Single-molecule Analysis of Protein-DNA Complexes Formed during Partition of Newly Replicated Plasmid Molecules in Streptococcus pyogenes*

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The Streptococcus pyogenes pSM19035 partition locus is ubiquitous among plasmids from vancomycin- or methicillin-resistant bacteria. An increasing understanding of this segregation system may highlight novel protein targets that could be blocked to curb bacterial proliferation. pSM19035 segregation depends on two homodimeric (δ2 (ParA) and ω2 (ParB)) proteins and six cis-acting centromeric noncurved parS sites. In the presence of ATP-Mg2+, δ2 (δATP-Mg2+)2 binds DNA in a sequence-independent manner. Protein ω2 binds with high affinity and cooperatively to B-form parS DNA. Atomic force microscopy experiments indicate that about 10 ω2 molecules bind parS, consisting of 10 contiguous iterons. Protein (δATP-Mg2+)2, by interacting with the N terminus of ω2 bound to parS, loses its association with DNA and relocalizes with ω2parS to form a ternary complex (δATP-Mg2+)2ω2parS with the DNA remaining in straight B-form. Then, the interaction of two (δATP-Mg2+)2ω2parS complexes via δ2 promotes pairing of a plasmid subtraction. (δδ60A+ATP-Mg2+)2, which binds but does not hydrolyze ATP, leads to accumulation of pairing intermediates, suggesting that ATP hydrolysis induces plasmid separation. We propose that the molar ω2:δ2 ratio regulates the different stages of pSM19035 segregation, pairing, and δ2 polymerization, before cell division.

Accurate segregation of newly replicated plasmids or bacterial sister chromosomes is achieved by evolutionarily distinct stabilization systems (for review, see 1–3). The mechanisms of stable maintenance of low copy number plasmids can be divided into those that function (i) by enhancing the resolution of oligomers into monomers, with the subsequent increase of the number of molecules to be segregated; (ii) by blocking proliferation of plasmid-free segregants; or (iii) by partitioning plasmid copies to daughter cells at cell division (4–6). The majority of the plasmid partition systems include two trans-acting homodimeric proteins (one binds a nucleotide cofactor and the other the centromere) and one or more cis-acting centromere-like sequences (for review, see 2, 3, 5). There are three types of nucleotide-binding proteins: type I or ParA ATPases, type II or ParM (actin-like) ATPases, and type III or TubZ (tubulin-like) GTPases. The mechanisms that underlie the accurate partitioning of plasmid DNA by the type I and type III motor proteins are still largely unknown (2, 3, 5). Centromere binding is performed by one of three well studied protein types. The large type Ia proteins recognize the cognate sequence via a helix-turn-helix domain and spread along the DNA up to several kilobases (7–9). The small type Ib and type II proteins recognize their cognate site via an antiparallel β-sheet of the ribbon-helix-helix fold and do not seem to spread from a cognate site into a nonspecific DNA sequence. The type Ib proteins (e.g. pSM19035-ω2) have an unstructured N-terminal domain required for activation of polymerization of ParA pSM19035-δ2 onto parS DNA (10), whereas the type II proteins have an extended C-terminal region involved in interaction with ParM (e.g. pSK41-ParR) (6). The presence of a centromere-binding protein and a centromere sequence in the type III partition system remains undetermined.

