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**REVIEW ARTICLE**

# Plasmid segregation: how to survive as an extra piece of DNA

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**Abstract**

Non-essential extra-chromosomal DNA elements such as plasmids are responsible for their own propagation in dividing host cells, and one means to ensure this is to carry a miniature active segregation system reminiscent of the mitotic spindle. Plasmids that are maintained at low numbers in prokaryotic cells have developed a range of such active partitioning systems, which are characterized by an impressive simplicity and efficiency and which are united by the use of dynamic, nucleotide-driven filaments to separate and position DNA molecules. A comparison of different plasmid segregation systems reveals (i) how unrelated filament-forming and DNA-binding proteins have been adopted and modified to create a range of simple DNA segregating complexes and (ii) how subtle changes in the few components of these DNA segregation machines has led to a remarkable diversity in the molecular mechanisms of closely related segregation systems. Here, our current understanding of plasmid segregation systems is reviewed and compared with other DNA segregation systems, and this is extended by a discussion of basic principles of plasmid segregation systems, evolutionary implications and the relationship between an autonomous DNA element and its host cell.

**Keywords:** *DNA segregation; plasmid; bacteria; ParA; ParM; cytomotive filaments; horizontal gene transfer*



## Contents



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## Introduction

### *Plasmids and their relationship to host cells*

Prokaryotes compensate for their necessarily small genomes by extensive lateral sharing of genetic material (Jain *et al.*, 1999; Dagan *et al.*, 2008; Koonin and Wolf, 2008; Isambert and Stein, 2009). Horizontal gene transfer occurs frequently between closely related cells and occasionally between more distant species, and is mediated by mobile genetic elements such as phages, plasmids and transposons. Widespread lateral genetic mobility, which can be contrasted with the comparatively static genomes of higher eukaryotes, has two implications. First, it allows for the preservation and dissemination of a greater number of genes than could be accommodated on the core genome. Second, the shift of non-essential genes onto extrachromosomal elements provides a forum for genetic experimentation, which is separate from the essential housekeeping roles of chromosomal genes and which is evidenced by the much greater variation amongst plasmid compared with chromosomal genes.

Plasmids are one of the major carriers for horizontal gene transfer and can generally be described as small, circular pieces of non-chromosomal DNA that are not essential to the individual host cell but may confer some advantage under certain growth conditions. Placed within the framework of a genetically dynamic population, plasmids can be considered as an intriguing combination of both parasite and sometimes benefactor to the individual cell as well as a repository for a shared genetic library within a population. Given the discrepancy between the levels at which the plasmid may provide benefit (at a population level) and impose cost (in the form of metabolic load on an individual host cell), plasmids have developed a number of strategies to ensure that they are not lost from their individual host cell during rapid cell growth (Gordon and Wright, 2000; Ebersbach and Gerdes, 2005; Ghosh *et al.*, 2006), and this will be the subject of this review.

### *General mechanisms for persisting in a growing cell population*

One can imagine a variety of general approaches that a non-essential, extra-chromosomal piece of DNA could adopt to ensure its propagation to all daughter cells upon division of the host cell. First, the DNA element could be maintained at such a high number of segregating units that the rate of formation of plasmid-free cells is very low. This could be supplemented with a second system to kill any daughter cells that happened to lack the DNA element. A more sophisticated third approach might be to develop a system reminiscent of eukaryotic mitosis that would actively segregate DNA molecules prior to cell division. Each of these general strategies has been adopted by plasmids, with the molecular details reflecting the requirements and properties of the individual plasmid.

All plasmids carry systems that regulate their copy number, usually by monitoring the rate at which replication initiation events are allowed (Nordström and Nordström, 1985). So-called high-copy number plasmids are maintained at between 20 and several hundred copies per chromosome equivalent, and include many cloning vectors such as pUC and pBR322-derived plasmids. It is generally assumed that these numbers are sufficiently high to ensure that plasmid-free cells will arise at a very low frequency. However, due to plasmid oligomerization and clustering, the actual number of segregating units that are present in the cell may be much lower than the copy number, possibly even as low as 1 or 2 (Eliasson *et al.*, 1992; Yao *et al.*, 2007). Therefore these high-copy plasmids may employ some additional, as yet unknown mechanism to ensure their presence in each daughter cell.

Plasmids have evolved a number of systems to eliminate plasmid-free cells from the host population (Ebersbach and Gerdes, 2005; Ghosh *et al.*, 2006; Schumacher, 2007). These systems support the plasmid-carrying host cell, which may otherwise suffer from reduced competitiveness due to the additional metabolic

load imposed by the plasmid, and may also provide an extra advantage to the host by killing a wide range of competitors. These mechanisms typically involve both a killing and an immunity factor, which can be protein or RNA based. One well-characterized system is the *hok*/*sok* mechanism encoded by the *parB* locus of plasmid R1 (Gerdes *et al.*, 1990; 1997). The *hok* gene encodes a 50-residue membrane-associated polypeptide that causes cell death by inducing loss of membrane potential. *sok* encodes a small *trans*-acting RNA, which blocks transcription of *hok* and thereby protects plasmid-carrying cells. The *hok*-encoding mRNA is relatively long lived and, through its translation to Hok, will persist to cause damage to newborn cells that have lost the R1 plasmid and can no longer produce the short-lived antidote RNA *sok*. This principle is somewhat similar to that employed to select for plasmid-carrying cells under laboratory conditions. Here cells are grown in the presence of lethal amounts of antibiotic, which will kill individuals that do not carry an antidote-encoding plasmid.

Alternatively, plasmids could ensure that they are retained during cell division by developing an active segregation apparatus that would accurately position plasmids at specific cellular sub-sites. This is generally the approach adopted by low copy number plasmids, which are maintained at fewer than 10 copies per chromosome equivalent and cannot rely on random segregation. Plasmids have evolved a number of different types of segregation machineries, but they all appear to share the basic design feature of using nucleotide-driven cytomotive filaments (Löwe and Amos, 2009) move and position replicated plasmids.

### *Typical active plasmid segregation systems*

The active segregation systems carried by most low copy number plasmids tend to be extremely small and simple in composition due to evolutionary pressures on plasmids to limit their genome size. In addition, partitioning systems are usually self-contained, meaning that they can be moved from one plasmid to another without loss of functionality (Møller-Jensen *et al.*, 2003; Ebersbach *et al.*, 2005), and they typically do not rely extensively on specific additional host-encoded factors to achieve basic segregation (Ebersbach and Gerdes, 2005). They are encoded by gene clusters that display a remarkable uniformity in general organization. Most partitioning systems studied to date comprise three components: a centromere-like region, a small DNA-binding adaptor protein, and a larger nucleotide-binding motor element that provides the force for plasmid movement (Figure 1). In general, the small adaptor protein binds to DNA repeats in the centromere to form a large nucleoprotein complex. This then somehow interacts with the motor to effect DNA movement to opposite poles of the cell such that each new daughter cell contains at least one plasmid upon cell division.

There are three major classes of segregation systems, exemplified by those encoded by F and P1 plasmids (Type I), R1 plasmid (Type II) and pXO1/pBtoxis (Type III). These classes are defined by their motor proteins, with Type I, II and III characterized by Walker A cytoskeletal ATPase proteins (WACA), actin-like proteins and tubulin-like proteins respectively (Schumacher, 2007; Ebersbach and Gerdes, 2005). These distinct families of proteins are unified by their propensity to form dynamic filaments in a nucleotide-dependent manner, and as such have been classed cytomotive filaments (Löwe and Amos, 2009). In principle, cytomotive filaments could achieve partitioning by pulling cargo attached to shrinking filaments, or by pushing cargo attached to growing filaments, and there has been some evidence for both modes of action (Garner *et al.*, 2007; Ringgaard *et al.*, 2009). A potential fourth family of plasmid segregation system has recently been identified, which differs from the other three in arrangement and which appears to require just a single protein to achieve segregation, although the details by which this might function are unknown (Simpson *et al.*, 2003).

The nomenclature of plasmid segregation systems can be confusing, since the same name is sometimes used to describe distinct proteins. This is particularly problematic in the case of Type I segregation systems, in which many but not all systems encode proteins called ParA and ParB. The denomination 'par' is used frequently and comes from the description of a partitioning system. Here, the elements will frequently be described as motor, adaptor or centromere and Table 1 gives the respective names of the components from important segregation systems.

## Active segregation mechanisms of low copy number plasmids

### *Type I/ParA-based systems – oscillating filaments and foci*

The challenge for an independent DNA segregation system can be considered in two parts: (i) the identification of specific regions in the cell space, and (ii) the placement of replicated DNA in those positions. Arguably, the second task is the more trivial since once a system for mapping the cellular topology has been established it becomes a simple problem of coupling DNA to this machinery. Most Type I systems studied to date appear to use a principle of dynamic oscillation to explore and mark the cellular environment. In the simplest case, this leads to the identification of two distantly located positions at the opposite ends of the bacterial nucleoid which are used to position newly replicated plasmids at these approximate quarter-cell positions. The situation

