes in both directions via the Brownian ratchet mechanism in a relatively small area [3, 16]. The eukaryotic cell must transport the sister chromatids a greater distance to either pole of the cell, making the pushing system less reasonable due to potential buckling. Thus, a pulling mechanism is employed that allows for force generation with decreased risk of buckling [6].

The issue posed for the larger eukaryotic cell, however, might be the greater distance that needs to be traveled by the segregating chromatids to ensure timely progression through mitosis. One strategy used in *Drosophila melanogaster* involves enzymatic severing and expedited depolymerization of MT plus and minus ends in processes called pacman and flux, respectively. Knockdown of the minus end MT-severing enzymes fidgetin and spastin or a plus end MT-severing enzyme, katanin, significantly retards chromatid-to-pole migration velocity. This experiment suggests that these enzymes may have evolved in certain eukaryotes to facilitate rapidity of chromatid segregation in anaphase [17].

The actin-like ParM and MT motility systems in *E. coli* and eukaryotes, respectively, are important for DNA segregation; furthermore, recent studies in fission yeast may represent an evolutionary link between non-spindle based prokaryotic and microtubule spindle-based higher eukaryotic chromosome segregation. Studies in fission yeast have revealed that spindle microtubules are dispensable in chromosome segregation. Interference with MT polymerization was shown to prevent mitosis only briefly; cells then undergo an abnormal nuclear division referred to as nuclear fission and subsequently enter S phase.

Intriguingly, inhibition of actin polymerization following MT polymerization disruption mitigates sister chromosome segregation, suggesting a more primal role of actin in driving nuclear division. This phenomenon may reflect an evolutionary transition from actin-dependent plasmid/chromosome segregation to a microtubule-driven process that may increase the precision and efficiency of segregation as size and complexity of the cell increased [18].

CONCLUSIONS

In this brief examination of the current literature, functional comparisons between prokaryotic and eukaryotic dynamically unstable filaments were examined. Similarities and requisite differences in dynamics, regulation and plasmid/chromosome segregation mechanisms were described to suggest that any filament capable of dynamic instability could serve as the molecular motor driving chromosome segregation. Finally, this review of the literature supports the notion that while functionally similar dynamically unstable filaments are present in both prokaryotes and eukaryotes, these filaments have evolved for optimal functioning in disparate systems.

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