To gain insight into the general mechanism that manages accurate ParAB-dependent partitioning of type Ib plasmids at cell division and to compare them with type II plasmids in bacteria of the Firmicutes phylum, the early stages of partitioning of the Streptococcus pyogenes plasmid pSM19035 were dissected in vitro. pSM19035, which has extraordinarily long inverted repeat sequences that comprise about 80% of the genome, has 2 ± 1 copies/cell and is stably maintained (11–13) (Fig. 1A). The par loci of pSM19035 encode two trans-acting proteins, found as homodimers in solution (δ2 (ParA type I) and ω2 (ParB type Ib)), and six cis-acting ω2 target sites (parS centromeres) (14, 15) (Fig. 1A). The parS centromeres overlap with the upstream region of the promoter (P) of δ (Pδ or parS1), ω (Pω or parS2) and copS (Pcop or parS3) genes and are present twice in the pSM19035 genome (14) (Fig. 1A). Each parS centromere (parS1, parS1', parS2, parS2', parS3, and parS3') consists of 9, 7, and 10 contiguous iterons, respectively, in direct or inverse orientation with sequence 5'-WATCACW-3'. The centromeric-binding protein ωδ binds one iteron or a non-parS control site with very low affinity (kD ~ 1000 nM); however, it binds two iterons (kD ~ 20 ± 2 nM) or full-length parS sites (kD ~ 5 ± 1 nM) with high affinity and cooperatively. By
FIGURE 1. Genome organization of plasmid pSM19035 and proposed structure of the \( \omega_2 \)-parS complex. A, pSM19035 duplicated sequences, which comprise ~80% of the molecule, indicated by a thick line and unique nonrepeated sequences (NR1 and NR2) by a thin line. The replication origins (yellow boxes), direction of replication (denoted by arrows), the six resolution sites (orange boxes), and the parS sites (red boxes) are indicated. The outer thin arrows indicate the organization of the genes. The plasmid is divided in regions that direct (repS) and control (copS) replication (Rep region). Plasmid resolution (\( \omega_1 \), \( \omega_2 \), and \( \gamma \) genes) (SegA) and postsegregation growth inhibition (\( \epsilon \) and \( \zeta \); brown arrows) and segregation (\( \delta \) and \( \omega \); red arrows) (SegB) are indicated. The resistance to erythromycin (erm1 and erm2) and the set of poorly characterized genes in blue and purple arrows are also indicated. The upstream region of the promoters of the copS, \( \delta \), and \( \omega \) genes constitute the six cis-acting centromere-like parS or parS\(-1\) sites. A parS site consists of a variable number of contiguous 7-bp heptad repeats (symbolized by \( \omega_2 \)). B, structural model of \( \omega_2 \) bound to parS1 DNA. The overall structure of \( \omega_2 \) forming a left-handed matrix around straight parS1 DNA based on the crystal structure determined for (\( \omega_2 \))\(_{19} \), DNA and (\( \omega_2 \))\(_{19} \) complexes (10, 17) is shown.
binding to the centromeric sequences, $\omega_2$ acts as a specific transcription factor, required for the correction of downward fluctuations of plasmid copy number control; the expression of the $\epsilon$ and $\zeta$ proteins needed for blocking proliferation of plasmid-free segregants (16) (see above); the accurate expression of $\delta_2$ and $\omega_2$ and ensuring plasmid partitioning (14, 15, 17) (Fig. 1A). Protein $\omega_2$ forms a nucleoprotein complex at the centromere sites without apparent distortion (14) (Fig. 1B). In the presence of ATP-Mg$^{2+}$, $\delta_2(\delta$-ATP-Mg$^{2+})_2$ is an ATPase with sequence-independent DNA binding activity ($k_{50}$ of $\sim 170 \pm 20$ nm) (10). Protein $\delta(\delta$-ATP-Mg$^{2+})_2$ does not appear to participate in regulating the par locus, leaving this function entirely to $\omega_2$ (14).

The first 19 N-terminal residues of $\omega_2 (\omega_2,AN19)$ are dispensable, both in vivo and in vitro, for binding to parS DNA (18). The structures of $\omega_2,AN19$ alone or in complex with two iterons in direct (pentamers) or inverted (inverted pentamers) orientations have been determined (17, 19). Extrapolating from these structures, we predict that $\omega_2$ molecules assemble as a left-handed protein helix to wrap around the parS sites on B-form DNA (see Fig. 1B). To test the crystal structure model (Fig. 1B) and to gain insight into the architectural requirement, AFM$^3$ studies were performed. Here, we report the visualization of nucleoprotein complexes formed by $\omega_2$ on linear or circular parS DNA and its dynamic interaction with $\delta(\delta$-ATP-Mg$^{2+})_2$ to form the partition complex. The $\delta(\delta$-ATP-Mg$^{2+})_2$, $\delta(\delta$-ATP-Mg$^{2+})_2$ interaction from two straight partition complexes mediates site-specific pairing and formation of large superstructures on DNA. The topographies of those ParAB complexes were compared with the architecture of the ParRM partition system.

**RESULTS**

$\omega_2$ Forms a Nucleoprotein Complex without Apparent Distortion of parS DNA—To study the type of nucleoprotein complexes formed by wild-type $\omega_2$ with full-length parS site on linear or supercoiled DNA, AFM experiments were performed. The 70-bp parS DNA consists of 10 contiguous iterons (heptads) organized in three blocks of two 7-bp direct repeats and one 7-bp inverted repeat, plus one direct repeat (Fig. 1A). The measured length of the linear DNA agreed with the expected value ($\sim 966$ nm) for a 2927-bp DNA with a 0.33-nm rise/bp. The parS site was located 120 bp from one end. No end showed any apparent curvature.