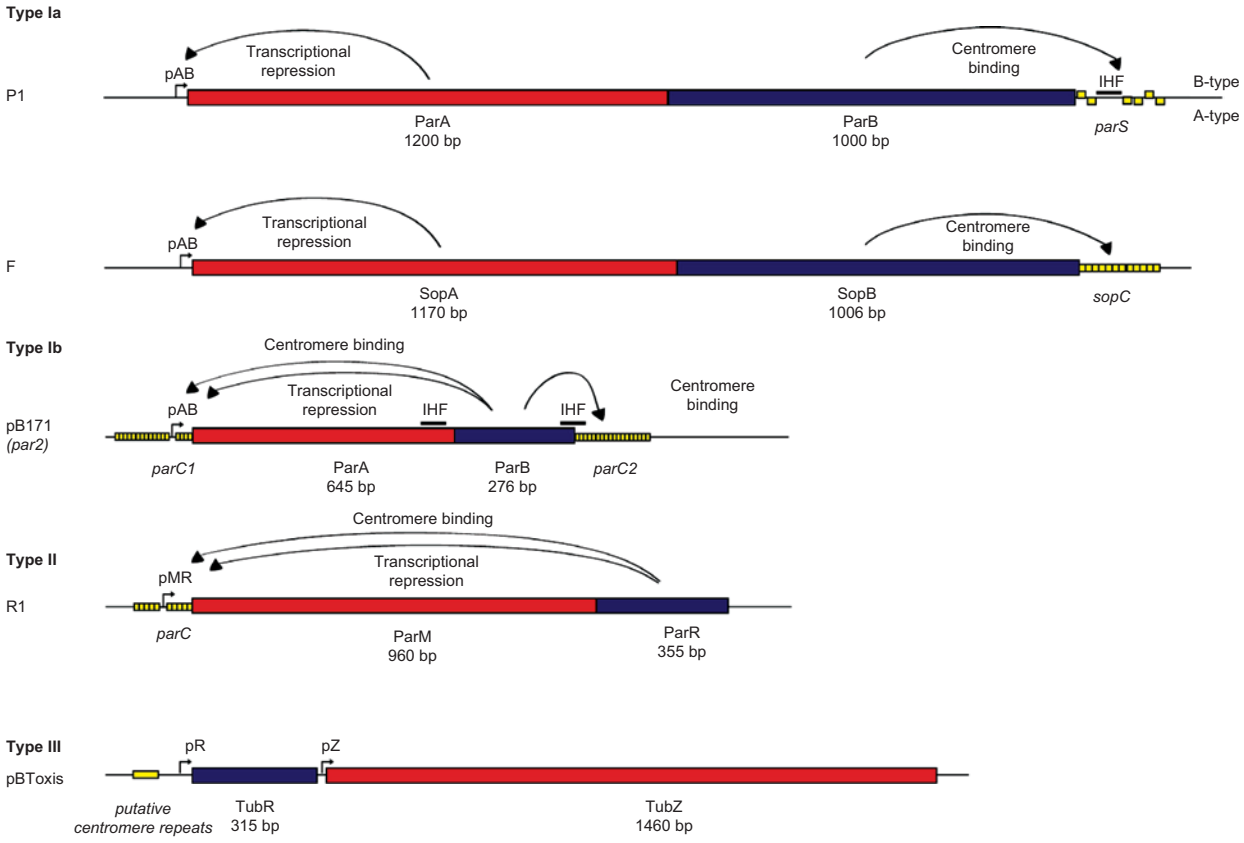


**Figure 1.** Genetic arrangement of Type I, II and III segregation systems. The arrangement of representative Type Ia (F and P1 plasmid), Ib (pB171 plasmid *par2)*, Type II (R1 plasmid) and Type III (pBtoxis/pXO1 plasmid) are shown. Plasmid names are given on the left and motor, adaptor and centromere elements are given in red, blue and yellow respectively. Binding of protein products to centromere and promoter regions is indicated. Genes are shown to scale, with sizes given below. IHF = Integration Host Factor binding site, $_{p}$XY = promoter for genes X and Y.

**Table 1.** Motor, adaptor and centromere elements of key plasmid and chromosome DNA segregation systems.

| Plasmid | Type | Motor | Adaptor | Centromere |
|---|---|---|---|---|
| P1 | Ia | ParA | ParB | *parS* |
| F | Ia | SopA | SopB | *sopC* |
| pB171 (*par2*) | Ib | ParA | ParB | *parC* |
| TP228 | Ib | ParF | ParG | *parH* |
| R1 | II | ParM | ParR | *parC* |
| pB171 (*par1*) | II | ParM | ParR | *parC* |
| pBToxis | III | TubZ | TubR | Unconfirmed |
| *B. subtilis* | Chromosomal | Soj | SpoOJ | *parS* |
| *C. crescentus* | Chromosomal | ParA | ParB | *parS* |

becomes more complex in cells containing multiple plasmid foci, which are usually positioned equidistant from one another within the region of the nucleoid (Niki and Hiraga, 1997; Ebersbach and Gerdes, 2001; Gordon *et al.*, 2004; Adachi *et al.*, 2006; Ebersbach *et al.*, 2006; Hatano *et al.*, 2007; Sengupta *et al.*, 2009).

In principle, a continuous self-organized oscillatory pattern can be achieved using just one protein and a surface, and simply requires a mechanism to ensure that the oligomeric assembly and disassembly of the protein on the surface is regulated to occur at different rates. For example, assume that protein A polymerizes on a surface at low concentrations, but that at high concentrations this induces a conformational change that causes rapid disassembly of the oligomers and release from the surface. Place protein A molecules within an enclosed sphere, and a continuous self-organized oscillatory pattern will be established (Figure 2A). A single point of nucleation will cause monomers to assemble over the surface, and new molecules to be recruited and localized within that position. Once a certain local concentration has been reached, a stochastic switch will occur, the oligomers will disassemble, and protein A molecules will diffuse away to reassemble on a free surface at the opposite position on the sphere and the cycle will begin again. An immediate implication of this system is that the continuous oscillation is strictly dependent on the relationship between the concentration at which the stochastic switch in protein A occurs, and the total concentration of protein A in the system. Accordingly, Type I segregation systems are highly sensitive to changes in concentration of any one component (Abeles *et al.*, 1985; Davey and Funnell, 1994; 1997).

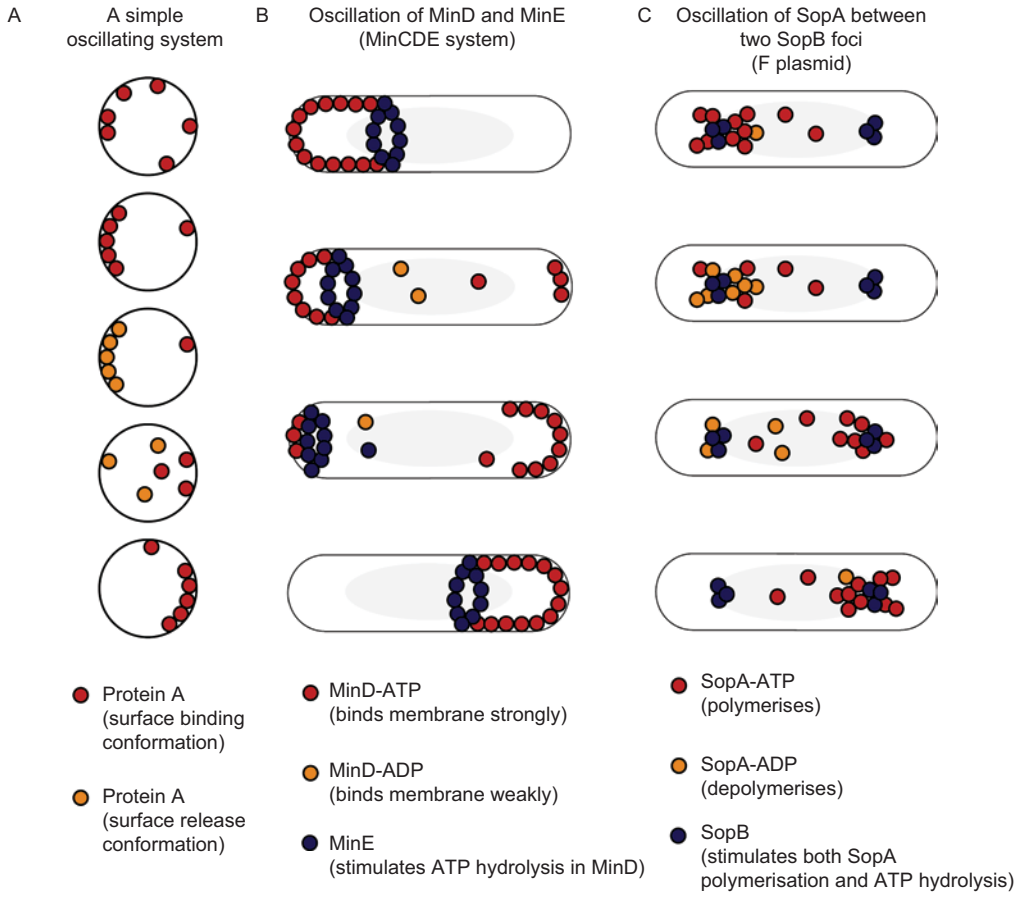


**Figure 2.** Establishing a pattern of continuous oscillation. (A) A simple system for establishing continuous oscillation. Protein A binds to the surface strongly at low concentrations (red molecules) and recruits further protein A molecules. At high local concentrations a stochastic switch occurs that converts protein A to a conformation that binds the surface weakly (orange molecules). Protein A dissociates, converts back to a strong surface binding conformation (red) and reassembles on the opposite surface where the cycle begins again. (B) Oscillation of MinD and MinE from the *E. coli* MinCDE system. MinD-ATP binds membrane strongly and recruits a ring of MinE. MinE stimulates ATP hydrolysis in MinD, and MinD-ADP dissociates from the membrane. MinD-ADP rebinds ATP and assembles as MinD-ATP on the membrane at the opposite pole. This causes recruitment of a new ring of MinE and the cycle begins again. (C) Oscillation of SopA between two SopB foci. SopB (bound to *sopC*) is localized in two foci at opposite ends of the nucleoid. SopB stimulates SopA-ATP polymerization and recruits asters of SopA-ATP filaments at one foci. SopB also stimulates ATP hydrolysis in SopA filaments, and this causes SopA-ADP to dissociate from the filaments. Released SopA rebinds ATP and reassembles at the SopB focus at the opposite pole, and the cycle begins again.

The best understood self-organized oscillatory system in bacteria is that established by the MinCDE system in *E. coli* (Lutkenhaus, 2007) (Figure 2B). The motor for this system, MinD, is closely related to the motor from Type I segregation systems, ParA, and despite being functionally distinct the two systems are mechanistically analogous (Cordell and Löwe, 2001; Raskin and de boer, 1999a and b; Lutkenhaus 2007). Both employ an oscillation between surfaces at opposite poles of the cell, although whilst MinD oligomerizes over the surface of the cell membrane, ParA is thought to assemble on the surface of the nucleoid (Raskin and de Boer, 1999a; 1999b; Ebersbach and Gerdes, 2001). Both systems establish a time-averaged bilobed map of the cell, although whilst ParA uses this to positively position plasmid DNA, the MinCDE system uses this to block assembly of the chromosomally encoded bacterial tubulin-like protein FtsZ and restrict its polymerization to the centre of the cell. MinD is a Walker-type cytoskeletal ATPase (WACA) protein with a small amphipathic helix that allows it to assemble over the surface of the membrane (Cordell and Löwe, 2001; Lutkenhaus, 2007). Oscillation of MinD is mediated by the regulator protein MinE, which stimulates its ATP hydrolysis and membrane release. MinC is the negative regulator of FtsZ and rides the MinDE oscillatory machine to block FtsZ assembly everywhere but at the midcell. Thus, in the same way that an independent DNA segregation systems needs (i) to establish oscillation and (ii) to couple this to DNA, the MinCDE system (i) establishes oscillation using mutual interactions between MinD and MinE, and (ii) uses this to localize MinC at the poles of the cell and thereby negatively regulate FtsZ assembly.