Increasing concentrations of $\omega_2$ incubated with a fixed concentration of linear parS (1–20 $\omega_2$ molecules/DNA molecule) gave rise to an increased number of similar protein-DNA complexes, with a concomitant decrease in the number of protein-free DNA molecules (data not shown). The optimal ratio for limiting $\omega_2$ concentrations and lower amount of protein-free DNA molecules was observed in the presence of $\sim 7$ $\omega_2$ molecules/parS site (7.2 $\omega_2$/DNA molecule) (Fig. 2A). A visual inspection of the images revealed that DNA molecules with bound $\omega_2$ contained only one discrete straight complex at the parS site (Fig. 2, A and B). The distance between the $\omega_2$/parS complex and the nearest end had a mean value of 31.2 $\pm$ 5.0 nm (Fig. 2C). This was consistent with a length of 120 bp (theoretical value of 39.6 nm) if a minor $\omega_2$ spread ($<15$ bp toward the 5’ end of the repeats) was taken into consideration (see 15). Similar results were observed at $\sim 20$ $\omega_2$/DNA molecule (data not shown).

The interaction of $\omega_2$ with supercoiled parS DNA was examined (Fig. 2D). Similar results were observed when $\omega_2$ was bound to supercoiled DNA or when the DNA was nicked prior to examination; the latter results, which simplified the interpretation, are shown. In the presence of 7.2 $\omega_2$/DNA molecule, one nucleoprotein complex/circular DNA molecule could be clearly identified (Fig. 2D). Inspection of the images revealed that the DNA molecule with bound protein contained one discrete $\omega_2$/parS complex without apparent distortion of parS (Fig. 2D).

$^3$The abbreviation used is: AFM, atomic force microscopy.
The structure of the parS complex was analyzed by measuring the length and width of ω2 bound to linear or circular DNA (n = 70 and 100, respectively). The mean height of the DNA was 0.23 nm (data not shown), which deviated from the theoretical height for double-stranded DNA (2 nm), confirming that DNA is usually seen smaller than the sample itself in AFM images (25–27). The apparent length (26.3 ± 3.3 nm on linear and 25.8 ± 3.4 nm on circular DNA) of the complexes observed in AFM images is consistent with the length of the parS centromere (70 bp) covered by ω2 (Fig. 3, A and B). To calculate the width of the complexes, the width of the DNA was plotted against the width of the protein-DNA complexes in the same image. On average, the analyzed complexes were 4.05 ± 0.83 nm and 4.89 ± 1.18 nm wide for linear and circular DNA, respectively (n = 39). The structure of ω2 bound to mini-parS DNA shows that the protein wraps around the DNA (17), forming a cylindrical structure with similar height and width. Therefore, the volume was also calculated assuming a cylindrical shape of the complex and using the width value obtained as described above. The volume of ω2-parS DNA complexes calculated in this way was 180–320 nm³ (n = 100), consistent to the expected (~235 nm³ assuming that the theoretical volume of ω2 was 19.8 nm³ plus the DNA contribution).

To validate the results of volume analysis further with a second methodology, Image SXM 169 software was used. The average height and area of a manually defined nucleoprotein complex and the adjacent region containing only DNA were subtracted to determine the volume of the complex. The volume obtained by this method was 212 ± 42 nm³ for the ω2-parS complex on linear and 235 ± 56 nm³ on circular parS DNA (n = 36). Hence, it is likely that ω2 binds to full parS DNA with a stoichiometry of ~1 ± 0.2 ω2/iteron. This is consistent with stoichiometry experiments showing (i) minor ω2 spreading.
(<15 bp) toward the 5′ end of the contiguous iterons (see 15); (ii) 2.2, 3.1, and 4.5 ω₂ molecules binding DNA segments containing two, three, and four heptads, respectively (15); and (iii) the co-crystal structure of the ω₂ΔN19-mini-parS complexes (17).

ω₂ Bound to parS Promotes δ₂ Recruitment and Plasmid Pairing—At a ratio of 100 ω₂/DNA molecule, ω₂ binds to parS regions on linear DNA and mediates site-specific pairing with very low efficiency (~1% of the total molecules analyzed by electron microscopy in the presence of glutaraldehyde fixation) (10). At 7.2 ω₂/parS DNA molecule, ω₂ bound to linear or circular parS DNA but failed to promote any site-specific plasmid pairing (Fig. 2).