There are three major classes of ParA-based DNA segregation systems (Gerdes *et al.*, 2000; Hayes, 2000). The first two, Type Ia and Type Ib, are found on low copy number plasmids and are distinguished by the size of their ParA-like protein as well as the overall genetic arrangement of the segregation locus (Figure 1). The Type Ia subgroup contains both the ParABS system from plasmid P1 (Li and Austin, 2002a; 2002b; Li *et al.*, 2004; Edgar *et al.*, 2006; Sengupta *et al.*, 2009) and SopABC from plasmid F (Adachi *et al.*, 2006; Bouet *et al.*, 2007; Castaing *et al.*, 2008), whilst the Type Ib subgroup includes ParABC from plasmid pB171 (Ebersbach and Gerdes, 2001; Ringgaard *et al.*, 2007a; Ringgaard *et al.*, 2009) and ParFGH from plasmid TP228 (Hayes, 2000; Golovanov *et al.*, 2003; Barillà *et al.*, 2007). There is also a third class of ParA-based segregation systems, and these are chromosomally rather than plasmid encoded. Although these are widely distributed amongst bacterial and archaeal chromosomes, their exact significance is unknown and they are likely to be complemented by a number of additional mechanisms for segregating chromosomes (Ghosh *et al.*, 2006; Hazan and Ben-Yehuda, 2006). The best characterized of these chromosomal segregation systems are the Soj/SpoOJ systems from *Thermus thermophilus* (Leonard *et al.*, 2004) and *Bacillus subtilis* (Lin and Grossman, 1998; Webb *et al.*, 1998; Murray and Errington, 2008; Gruber and Errington, 2009), the ParABS system from *Caulobacter crescentus* (Mohl and Gober, 1997; Mohl *et al.*, 2001) and the ParAI/BI/SI system from *Vibrio cholerae* (Fogel and Waldor, 2006).

**Mutual modulations: how to regulate and ride an oscillating motor**

In order to convert a simple oscillating pattern into a sophisticated system for positioning DNA, Type I segregation systems have developed an intricate system of mutual modulations in which all the components mediate various behaviors of one another in a concentration and localization dependent manner. There are five main components to be considered in the network of mutual modulations. The first three comprise the core elements of the segregation system: motor, adaptor and centromere. The adaptor and centromere affect the polymerization, DNA binding and nucleotide hydrolysis of the motor, whilst the motor affects adaptor binding both to the centromere region and to non-specific DNA. The interactions between these three basic components are fine-tuned by two further generic elements: non-specific DNA, and nucleotides. Nucleotide binding and hydrolysis in the motor induces conformational changes that modulate both DNA and adaptor binding, as well as polymerization. Reflecting the complexity of these multiple interactions, as well as subtle but significant differences between Type I model systems, there is currently no comprehensive, universal model to describe exactly how the oscillation of the motor is regulated and coupled to plasmid localization. Important questions include how motor assembly is regulated, how this leads to its dynamic repositioning in the cell and how this is coupled to plasmid DNA, and these will be discussed in turn.

How does the adaptor/centromere complex modulate the activity of the motor and cause its dynamic localization? First, the motor ParA is thought to polymerize over the surface of the nucleoid. A number of ParA proteins have been shown to interact with non-specific DNA (including ParA from P1 (Davey and Funnell, 1994; 1997) and pB171 plasmids (Ebersbach and Gerdes, 2004), and SopA from F plasmid (Castaing *et al.*, 2008)), whilst many have been reported to exhibit ATP-dependent polymerization although this does not necessarily involve nucleotide hydrolysis (Barillà *et al.*, 2005; 2007; Lim *et al.*, 2005; Ebersbach *et al.*, 2006; Bouet *et al.*, 2007; Hatano *et al.*, 2007; Dunham *et al.*, 2009). Interestingly, the presence of non-specific DNA has been shown both to enhance and to inhibit polymerization of different ParAs, reflecting possible differences between systems (Bouet *et al.*, 2007; Castaing *et al.*, 2008; Ah-Seng *et al.*, 2009). Second, the polymerization of ParA over the surface of the nucleoid, as well as the rate of nucleotide hydrolysis, is moderated by the presence of the adaptor/centromere complex, probably through an arginine finger motif located in the flexible tail of the adaptor proteins (Barillà *et al.*, 2007). Again, there are differences in the literature as to whether this effect is positive or negative (Davey and Funnell, 1997; Barillà *et al.*, 2005; 2007; Bouet *et al.*, 2007; Ringgaard *et al.*, 2009), and in certain cases opposite effects can be achieved using the same components depending on the relative concentrations (Barillà *et al.*, 2005; 2007). In fact, one of the incredible features to emerge from a comparative analysis of several Type I systems is the apparent variation that can be tolerated in the regulation of related ParA motors whilst still achieving the same basic task of dynamic oscillation and plasmid localization.

Modulation of the activity of ParA can lead to a dynamic oscillatory pattern. This has been reported using fluorescently tracked ParA proteins from F plasmid (SopA) (Lim *et al.*, 2005; Adachi *et al.*, 2006; Hatano *et al.*, 2007) and the *par2* system of pB171 plasmid (ParA) (Ebersbach *et al.*, 2006; Ringgaard *et al.*, 2009), as well as some chromosomally encoded ParA proteins (Mohl and Gober, 1997; Fogel and Waldor, 2006). In contrast, ParA from P1 plasmid is apparently localized evenly over the surface of the nucleoid (Sengupta *et al.*, 2010), although it is not yet possible to distinguish polymerized from unpolymerized protein using these techniques and therefore there may be oscillations in conformation if not concentration. Equivalent to the MinE-regulated oscillation of MinD, it is possible to imagine how an adaptor/centromere regulation of ParA could establish oscillation using the F plasmid SopABC systems as an example. SopA oscillates

between two SopB/*sopC* foci, which are located at opposite ends of the nucleoid (Lim *et al.*, 2005; Adachi *et al.*, 2006; Hatano *et al.*, 2007). There is an additional weak SopA density over the surface of the nucleoid, and this results from a non-specific interaction between SopA and DNA. SopB/*sopC* both (i) stimulates SopA polymerization and (ii) induces ATP hydrolysis and release from DNA (Bouet *et al.*, 2007; Castaing *et al.*, 2008; Ah-Seng *et al.*, 2009). Thus, SopB/*sopC* will initially nucleate polymerization of SopA and cause the formation of SopA asters emanating from plasmid foci (Lim *et al.*, 2005). Slow ATP hydrolysis, stimulated by the SopB/*SopC* complex, will cause release from the DNA. SopA will diffuse away, rebind the surface of DNA at some distance from the foci, and polymerize in the presence of another SopB/*sopC* complex at the opposite end of the nucleoid, and so the cycle beings again.

How does the oscillating ParA motor modulate the activity of the adaptor/centromere complex, and how can these mutual modulations lead to plasmid segregation? In all tested cases the ParA motor stimulates dissociation of the adaptor from the centromere, at least at high concentrations (Bouet and Funnell, 1999; Barillà and Hayes, 2003). This is almost certainly required for the initial uncoupling of pairs or clusters of plasmids at the mid-cell position. Conversely, there are apparent differences in the mechanism by which plasmids are coupled to the ParA motor, since colocalization between plasmid and ParA reveal differences in their relative positions (Adachi *et al.*, 2006; Ringgaard *et al.*, 2009). This mirrors the subtlety in concentration-dependent effects of the adaptor/centromere complex on the DNA binding, nucleotide hydrolysis and polymerization of the motor. The most intuitive model for plasmid coupling to a ParA protein comes from a recent study on the *par2* system from pB171 (Ringgaard *et al.*, 2009). In this, the ParB/*parS* adaptor/centromere complex stimulates ATP hydrolyis and depolymerization of ParA. Thus, ParA assembles on the nucleoid in the ParB/*parS*-free regions. When a plasmid-bound complex is encountered, ATP hydrolysis and depolymerization is stimulated and the ParA focus retracts. An affinity between ParB/*parS* and ParA ensures that plasmids somehow stay attached to the ends of the depolymerizing filaments until they reach the end of the nucleoid and ParA filaments depolymerize completely. Again, the released ParA will diffuse away and reassociate

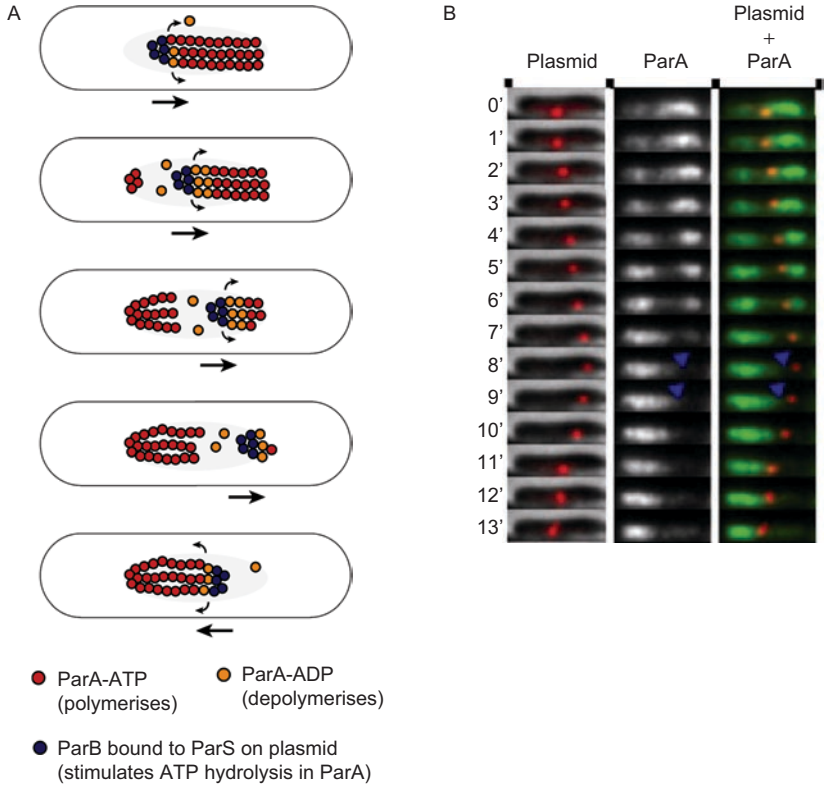


**Figure 3.** Coupling plasmids to oscillating filaments: the ParABS *par2* system of pB171 plasmid. (A) Schematic showing ParB-bound plasmids following retracting ParA filaments. ParB bound to *parS* binds to the ends of ParA filaments, and stimulates ATP hydrolysis and ParA depolymerization. Released ParA monomers rebind ATP and repolymerize in the ParB-free region, which appears in the wake of the depolymerizing filament. (B) Fluorescently tagged plasmids following fluorescently tagged depolymerizing ParA filaments in *E. coli* cells carrying a plasmid with the *par2* system of pB171. Plasmids are labeled using TetR-mCherry bound to an array of *tetO* repeats, and ParA is labeled using a ParA-GFP fusion protein. The time in seconds is given on the left, plasmids are shown in red and ParA in green or white. Image taken from Ringgaard *et al.*, 2009.

on a free region of nucleoid ready for the next round of ParB/*parS* stimulated depolymerization (Figure 3).

**Interactions with DNA**

Type I segregation proteins employ extensive interactions with DNA in order to achieve a number of ends. First, the entire process of plasmid separation and segregation is thought to take place at the nucleoid, probably close to the surface. This is not a prerequisite for the oscillating system since the related chromosomal MinD protein localizes on the cell membrane (Raskin and de Boer, 1999b; Hsieh *et al.*, 2010), and it may reflect the fact that a significant force would be required to separate plasmid DNA from the nucleoid mass and bring it out to the cell membrane. This nucleoid localization of plasmid DNA may be partly facilitated by interactions with general DNA binding proteins such as replication and transcription machinery or DNA compaction proteins such as MukB, HU and Fis. Localization of the segregation complex to the nucleoid is achieved through an affinity of both ParA and ParB for non-specific DNA (Bouet *et al.*, 2007; Castaing *et al.*, 2008; Ah-Seng *et al.*, 2009).

Second, the adaptor protein binds specifically to its cognate centromere DNA site and so couples the plasmid to the motor element. The exact interactions between adaptor and centromere are highly variable between segregation systems, and this may have evolved to minimize cross talk between different plasmids (Fothergill *et al.*, 2005). The best understood Type I adaptor/centromere complex is that encoded by the P1 plasmid, which has been cocrystallized with its centromere DNA (Schumacher and Funnell, 2005; Schumacher *et al.*, 2007b). The ParB adaptor protein has a helix-turn-helix DNA binding fold that binds as a dimer to two different classes of DNA repeat in the centromere. In one of the few examples of a known contribution of a specific host-encoded factor to plasmid segregation, the P1 *parS* site is interrupted by a small 29-bp DNA sequence to which the small heat-stable integration host factor (IHF) binds (Funnell, 1988a; 1988b). This causes bending of the DNA and greatly increases the affinity of ParB for *parS.* Sequence alignments predict that other Type Ia adaptor proteins are closely related and also have a helix-turn-helix fold. In contrast, Type Ib adaptor proteins are highly divergent but may be characterized by a ribbon-helix-helix fold that was observed in the crystal structure of ParG from TP228 (Golovanov *et al.*, 2003), and which are also typical of Type II plasmids such as R1 (Møller-Jensen *et al.*, 2007; Schumacher *et al.*, 2007a). Once bound to their centromere site, most adaptor proteins continue to bind oligomerically to adjacent regions of DNA on either side. This could contribute to long-distance gene silencing and plasmid supercoiling, although the extent to which this is significant in the cell remains to be shown (Lynch and Wang, 1995; Rodionov *et al.*, 1999; Surtees and Funnell, 1999; 2001; Bouet *et al.*, 2000; Pratto et al., 2008). The multimeric adaptor/centromere complex can in fact form across separate molecules of DNA, leading to pairing of plasmids through their centromere sites. This has been demonstrated both *in vitro* (Ringgaard *et al.*, 2007b; Pratto et al., 2008) and *in vivo* (Edgar *et al.*, 2006; Ringgaard *et al.*, 2009; Sengupta *et al.*, 2010) and may play an important role in the segregation process.

Finally, the DNA binding activity of Type I segregation proteins is exploited for transcriptional autoregulation. Any self-organized dynamic oscillation pattern is critically sensitive to the concentration of individual components, and the segregation system has developed elegant strategies to autoregulate protein levels. Type Ib systems resemble the overall organization of Type II systems, with the promoter for both motor and adaptor placed within or close to the centromere site (Figure 1). Thus, the formation of the adaptor/centromere nucleoprotein complex for plasmid segregation will have the additional effect of inhibiting protein expression (Golovanov *et al.*, 2003). In contrast, Type Ia systems place the centromere downstream of the motor and adaptor protein and use the motor rather than adaptor protein as a transcriptional autorepressor (Davis *et al.*, 1992; Davey and Funnell, 1994; 1997). Accordingly, Type Ia ParA proteins have a unique additional N-terminal domain that binds specifically to their promoters in an ADP-bound conformation, and the adaptor protein positively regulates this interaction (Dunham *et al.*, 2009).

**A description of ParA proteins**

Phylogenetic analysis classifies the superfamily of prokaryotic ParA-like proteins into three related but distinct clades, with many but not all known members involved in DNA segregation (Figure 4) (Gerdes *et al.*, 2000; Hayes, 2000). The first group includes the Type Ia plasmid segregation ParAs, as well as a number of plasmid-encoded RepA proteins which are involved in replication and stability. The second group is primarily composed of the chromosomally encoded ParA-like proteins, but also includes a few plasmid proteins such as the segregation IncC protein from the broad-host range RK2 plasmid. The final group includes both Type Ib plasmid segregation ParAs, and the closely related MinD, suggesting a possible distinct origin for Type Ib ParA compared with Type Ia ParA.

Crystal structures are available for at least one protein from each of the three groups (Cordell and Löwe, 2001; Leonard, 2005b; Pratto et al., 2008; Dunham *et al.*, 2009). A comparison of ParA from the Type Ia P1 and P7 plasmids, the chromosomal ParA-like protein Soj from *T. thermophilus*, and the Type Ib ParA-like protein MinD from *E. coli* reveals a very similar ~300 residue deviant Walker-fold that contains the nucleotide-binding domain

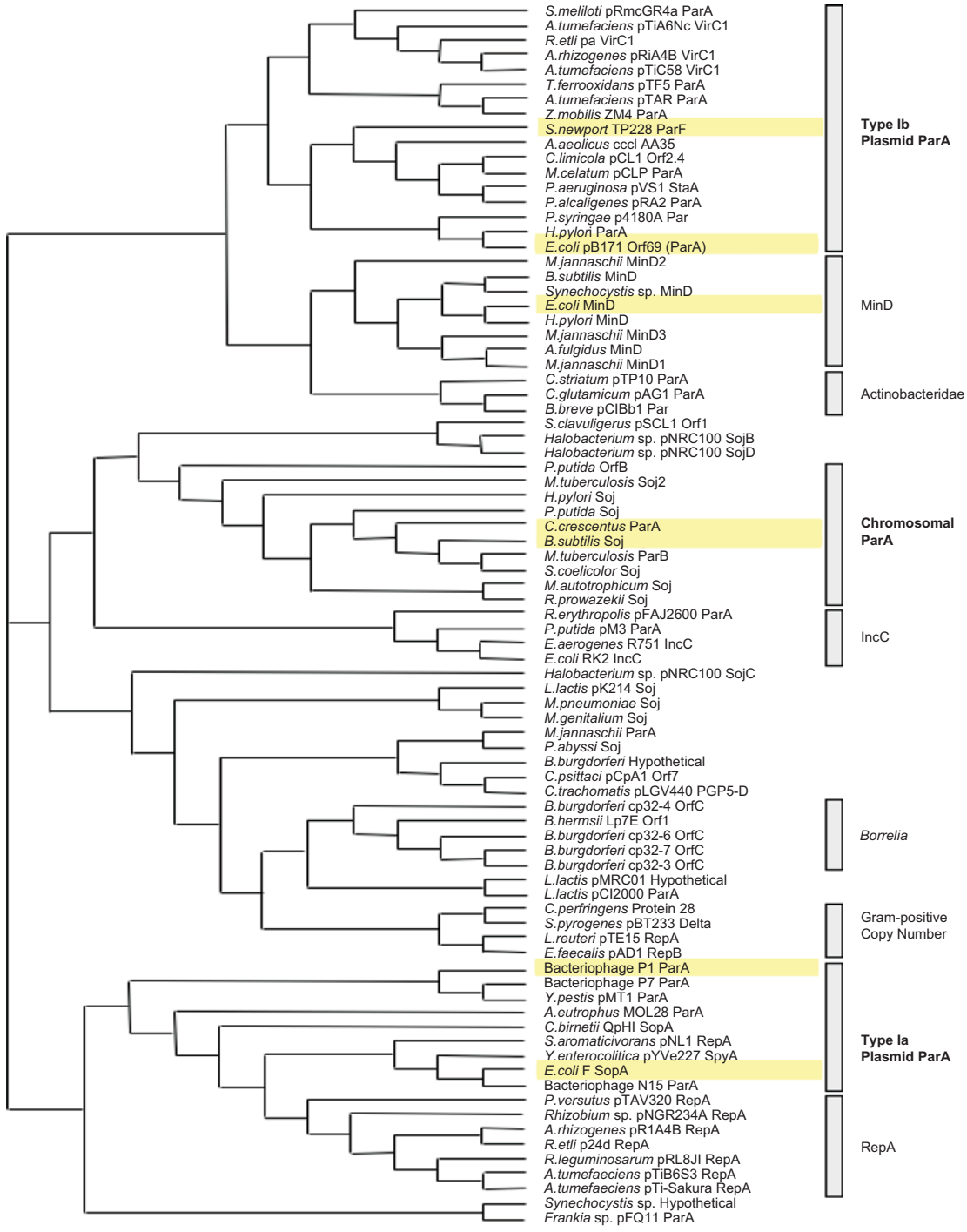


**Figure 4.** A phylogenetic analysis of Type I ParA-like proteins. An unrooted cladogram of a non-exhaustive set of 83 members of the ParA superfamily. The lengths of horizontal bars do not represent evolutionary distance. ParA-like proteins fall into three main clades, exemplified by plasmid encoded Type Ib ParAs; chromosomal ParAs (Soj) and plasmid encoded Type Ia ParAs. MinD is closely related to Type Ib ParA-like proteins. ParA-like proteins mentioned in the text are highlighted in yellow. Adapted from Hayes 2000.

(Figure 5). MinD carries an additional small C-terminal amphipathic helix that recruits it to the membrane and that would not be expected in the closely related Type Ib plasmid-segregating ParA-like proteins. The major difference between the three proteins is the addition of a 100-residue N-terminal structure in P1 and P7 ParAs, that comprises both an extended α-helix and a winged-HTH structure and that is likely to be involved in promoter binding and autorepression (Figure 5A) (Dunham *et al.*, 2009).

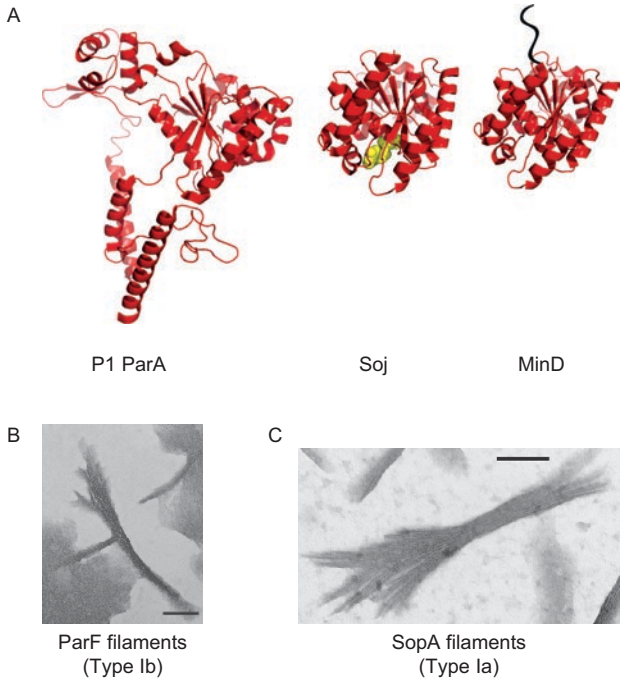


**Figure 5.** Structure of the Type I ParA protein. (A) Crystal structures of *E. coli* P1 ParA (Apo form, PDB accession number 3EZ7, Dunham *et al.*, 2009), *T. thermophils* Soj (ADP-bound form, PDB accession number 2BEJ, Leonard *et al.*, 2005b) and *A. fulgidus* MinD (Apo form, PDB accession number 1HYQ, Cordell and Löwe, 2001). Protein is shown in red and nucleotide in yellow. The black line indicates the membrane-binding amphipathic helix of MinD that is not present in this structure and that extends from the C-terminus. B. Negatively stained electron micrograph of ATP-polymerized ParF protein from plasmid TP228. Taken from Barilla et al. (2005). (C) Negatively stained electron micrograph of ATP-polymerized SopA protein from plasmid F. Taken from Bouet *et al.* (2007). Scale bar = 100 nm.