Protein (δATP-Mg²⁺)₂ (at 7 δ₂/DNA molecule) bound DNA in a sequence-independent manner (supplemental Fig. S1), but such protein-DNA complexes were not observed when ATP was omitted (data not shown). We failed to detect any site-specific intermolecular pairing (supplemental Fig. S1).

At 7.2 ω₂/DNA molecule, ω₂ promoted relocalization of (δATP-Mg²⁺)₂ from any position on the supercoiled DNA toward the ω₂-parS complex in more than 90% of the molecules with subsequent increase in the protein-DNA volume. A ternary complex ((δATP-Mg²⁺)₂;ω₂-parS) was observed in ~67% of the molecules with site-specific intermolecular pairing in the remaining fraction (Fig. 4A). When linear parS DNA was used, ω₂ bound to parS promoted redistribution of (δATP-Mg²⁺)₂ to relocalize with ω₂-parS to form the ternary complex. Similar results were observed when limiting protein concentrations were used (data not shown).

In the presence of subsaturating amounts of ω₂ and (δATP-Mg²⁺)₂ at 1:1 molar ratio, site-specific intermolecular pairing in a subfraction (~22%, n = 160) of the molecules was observed (Fig. 4A). Similar results were reported previously in the presence of an ~14-fold excess of both ω₂ and (δATP-Mg²⁺)₂ (at 1:1 ratios) proteins per linear DNA molecule when analyzed by electron microscopy in the presence of glutaraldehyde fixation (10).

The parS DNA region on the pairing complex was not significantly distorted by ω₂ binding or (δATP-Mg²⁺)₂ interaction with ω₂ (Fig. 4B). We could hypothesize that ω₂-mediated centromere pairing but the pairing complex is unstable, and (δATP-Mg²⁺)₂ was required to “activate” ω₂ to promote stable plasmid pairing. Because the presence of higher (δATP-Mg²⁺)₂ concentrations (14 or 28 δ₂/DNA molecule) under limiting ω₂ concentrations led to the formation of high order superstructures, with three or more ω₂-parS complexes paired by (δATP-Mg²⁺)₂ (Fig. 4A), we do not favor this hypothesis. Indeed, at }

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and site-specific pairing of supercoiled DNA (<1% of the analyzed particles, n = 138) were not observed (supplemental Fig. S2A). This is consistent with data showing that ω₂ΔN19, which lacks the first 19 residues, binds to parS DNA and represses promoter utilization with affinity similar to wild type (18) but fails to interact with (δ·ATP·Mg²⁺)₂ and to promote pairing of linear DNA by electron microscopic examination upon glutaraldehyde fixation at saturating concentrations of the protein (100 ω₂ΔN19/DNA molecule) (10).

The ω₂T29A mutant binds DNA with low affinity and without sequence specificity (18). In the presence of a 10-fold excess of ω₂T29A/DNA molecule, ω₂T29A formed discrete complexes on DNA. At 2.5:1 ω₂T29A:δ, molar ratios, ω₂T29A promoted relocation of (δ·ATP·Mg²⁺)₂ from any position on the DNA toward the ω₂T29A-DNA complex in ~75% of the molecules, and pairing of multiple plasmid in only ~8% (n = 136) of the analyzed particles was observed (supplemental Fig. S2B). Protein (δ·ATP·Mg²⁺)₂ seems to interact with all ω₂T29A-DNA complexes, forming large protein-DNA complexes.

Relative Stoichiometry of the Partition Complex—To study the apparent stoichiometry of all three components of the partition complex, we compared the volumes of ω₂-parS complex with the ternary (δ·ATP·Mg²⁺)₂·ω₂·parS partition complex and

FIGURE 4. Structure of the parS·ω₂·(δ·ATP·Mg²⁺)₂ complex. A, number of plasmid molecules/complex observed when circular parS DNA is incubated with ω₂ and increasing concentrations of δ. Column 1 shows the number of monomeric DNA molecules (ternary complex); columns 2, 3, 4, and 5+ denote the percentage of complexes formed with 2, 3, 4 or more plasmid molecules (higher order complexes). B, summary of AFM images. Panel a, single pair mediated by a large nucleoprotein complex. Panel b, intermolecular pair showing two (panels c–e) or three or more (panel f) distinct nucleoprotein complexes interacting.
**pSM19035 Partition Complex**

**A**

![Graphs showing the number of molecules per complex](image)

**B**

![Images showing the structure of the paired complex](image)

**FIGURE 5. Structure of the parS-\(\omega_2(\delta D60A-\text{ATP-Mg}^{2+})_2\)_2 complex.** A, quantification of plasmid molecules/complex observed when circular parS DNA (25 nM) was incubated with 180 nM \(\omega_2\) and increasing concentrations of \(\delta D60A\) (175 and 350 nM). The column indication is as in Fig. 4. B, two representative images showing two and three plasmids paired.