Most ParA-like proteins, including Soj and MinD, have been shown to polymerize at least weakly in the presence of ATP (Suefuji and RayChaudhuri, 2002; Barillà *et al.*, 2005; 2007; Leonard, 2005b; Lim *et al.*, 2005; Ebersbach *et al.*, 2006; Bouet *et al.*, 2007; Hatano *et al.*, 2007; Dunham *et al.*, 2009) (Figure 5B and C). This may be stimulated by DNA although it is unclear how DNA binding would affect the structure of the filaments. There is currently no detailed structural information on any WACA filaments, partly because they are typically bundled and highly heterogeneous by electron microscopy (Figures 5B and 5C). Most ParAs form dimers in solution, some of which are regulated by nucleotide binding (Davey and Funnell, 1994; Leonard, 2005b; Pratto et al., 2008; Dunham *et al.*, 2009), and it is assumed that these will form the basic unit for assembly into ATP-dependent polymers.

### A possible role for cellular components

It remains unclear whether Type I plasmid segregation systems have any requirement for specific host encoded factors to tether newly separated plasmids to the quarter cell positions, or to couple segregation to the cell cycle. Chromosomally encoded systems that are analogous to Type I segregation systems are known to act together with other factors to regulate and achieve chromosome segregation. For example, newly replicated chromosomes in *C. crescentus* are anchored at the cell poles by an extended network of the origin-binding protein PopZ (Bowman *et al.*, 2008; Ebersbach *et al.*, 2008; Ramamurthi and Losick, 2008), whilst the Soj/SpoOJ system from *Bacillus subtilis* functions in concert with both the replication initiator protein DnaA and the condensin complex Smc/ScpAB (Murray and Errington, 2008; Gruber and Errington, 2009). However, it is to be expected that chromosome segregation systems have a higher degree of complexity than their plasmid-encoded counterparts due to less restriction on genome space, the higher cost of failure of segregation, and the larger DNA cargo requiring transport.

Given that many chromosome origins also segregate to the quarter cell positions, for example in *E. coli* (Sherratt, 2003), it is possible that plasmids hijack a common host-encoded tethering complex. However, a number of genetic screens have tested for dependence on host factors, but no candidates required for segregation (as opposed to recombination and replication) have yet emerged (Biek and Cohen, 1986; Niki *et al.*, 1988; Ingmar *et al.*, 1998; Slavcev and Funnell, 2005). Additionally, different Type I plasmids within the same cell do not superimpose each other precisely at the quarter cell position (Ho *et al.*, 2002; Ebersbach *et al.*, 2005), and quarter-cell positioned plasmids display significant lateral movement (Sengupta *et al.*, 2010), both suggesting the absence of a fixed and universal cellular tether. Finally, segregation systems can be moved onto unrelated plasmids and confer localization typical of the segregation locus, even in unrelated hosts (Lin and Grossman, 1998; Yamaichi and Niki, 2000; Godfrin-Estevenon *et al.*, 2002; Møller-Jensen *et al.*, 2003). In the most extreme case, the chromosomal Soj/SpoOJ segregation locus from *B. subtilis* was used to accurately partition an unstable plasmid in *E. coli* cells (Yamaichi and Niki, 2000). Taken together, these experiments posit a role for either a universal cellular tether conserved across both Gram-positive and Gram-negative species, or for an independence from host-encoded DNA tethers.

### *Type II/ParM-based systems – actin-like proteins drives a mitotic-like mechanism*

Type II plasmid segregation systems are characterized by ATP-driven actin-like motors, in contrast to the WACA family ParAs of Type I systems. The general organization and principle of plasmid segregation is conserved: an adaptor protein interacts with its cognate centromere site to form a nucleoprotein complex,

and adjacent complexes are separated and localized by force generated by the motor protein (Figure 1). The best-characterized Type II segregation system, in fact the best characterized of any plasmid segregation system, is that encoded by the *Escherichia coli* R1 low copy number plasmid. This understanding results from both the intuitive simplicity of the system, and several decades of intense research (Gerdes and Molin, 1986; Jensen and Gerdes, 1997; Møller-Jensen *et al.*, 2003). Two studies have recently led to the identification of a number of other actin-like proteins, which show only a low sequence identity with ParM but which are also involved in plasmid segregation (Derman *et al.*, 2009; Polka *et al.*, 2009). While the molecular details of these novel Type II systems are yet to be characterized in detail, they appear to be unified by the use of dynamic actin-like filaments to generate DNA movement and are likely to be based on a similar general principle of adaptor/centromere-regulated filament dynamics. The R1 plasmid segregation system will be described in some detail, followed by a brief description of novel and diverse Type II systems.

Movement of the R1 plasmid is driven by dynamic instability in actin-like ParM filaments (Garner *et al.*, 2004; 2007). Light microscopy reveals dynamic ParM filaments running from pole to pole in a rod-shaped *Escherichia coli* cell, with clusters of R1 plasmid located at either end (Møller-Jensen *et al.*, 2003; Campbell and Mullins, 2007) (figure 7A). Purified ParM forms short and unstable filaments in the presence of ATP, and these filaments are stabilized by the addition of the adaptor ParR together with the centromere *parC* (Garner *et al.*, 2007). This leads to plasmid segregation though a mechanism of insertional polymerization: ParM forms short and unstable filaments throughout the cell until it encounters two plasmid-bound ParR/*parC* complexes, one at either end. Thus stabilized, ParM forms long and stable filaments and this causes bidirectional movement of plasmids, which are attached to the elongating tips (Møller-Jensen *et al.*, 2003; Campbell and Mullins, 2007; Garner *et al.*, 2007; Salje and Löwe, 2008).

**Structure and properties of ParM**

The three-dimensional structure of ParM is very similar to that of actin, despite a notably low sequence alignment (< 15%) (van den Ent *et al.*, 2002) (figure 6). Additionally, ParM forms polar, double-stranded filaments with an overall twist and rise very similar to actin although the handedness is reversed to left (van den Ent *et al.*, 2002; Orlova *et al.*, 2007; Popp *et al.*, 2008; 2009). In spite of an overall similarity in structure, the dynamics of ParM filaments differ significantly: whereas the two ends of an actin filament exhibit different rates of assembly/disassembly that result in a treadmilling movement, ParM exhibits equal activity at both tips (Garner *et al.*, 2004). Furthermore, ParM filaments with uncapped ends exhibits ATP-driven dynamic instability (Garner *et al.*, 2007), an activity that had previously only been observed in microtubules but has since also been described for other Type II actin-like plasmid segregating filaments (Derman et al., 2009; Polka *et al.*, 2009).

**The interaction between ParM and ParR/parC**

The exact molecular mechanism by which ParR/*parC* protects ParM caps from disassembly remains enigmatic but is key to understanding this system. X-ray crystallography and electron microscopy analysis of oligomeric ParR bound to *parC* from R1, pB171 (*par1)* and pSK41 plasmids revealed that each type of nucleoprotein complex assembles as a large continuous open helix, or solenoid structure (Møller-Jensen *et al.*, 2007; Schumacher *et al.*, 2007a) (figure 6). ParR binds to *parC* as a dimer via its ribbon-helix-helix (RHH2) motif in the N-terminal domain, while the C-termini of ParR are disordered and face into the centre of the solenoid. It has been shown that a single nucleoprotein complex interacts with the tip of a single ParM double-stranded filament, and further that one ParM filament can interact with one ParR/*parC* complex at either end (Choi *et al.*, 2008; Salje and Löwe, 2008). This is important as it means that a single pair of plasmids could be separated by a single ParM filament. This is composed of two ParM protofilaments aligned in a parallel orientation, and the question of how the same nucleoprotein structure can interact with both non-identical ends of a polar filament remains one of the key puzzles in understanding this molecular mechanism. The disordered C-terminal tails of ParR interact with loops on the outside surfaces of the double-stranded ParM filament and somehow act to protect the filament from disassembly as well as staying faithfully associated with the growing tip (Schumacher *et al.*, 2007a; Salje and Löwe, 2008). This principle is reminiscent of the way in which formins ride the barbed ends of actin filaments and promote their elongation (Schonichen and Geyer, 2010), although there is no evidence of any evolutionary relationship between formins and ParR.

**Cellular position of segregating plasmids**

R1 plasmids are localized close to the poles of growing cells in contrast to the approximate quarter-cell position employed by Type I plasmids (Weitao *et al.*, 2000; Adachi *et al.*, 2006; Ringgaard *et al.*, 2009). Furthermore, plasmid separation is apparently uncoupled to the host cell cycle and simply proceeds through cycles of ParM elongation and dissociation (Campbell and Mullins, 2007). Newly replicated plasmids are located at mid-cell until ParM elongates between them and moves them to their pole-proximal positions, at which point ParM depolymerizes in response to an unknown trigger. Plasmids remain at their pole-proximal positions for a short period before

randomly diffusing away through the cell. If two foci encounter one another they remain associated for a short time before ParM elongates between them and the cycle begins again (Campbell and Mullins, 2007). The reported copy number of R1 plasmid is somewhat higher than F and P1 plasmids, at 4–6 copies per chromosome equivalent compared with 1–2 (Nordström *et al.*, 1980), although these values are dependent on growth conditions. Like many bacterial plasmids, R1 is thought to be generally present as small clusters of plasmids. The exact position of plasmids and ParM filaments within the cell relative to the nucleoid and cell membrane is difficult to determine using light microscopy (figure 7). Importantly, it is unclear whether pole-positioned plasmid clusters remain associated with or extend beyond the nucleoid, or whether there is any membrane association that might serve as an anchor. A recent study by this author in which cryoelectron microscopy was used to study ParM filaments in thin sections of cells revealed snapshots of filaments running close to the edge of the nucleoid (Salje *et al.*, 2009) (Figure 7), but it is not clear whether this positioning is attributable to a weak association of ParM for the DNA, an interaction between the plasmid and the nucleoid, or simply the impenetrability of the nucleoid to growing ParM filaments. The images showed that ParM is present in the cell as small tightly packed bundles of 3–5 filaments (Figure 7B), and the close match between the plasmid copy number and the filament number supports the notion that each double-stranded filament might be attached to just one plasmid within a cluster at either end.

The initial stages of the ParMRC segregation process are poorly understood, and involve the capture of ParM filaments to plasmid-bound ParR/*parC* sites as well as physical separation of clustered plasmids. Newly replicated plasmids are probably linked through host-encoded DNA-binding proteins and DNA concatenation, and the release of this linkage may provide an additional regulatory step for the segregation process. Specific plasmid coupling though ParR-bound *parC* sites has been demonstrated *in vitro* (Jensen *et al.*, 1998; Møller-Jensen *et al.*, 2007), and it is possible that this may occur in the cell and facilitate the recruitment of ParR/*parC* complexes to both ends of ParM filaments.

## A comparison of Type II (ParM-based) plasmid segregating proteins

A recent extensive phylogenetic study identified over 35 families of previously uncharacterized actin-like proteins in bacteria (Derman *et al.*, 2009), a number of which appear to be components of plasmid segregation systems and can thus be classified as novel Type II segregation

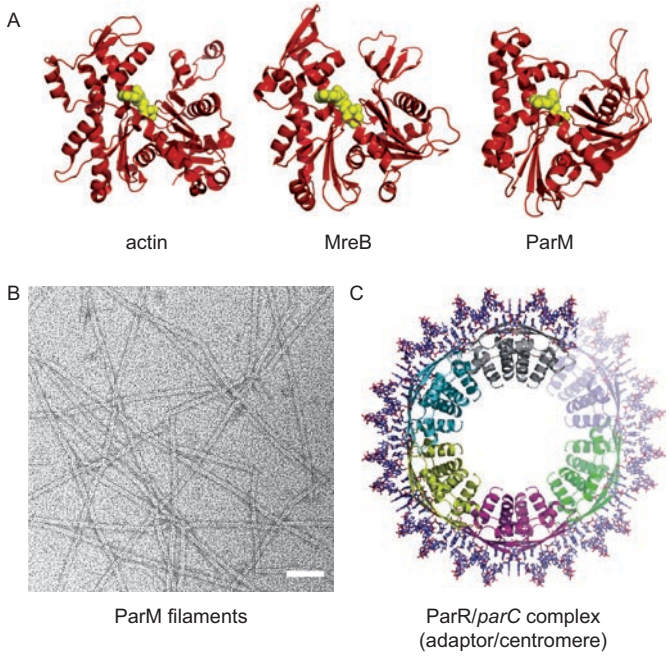


**Figure 6.** Structure of ParM and the ParR/parC complex. (A) Crystal structure of rabbit contractile muscle actin (ADP-bound form, PDB accession number 1J6Z, Otterbein *et al.*, 2001), *T. maritima* MreB (ADP-bound form, PDB accession number 1JCG, van den Ent *et al.*, 2001) and *E. coli* R1 ParM (ADP-bound form, PDB accession number 1MWM, van den Ent *et al.*, 2002). Nucleotide is shown in yellow. (B) Negatively stained electron micrograph of ParM polymerized in the presence of ATPγS. From Jakob Møller-Jensen, personal communication. Scale bar = 100 nm. (C) Crystal structure of pSK41 ParR bound as multiple dimers to *parC* DNA. Adapted from Schumacher *et al.* (2007a).

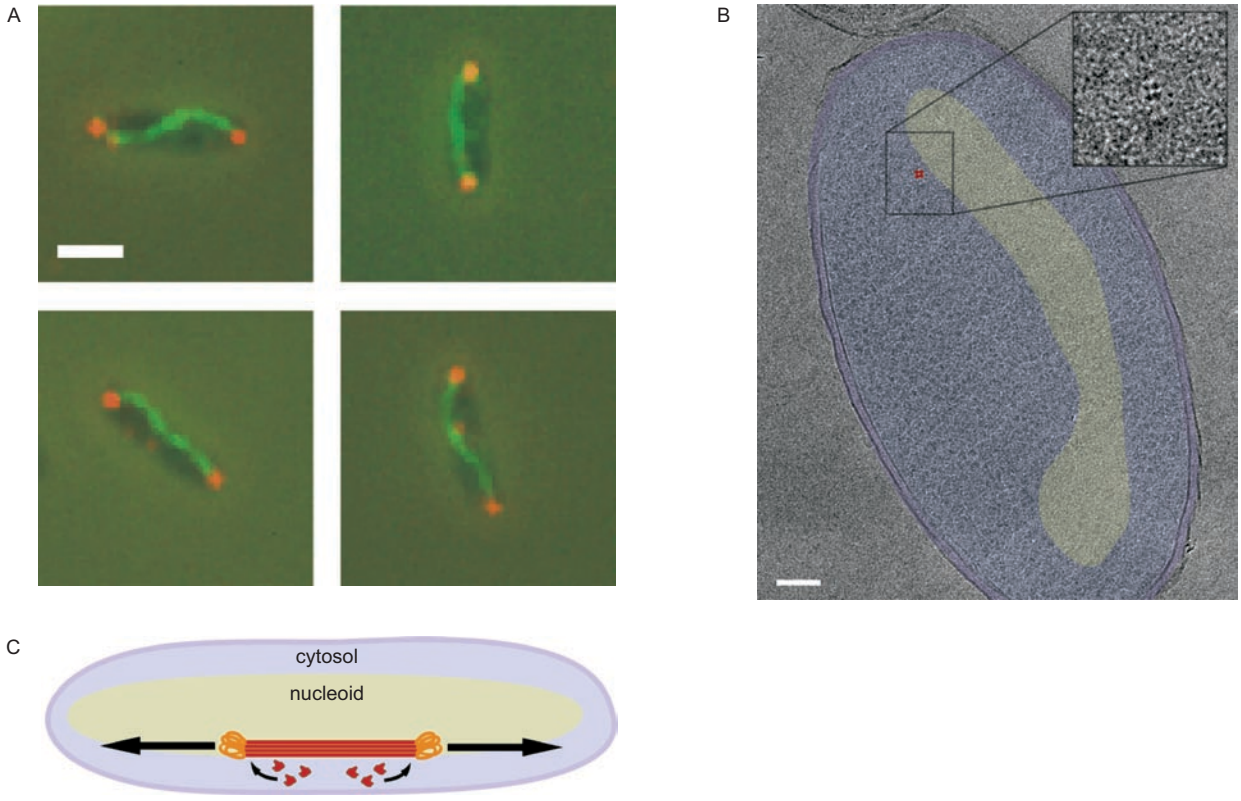


**Figure 7.** A cellular description of the ParMRC system. (A) Immunofluorescent labeling of ParM (green) and plasmids (red) in *E. coli* cells carrying plasmids with the R1 ParMRC segregation system. Taken from Møller-Jensen *et al.* (2003). Scale bar = 1 μM. (B) Cryoelectron micrograph of a thin section of a frozen *E. coli* cell harbouring the R1-derived plasmid pKG491. Inset and red spots show a small bundle of ParM filaments. The nucleoid is indicated in yellow, the cytosol in blue and the cell wall in purple. Scale bar = 100 nm. Taken from Salje *et al.* (2009). (C) Schematic indicating the possible cellular position of plasmid-bound elongating ParM filaments. Plasmids are shown in yellow and ParM filaments in red. From Salje *et al.* (2009).

systems. These share a very low sequence identity with both actin and with ParM, and could only be identified using multiple rounds of BLAST searches based on a signature nucleotide-binding motif. However, this low similarity with ParM at the sequence level does not necessarily imply that they originated independently, since genes carried on plasmids are free to diverge much more rapidly than on core genomes. It remains to be shown whether there are significant mechanistic differences in the molecular mechanisms of these divergent Type II systems, and this is an area of active research. Two novel ParM-like proteins have been studied in some detail: AlfA, which had previously been identified as the motor component of the partitioning system of the *Bacillus subtilis natto* plasmid pBET131 (derived from pLS32) (Becker *et al.*, 2006), and Alp7A from *Bacillus subtilis natto* plasmid pLS20, which was identified in the recent bioinformatics screen (Derman *et al.*, 2009). Both proteins have been shown to form dynamic pole-to-pole filaments in cells, which are required for plasmid segregation (Becker *et al.*, 2006; Derman *et al.*, 2009). Two separate *in vitro* studies suggested that the dynamics of AlfA differ from that of R1 ParM (Polka *et al.*, 2009; Popp *et al.*, 2010a), although it remains to be seen whether these differences are significant at the cellular level. A recent study on AlfB, the ParB equivalent from this pBET 131 plasmid, concluded that the centromere-binding and transcriptional regulation were functionally analogous to that from the classical R1 ParMRC system (Tanaka, 2010). Time-lapse microscopy of cellular GFP-tagged Alp7A, from the second family of divergent ParMs, revealed dynamically unstable filaments strongly reminiscent of R1 ParM, although the filaments were also found to exhibit treadmilling when fully extended between the two cell poles for some period of time (Derman *et al.*, 2009). There are currently no crystal structures available for any of these proteins, although low-resolution electron microscopy and X-ray fiber diffraction studies of AlfA have suggested that there are certain structural differences in filament formation (Polka *et al.*, 2009; Popp *et al.*, 2010a).

### *Type III/TubZ-based systems – a role for tubulin-like proteins*

One of the notable features of plasmid-segregation systems is their propensity to borrow and adapt chromosomal

proteins for their own purposes. Indeed, it is remarkable just how adaptable a filament-forming protein such as actin appears to be since one might naively expect that the structural dictates on polymerization would be strict. Most bacterial chromosomes carry at least one homolog of the two major eukaryotic cytomotive filament-forming proteins actin and tubulin (Graumann, 2004; Michie and Löwe, 2006; Erickson, 2007), and it was with considerable excitement that two separate groups recently reported the first plasmid-encoded tubulin-like proteins, both in *Bacillus* species (Berry *et al.*, 2002; Tang *et al.*, 2006; Tinsley and Khan, 2006; Larsen *et al.*, 2007). Both TubZ-bt from *Bacillus thuriengensis* plasmid pBtoxis and TubZ-ba (previously RepX) from *Bacillus anthracis* plasmid pXO1 encode tubulin/FtsZ-like GTPases, which are required for plasmid stability. They are found in a putative segregation locus together with a smaller protein that may be analogous to a ParB-like adaptor protein and which is called TubR, as well as a possible centromere-like repeat region upstream (Figure 1, Table 1). TubZ forms dynamic filaments that exhibit treadmilling action and require TubR for formation (Larsen *et al.*, 2007; Margolin, 2007; Chen and Erickson, 2008). These dynamic filaments do not appear to require any additional host-encoded factors since they could also be observed when just TubZ and TubR were coexpressed in *E. coli* (Larsen *et al.*, 2007). In *B. thuriengensis* TubZ-GFP forms filaments that move along one side of the cell and are able to follow the curve of the membrane at the poles and grow back along the opposite side of the cell. How this movement acts to separate plasmids is unclear, but likely requires some mechanism to attach TubZ filaments to the plasmid through the TubR protein.