The mechanisms that have evolved to segregate genomes of Firmicutes differ between plasmids of a discrete structure without significant spreading or compaction, shortening, or distortion of the DNA. This is consistent with (i) cooperative binding of \(\omega_2\) to 7–10 contiguous iterons (parS sites) with a marginal spreading onto non-parS DNA, as shown in DNase I protection studies where \(\omega_2\) spreads \(<15\) bp, if at all; (ii) DNA titration of increasing numbers of heptads, indicating that \(\omega_2\) binds with a \(\omega_2\):heptad stoichiometry of 1:1 (15); (iii) each iteron recruiting one \(\omega_2\) molecule, implying that the fully bound parS site is cooperatively coated by \(\sim10\) \(\omega_2\) molecules (15, 17); and (iv) the homotetrameric structure of \(\omega_2\) binding to mini-parS, which forms a left-handed wrap around ideal B-form DNA (17). The topography of \(\omega_2\) (ParB Ib) bound to parS differs from P1-ParB or F-SopB (ParB Ia) bound to parS or sopC. Both P1-ParB and F-SopB bind and spread up to several kilobases of DNA in a centromere-dependent manner. In the case of P1, the DNA might wrap around a multimeric protein core (28), whereas F-SopB wraps DNA in a right-handed manner (29).

The mechanisms that have evolved to segregate genomes of Firmicutes differ between plasmids...
straight DNA, and (δ-ATP-Mg$_{2+}$)$_2$ interacts with DNA in a sequence-independent manner or interact with $\omega_2$. However, such protein-protein interaction could be “unstable” because we failed to detect the accumulation of such intermediates. Second, (δ-ATP-Mg$_{2+}$)$_2$ bound to DNA interacts with the unstructured N-terminal domain of $\omega_2$ bound to parS DNA. Indeed, $\omega_2$ΔN19 bound to parS DNA failed to interact with (δ-ATP-Mg$_{2+}$)$_2$. Third, the interaction of $\omega_2$ bound to parS with (δ-ATP-Mg$_{2+}$)$_2$ promotes its relocalization from any DNA site to the $\omega_2$parS complex. This is consistent with data showing that addition of $\omega_2$ to preformed (δ-ATP-Mg$_{2+}$)$_2$-DNA led only to the formation of discrete ternary complexes (parS$\omega_2$(δ-ATP-Mg$_{2+}$)$_2$) in the majority of cases (∼67% of total molecules) (see Fig. 4A). Fourth, (δ-ATP-Mg$_{2+}$)$_2$ mediates site-specific pairing, quaternary parS$\omega_2$(δ-ATP-Mg$_{2+}$)$_2$parS complexes, in ∼22% of the cases without apparent distortions in the DNA. However, when (δ-ATP$\gamma$S-Mg$_{2+}$)$_2$ was used, the amount of ternary complexes markedly increased to levels comparable with (parS$\omega_2$(δ6D60A-ATP-Mg$_{2+}$)$_2$)$_2$parS complexes (see below). Fifth, the formation of quaternary parS$\omega_2$(δ-ATP-Mg$_{2+}$)$_2$parS complexes between the six different parS sites should condense the plasmid molecule. Like protein $\delta_2$ (ParA-like) in vitro, P1-ParA facilitates pairing (blocked supercoiled diffusion) of P1-ParB bound to a parS site in two plasmid monomers in vivo (33). Finally, (δ6D60A-ATP-Mg$_{2+}$)$_2$ was sufficient to induce pairing, although dislodging the paired molecules might require ATP hydrolysis (compare Figs. 4 and 5). The topology of the ternary complex differs markedly from the ParM-ParRparC complex, where the oligomeric right-handed ParR ring, with parC wrapped around, acts through its C-terminal domain as an anchor on the ends of the elongating ParM filaments (31, 32, 34–37).