## A comparison with other systems

### *Eukaryotic plasmids*

Plasmids are widespread amongst prokaryotes, but a number have also been described amongst the lower eukaryotes. Plasmids have not been found in animals although extrachromosomal DNA elements exist in the form of viruses. A few plasmids have been described in plants, some of which are transferred from pathogenic bacteria, but the large majority of eukaryotic plasmids are found in fungi (Meinhardt *et al.*, 1990; Griffiths, 1995).

The best-characterized eukaryotic plasmid is the 2-μm circle plasmid of *Saccharomyces cerevisiae* (Jayaram *et al.*, 2004). This plasmid contributes no observable advantage to the host cell, and as such is often described as a selfish DNA element (Velmurugan *et al.*, 2003). The small 6 kb plasmid is present at approximately 60 copies per cell, and similar to prokaryotic plasmids it is organized into one or two clusters. The plasmid clusters are located within the cell nucleus, where the plasmid harnesses the replication and mitotic machinery of the host cell (Velmurugan *et al.*, 2000; Yang *et al.*, 2004). Thus the major difference between the segregation approach adopted by the 2-μm plasmid and that of low copy number prokaryotic plasmids described in this review is that the former lacks the independence of the latter. This may result from the evolutionary origin of the yeast plasmid, or from the fact that the cell cycle in eukaryotes is tightly regulated resulting in pressure to synchronize with the host. The stability locus of the 2-μm plasmid has a similar general arrangement to the prokaryotic plasmid systems, encoding two proteins (Rep1p and Rep2p) and a DNA region (*STB*) (Jayaram *et al.*, 2004), although there is no evidence that either of the two proteins is a force generating nucleotide-binding protein analogous to the prokaryotic motor elements. In contrast, Rep1p and Rep2p bind to the *STB* locus and recruit it to the centromere-binding mitotic motor-protein Kip1p (Cui *et al.*, 2009). Thus attached, plasmids are able to share the host segregation machinery and move through the predivisional cell together with the separating mitotic chromosomes.

### *Eukaryotic mitosis*

Eukaryotic mitosis involves a complex and sophisticated set of mechanisms to move newly replicated DNA apart, parts of which may resemble prokaryotic systems but many parts of which appear to exist only in eukaryotes. Even a relatively simple organism such as yeast employs tens or even hundreds of proteins to regulate and execute the wonderfully synchronized dance that involves condensing the replicated DNA, moving and positioning it in pairs at the mid-cell, uncoupling the paired chromatids and then bringing them to opposite poles prior to cytokinesis (Kline-Smith *et al.*, 2005; Nasmyth, 2005; Westermann *et al.*, 2007). However, despite the fact that this process involves several more orders of complexity than prokaryotic plasmid segregation, the basic requirements and principles remain the same. The system needs to distinguish between two (or more) molecules of DNA, pair them and then separate the pairs, bring them to the opposite pole positions, and then ensure that they remain there at the time of cell division. Eukaryotic mitosis has the advantage that the cell cycle is tightly regulated. Consequently, the process of separation is finely timed, and occurs only once. In contrast, most plasmid partitioning mechanisms are based on cycles of activity that iteratively test the positions of the plasmids and ensure that they are positioned distant (or equidistant) from one another.

Both mitosis and plasmid segregation employ nucleotide-driven filaments (Löwe and Amos, 2009; van der Vaart *et al.*, 2009), although the force for eukaryotic mitotic motility is supplemented by the activity of motor proteins which have not been reported in bacteria. This

difference is likely to result from the relatively large distance that mitotic DNA has to travel though the eukaryotic cell compared with that through the prokaryote, and the larger size of DNA cargo that is transported. Whereas mitosis always uses microtubules made from tubulin to move DNA, plasmid segregation systems are variable and based on at least three different kinds of filaments (Gordon and Wright, 2000; Ebersbach and Gerdes, 2005; Ghosh *et al.*, 2006; Gitai, 2006). Thus, an increase in complexity is countered by some loss of diversity, which is imposed by the large number of interacting and interdependent elements in a complex system.

### *Bacterial chromosome segregation*

Our understanding of bacterial chromosome segregation lags behind that of both eukaryotic mitosis and bacterial plasmid segregation systems (Gordon and Wright, 2000; Sherratt, 2003; Leonard *et al.*, 2005a). It was historically assumed that prokaryotic chromosome segregation occurred concomitantly with lateral growth of the cell wall, through insertion of newly replicated origins into the cell membrane (Jacob *et al.*, 1966). However, it is now clear that newly replicated origins are in fact actively transported to specific positions at opposite poles of the cell, and that the speed of transport is much faster than that of cell growth (Lau *et al.*, 2003; Sherratt, 2003; Wang *et al.*, 2008). The exact mechanism by which this occurs is not understood, and may vary significantly between organisms and some may have multiple mechanisms. The chromosomally-encoded bacterial actin MreB may be involved in this process in *C. crescentus* (Gitai *et al.*, 2005), although it is now clear that it is not required in *E. coli* or *B. subtilis* (Karczmarek *et al.*, 2007). As described above, a large number of bacterial and archaeal chromosomes encode putative partitioning systems analogous to the Type I systems close to their origins of replication, with the notable exception of *Escherichia coli.* The extent to which these systems are actually involved in chromosome segregation is unknown, and in some cases the partitioning systems are likely redundant since they can be deleted with no significant deleterious effect at least under certain laboratory growth conditions (Yamaichi and Niki, 2000). However, there are species in which these systems are known to be critical for accurate chromosome segregation, and the best studied of these are the Soj/SpoOJ system of *Bacillus subtilis* (during spore formation) (Ireton *et al.*, 1994; Marston and Errington, 1999; Pogliano *et al.*, 2002; Lee and Grossman, 2006; Gruber and Errington, 2009), the ParABS system from *Caulobacter crescentus* (Mohl and Gober, 1997; Mohl *et al.*, 2001; Figge *et al.*, 2003; Toro *et al.*, 2008) and the ParAI/BI/SI system from *Vibrio cholerae* (Fogel and Waldor, 2006; Saint-Dic *et al.*, 2006).

How similar are these chromosomal partitioning loci to those of the Type I plasmids? An extensive phylogenetic analysis based on genetic organization and ParA sequence showed that the partitioning systems fall into three related but distinct clades, and that these are generally encoded on either chromosomes or plasmids (Gerdes *et al.*, 2000; Hayes, 2000). Interestingly, a number of organisms which carry two chromosomes were shown to encode the chromosome-like partitioning locus on one (usually the larger) and a non-chromosomal-like system on the other (usually the smaller) suggesting a plasmid origin for the second chromosome (Gerdes *et al.*, 2000). The mechanism by which chromosomal ParA (or Soj) drives DNA segregation is unclear but shares many features with that of Type I plasmid ParAs. ParA (Soj) undergoes dynamic redistribution through the cell (Marston and Errington, 1999; Sullivan and Maddock, 2000; Fogel and Waldor, 2006), forms ATP-dependent filaments (Leonard, 2005b), interacts non-specifically with DNA (Leonard, 2005b; Hester and Lutkenhaus, 2007) and binds to the ParB/*parS* complex.

## Discussion

### *Thinking about plasmids in cells*

Unlike the simplified models for low copy plasmid segregation discussed so far, plasmids actually replicate and segregate within a complex and crowded cellular environment. While it is impossible to predict and account for all the relevant variables within a living cell, we can consider the two following problems. First, to what extent does the plasmid depend on its host cell to survive and second, to what extent does the cellular environment affect the models presented here? These problems are closely related but will be addressed separately for clarity.

The extent to which a plasmid borrows the machinery of its host cell varies, although basic functions such as replication, transcription and translation are almost always encoded by the host. There is also a spectrum of variation in the specificity of host cell that the plasmid can inhabit, with something approximating a non-curable mini-chromosome at one end and something closer to a promiscuous bacteriophage at the other. So-called broad host-range and narrow host-range plasmids occupy different positions along that scale, and the specificity of the origin of replication is an important determinant of promiscuity (Kues and Stahl, 1989; van Belkum and Stiles, 2006). Low copy plasmid segregation systems typically do not depend heavily on specific chromosomally encoded factors, and this probably provides the competitive advantage of being able to move between different cells and to adapt to changes within the cell's chromosome. Nevertheless, plasmid segregation systems have evolved to take advantage of generic cell structures, most strikingly the surface of the bacterial nucleoid thought to be used by Type I ParAs.

Plasmids, once segregated, often localize to reproducible positions in the cell (Weitao *et al.*, 2000; Li and Austin, 2002a; Gordon *et al.*, 2004; Ebersbach *et al.*, 2005) and it remains unclear whether this is a secondary effect of the interaction between segregation systems and the cellular topology, or whether some specific and as yet unidentified cellular factor is involved in tethering the DNA.

The second problem concerns the complexity in thinking about these plasmid segregation systems (or indeed any molecular systems) once they are within the cellular environment. The biochemical activities of individual components as measured *in vitro* are modulated by the crowding, pH, ionic strength and temperature within the cell, not to mention any specific interactions with proteins, membrane or ligands. The beauty of plasmid segregation systems lies in their simplicity. However, any plasmid segregation system is necessarily connected to a large DNA molecule that has molecular partners and steric demands of its own. The replication factory will have an anchoring effect on the plasmid during replication, as well as exerting a small but significant extrusion force on the newly replicated DNA molecules. Concatenation following replication will need to be resolved through the action of topoisomerases and has been proposed to play a role in the regulation of segregation (Martinez-Robles *et al.*, 2009; Witz and Stasiak, 2009). Cellular plasmid DNA is supercoiled, and this both affects and is affected by the DNA binding activity of segregation proteins. It is not known whether newly replicated DNA is anchored to the membrane at any time through DnaA protein, like the host chromosome (Aranovich *et al.*, 2006; Kaguni, 2006), and how this might affect plasmid segregation (Banack *et al.*, 2000; Kim *et al.*, 2000). Finally, it is unclear the extent to which the physical properties of the DNA molecules – size, compaction and charge – together with the crowded cellular environment, will impact the polymerization of filaments within the cell.

### *Plasmid segregation systems as a self-organized assembly*

Plasmid segregation systems provide an excellent example of dynamic self-organization. This describes an emergent ordered behavior, which results from an input of energy (in this case from nucleotide binding and/or hydrolysis) and the specific properties and resulting interactions of the individual components within their parameter space. No extra input of information is required and in the case of the R1 plasmid this emergent dynamic behavior can be reconstituted *in vitro* (Garner *et al.*, 2007). Self-organization can be contrasted with self-assembly which leads to a static final product. Whilst plasmid segregation describes a simple and controlled example of self organization, this phenomenon can be described on many levels in biology and indeed an entire living cell or organism can be considered as a self organized system with dynamic organization emerging from the interaction of gene products combined with an input of energy.

### *The elegance of plasmid segregation systems*

Low copy number segregation systems are under evolutionary pressure to occupy as little genome space as possible. Accordingly, they have developed strategies to build a complex and highly controlled system from a remarkably small amount of genetic real estate. They have adopted a number of principles to increase efficiency, including the following: (a) build large structures out of small subunits using extensive oligomerization; (b) modulate protein activity to get multiple functions from a single protein; and (c) use a cyclical rather than linear pattern of behavior, which is most easily achieved by binding, hydrolyzing and then releasing nucleotides.

All the plasmid segregation systems described here employ oligomerization to build large structures from small genes. The most obvious is the extensive use of filaments to cover large regions of the cell. More subtle, however, is the use of oligomeric DNA-binding proteins to build large nucleoprotein assemblies. For example, the ParR protein from R1 plasmid is encoded by a 393-bp gene, but binds as a group of 10 dimers to a 300-bp *parC* region of DNA to form a 260 kDa complex capable of binding and stabilizing ParM filaments (Jensen *et al.*, 1994; Breuner *et al.*, 1996; Møller-Jensen *et al.*, 2007; Schumacher *et al.*, 2007a). In contrast, eukaryotic formins, which perform an equivalent function of capping and stabilizing actin filaments, are encoded by at least two separate 3000-bp genes to form a dimeric 120–220 kDa protein, which are additionally assisted by cofactor proteins such as profilin (Paul and Pollard, 2009; Schonichen and Geyer, 2010).

Proteins encoded by low copy number plasmids often have multiple functions, which are finely regulated through interaction with other segregation proteins, nucleotide or DNA. Type Ia ParA proteins act as a transcriptional repressor when bound to specific promoter DNA in the presence of ADP, or as a plasmid anchoring and segregating complex when interacting in the ATP-bound conformation with non-specific DNA and ParB/*parS* (Davey and Funnell, 1994; Bouet and Funnell, 1999; Dunham *et al.*, 2009). Equally the R1 ParR protein behaves as an autorepressive transcriptional regulator when bound to *parC* (Jensen *et al.*, 1994; Møller-Jensen *et al.*, 2007; Schumacher *et al.*, 2007a; Salje and Löwe, 2008), while at the same time interacting with the ends of ParM filaments (Møller-Jensen *et al.*, 2003; Schumacher *et al.*, 2007a; Choi *et al.*, 2008; Salje and Löwe, 2008). Further, the interaction of ParR with ParM has two aspects: the C-terminal tails of ParR interact specifically

with the sides of the ATP-bound end subunits of ParM and somehow stimulate ATPase activity (Schumacher *et al.*, 2007a; Salje and Löwe, 2008), whilst the oligomeric ParR/*parC* complex forms a large structure that physically encloses ParM filaments (Møller-Jensen *et al.*, 2003; Schumacher *et al.*, 2007a; Salje and Löwe, 2008).

Finally, all the plasmid segregation systems described so far are based on a repeating cyclical pattern of assembly, compared with the one-off DNA segregation of eukaryotic mitosis. The advantage of such an approach is that it avoids the need for an extra regulatory system that would be required to sense and coordinate a one-off event. Further, there is an advantage to not being closely linked to the host cell cycle machinery as it facilitates propagation of the genetic element through horizontal transfer to other organisms.

### *A case for convergent evolution: diverse solutions to the same problem*

One of the curious features of all the plasmid segregation systems described here is the conserved pattern of genetic organization and overall principle for segregation (Figure 1). All systems encode one nucleotide-binding motor protein, one DNA-binding adaptor protein, and at least one centromere region of variable position and length. Accordingly, all systems are based on the principle of a filament-forming protein that interacts with a plasmid-bound nucleoprotein complex although this has not been fully shown for Type III systems. The motors of Type I, Type II and Type III plasmid segregation systems are unrelated. All, however, have a chromosomally encoded homolog from which they presumably originally evolved. The Type I motor (typified by ParA) is related to the cell division protein MinD, as well as the chromosome segregating protein Soj/ParA (Gerdes *et al.*, 2000; Hayes, 2000); the Type II motor (typified by ParM) is related to the cytoskeletal filament-forming protein MreB as well as the cell division protein FtsA (Gerdes *et al.*, 2000; van den Ent *et al.*, 2002), whilst the Type III motor (typified by TubZ) is related to the bacterial tubulin-like protein FtsZ that drives bacterial cell division (Larsen *et al.*, 2007; Margolin, 2007; Chen and Erickson, 2008). Additionally, the organization of Type I segregation systems is conserved in the chromosomally located ParABS or Soj/SpoOJ chromosome segregation system (Gordon and Wright, 2000; Ebersbach and Gerdes, 2005; Schumacher, 2007).

It is not clear whether the conserved pattern of organization between segregation systems reflects a common origin or if they evolved completely independently. In the former case, motor elements in the original gene cluster would have occasionally been replaced with different and preferable chromosomal filament-forming proteins. This would have occurred at least four times, with the adoption of Soj/ParA (Type Ia), MinD (Type Ib), MreB (Type II) and FtsZ (Type III). In this model, it would be expected that adaptor proteins could cluster together in a phylogenetic group not necessarily coincident with their respective motor proteins. In support of this model, both the adaptor proteins and the overall organization of Type Ib systems are much closer to that of Type II, although the Type Ib motor protein is closer to that of Type Ia (Hayes, 2000; Fothergill *et al.*, 2005). It may be, therefore, that Type Ia and Ib systems are in fact more distant from one another, and that Type Ib and Type II diverged by a single switch in motor protein. Alternatively, the different types of segregation systems may have originated quite independently, simply reflecting the success of combining DNA-binding proteins with filament-forming proteins to achieve DNA segregation.

### *A case for divergent evolution: the remarkable adaptability of filaments*

Monomeric ParM, actin and MreB are extremely similar in structure but form structurally and dynamically distinct filaments that have been adopted for different means (van den Ent *et al.*, 2002; Orlova *et al.*, 2007; Popp *et al.*, 2008; 2010b; van den Ent, 2001b; Garner *et al.*, 2004; Holmes *et al.*, 1990; Oda *et al.*, 2009). Using a similar monomeric structure, actin and ParM form double filaments with opposite handedness (Orlova *et al.*, 2007; Popp *et al.*, 2008), while MreB forms filaments that are uniquely flat rather than twisted (van den Ent *et al.*, 2001b). There have been reports of prokaryotic actin-like filaments that can exhibit both treadmilling (Kim *et al.*, 2006; Derman *et al.*, 2009; Popp *et al.*, 2010b) and dynamic instability (Garner *et al.*, 2004), the latter being a phenomenon previously only observed in microtubules. Bacterial cytoskeletal proteins typically display very low sequence identities with their eukaryotic analogs, and in fact this is one of the reasons why the bacterial cytoskeleton remained undiscovered for so long. The overall sequence identity between MreB and actin or MreB and ParM is less than 15% (van den Ent *et al.*, 2001a; 2001b; 2002), although the nucleotide-binding active site is significantly more conserved. Therefore, the same tertiary fold of these proteins can tolerate extensive changes in primary sequence resulting in an ease of genetic experimentation, and this led to the demonstrable emergence of dynamically and structurally distinct filaments.

This principle of diversity can also be applied to Type I ParAs, although the structural and physical properties of the filaments are not well characterized. The structures of P1 ParA, Soj and MinD are very similar (Cordell and Löwe, 2001; Leonard, 2005b; Dunham *et al.*, 2009) (Figure 5), and the addition of a small amphipathic helix is sufficient to recruit MinD to the membrane (Koonin, 1993; Cordell and Löwe, 2001) while the addition of an

extended N-terminal domain on Type Ia ParA confers specific promoter DNA binding (Dunham *et al.*, 2009). One of the most intriguing aspects of the Type I segregation systems is the incredible variation in the fine-tuning of closely related ParA proteins. Evidently, the system can tolerate prolific experimentation with rates of nucleotide hydrolysis and polymerization, and with DNA binding affinity. This mutual tinkering in motors and adaptors has resulted in a variety of distinct but delicately balanced mechanisms by which the same basic components act together to achieve the same final goal.

### *Key remaining questions*

The most pressing questions in the understanding of plasmid segregation systems fall into three broad groups: (i) molecular details; (ii) evolutionary questions; and (iii) understanding segregation systems in their cellular environment.

Outstanding questions remain concerning the mechanistic details of all three segregation systems. For Type I plasmids, it remains to be shown exactly how the various activities of adaptor proteins and DNA affect the polymerization, nucleotide hydrolysis and DNA binding of ParA proteins and how mutual modulations between all three components orchestrate plasmid segregation. There are currently no detailed structures of any ParA filaments, less any models for the interactions between the filaments and the adaptor/centromere complex. ParA filaments are thought to assemble over the surface of the nucleoid (Ebersbach *et al.*, 2006; Castaing *et al.*, 2008; Ringgaard *et al.*, 2009) and this is frequently compared to the assembly of MinD over the surface of the cell membrane. Unlike the continuous membrane surface, however, the nucleoid is actually composed of a compacted polymer and it is difficult to envisage how a continuous filament might polymerize over this discontinuous and uneven surface. In contrast, the components of the Type II R1 segregation system are well understood structurally and biochemically, although it remains to be seen exactly how the adaptor/centromere complex interacts with and modulates the activity of ParM filaments, and what causes dissociation of filaments once plasmids have been separated. The recently discovered motor of Type III segregation systems, TubZ, forms dynamic treadmilling filaments that are required for segregation, but it is unclear how this is coupled to plasmid movement or where plasmids are positioned within the cell.

Plasmid segregation systems can be studied individually as models for simple DNA segregating machines, or in large numbers as models for the evolution of cytomotive filaments and as indicators of horizontal gene transfer of genes and operons. Extensive phylogenetic analysis of plasmid segregation systems is impeded by the high sequence divergence in prokaryotic genomes and especially in prokaryotic extrachromosomal DNA elements, but in certain cases short, highly conserved regions can be sufficient to identify related proteins with low sequence identity (Derman *et al.*, 2009). An ideal genomic analysis would include highly divergent microbial genomes, such as those from environmental samples, as these may help to understand the frequency of transfer of a segregation system into unrelated hosts, as well as the coupling between segregation locus and origin of replication. Such an analysis will illuminate the evolutionary relationship between existing segregation systems, and may reveal novel solutions to the problem of segregation using the same core cytomotive filaments.

Finally, a complete understanding of any one DNA segregation system will require an appreciation of the segregation mechanism within its cellular environment. This exciting challenge necessitates an understanding of both specific and non-specific cellular factors. It is now clear that prokaryotic cells are highly organized in all four of their major structures: the periplasm, the cell membrane, the cytosol and the nucleoid. Plasmid segregation systems function within this context and thus a comprehensive model of plasmid DNA segregation will emerge only as rapidly as our understanding of prokaryotic cell structure.

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