Mechanistic Implications of the Pairing Complex—The differences in the structures of $\omega_2$parS (type Ib) and ParRparC (type II) complexes might indicate how they interact with their partners (δ$_2$ and ParM) and how they segregate plasmid molecules. Previous cytological, biochemical, and structural studies together with this work provide detailed sequential information about the mechanism of ParAB active plasmid partitioning (10, 15, 17). (δ-ATP-Mg$_{2+}$)$_2$, which shows a slow rate of ATP hydrolysis, binds DNA and interacts with $\omega_2$ bound to parS; however, neither δ$_2$K36A, which does not bind ATP, nor (δ-ATP-Mg$_{2+}$)$_2$ interacts with DNA (10). parS-bound protein $\omega_2$ interacts with (δ-ATP-Mg$_{2+}$)$_2$ bound to DNA and recruits it to the parS$\omega_2$ complex. Limiting concentrations of (δ-ATP-Mg$_{2+}$)$_2$ promotes pairing of two supercoiled plasmid molecules in a subfraction of the molecules (Fig. 4). Once the plasmids are paired, the local intracellular concentration of $\omega_2$ increases, the ATPase activity of $\delta_2$ increases, and plasmid pairing is lost. On the other hand, when (δ6D60A-ATP-Mg$_{2+}$)$_2$ was used, multiple pairing molecules were observed, suggesting that in the absence of ATP hydrolysis, paired intermediates accumulate (Fig. 5). In the nanomolar range, both proteins are needed for plasmid pairing (Fig. 4), but in the millimolar range, both proteins are needed for (δ-ATP-Mg$_{2+}$)$_2$ polymerization onto DNA (10). The mechanistic role of $\omega_2$ in promoting plasmid segregation by polymerization and depolymerization from the DNA, prior to cell division segregation, is poorly understood. After Leonard and co-workers (2), we proposed that $\omega_2$ bound to parS assists treadmilling of $\delta_2$. The processive disassembly of $\delta_2$ filaments could contract the (δ-ATP-Mg$_{2+}$)$_2$ spiral-like structure, observed both in vivo and in vitro, and move the cargo (individual $\omega_2$parS) stepwise outward along the cell axis (10). Recently, it has been shown that decreasing local intracellular concentration of $\omega_2$ leads to a decrease on ATP hydrolysis and a stimulation of polymerization of (δ-ATP-Mg$_{2+}$)$_2$ or $\delta_2$-ATP-Mg$_{2+}$ onto plasmid DNA, thereby generating one (minus) end of the nucleoprotein filament (parS$\omega_2$)$_{10}$((δ-ATP-Mg$_{2+}$)$_2$ or $\delta_2$-ATP-Mg$_{2+}$)$_{	ext{parC}}$. From the initial assembly site (δ-ATP-Mg$_{2+}$)$_2$ or $\delta_2$-ATP-Mg$_{2+}$, polymerization leads to the formation of a nascent $\delta_2$ filament that depends on the presence of ATP, but is independent of ATP hydrolysis, because $\delta_2$ polymerization was observed in the presence of ATP$\gamma$S-Mg$_{2+}$ (see 10). When the plus end of the filament reaches another ternary parS$\omega_2$ (δ-ATP-Mg$_{2+}$)$_2$ complex (see Fig. 1B), the $\omega_2$-δ$_2$ molar ratio decreases. This change in the $\omega_2$-δ$_2$ ratio stimulates the ATPase activity of $\delta_2$ (δ-ATP-Mg$_{2+}$)$_2$ dissociates from the nucleoprotein complex, proximal to the $\omega_2$-parS region. Alternatively, (δ-ATP-Mg$_{2+}$)$_2$, by promoting intramolecular pairing of the six different $\omega_2$parS complexes, condenses the plasmid DNA and plays an essential role in the early stages of plasmid segregation. Upon interaction with $\omega_2$parS, (δ-ATP-Mg$_{2+}$)$_2$ binds and polymerizes onto plasmid and/or chromosomal DNA. Protein (δ-ATP-Mg$_{2+}$)$_2$ polymerized onto chromosomal DNA moves the (ω$_2$parS) cargo passively toward the cell poles by an ω$_2$-δ$_2$ interaction. The proposed models for how $\omega_2$ wrapped around parS promoters, $\delta_2$-mediated pairing and subsequent polymerization of (δ-ATP-Mg$_{2+}$)$_2$ or $\delta_2$-ATP-Mg$_{2+}$ and depolymerization of (δ-ATP-Mg$_{2+}$)$_2$ differ markedly from the type II partition system. In the ParRM system, each pair of plasmids, anchored ParM at ParRPparC, drives apart the plasmid copies by ParM-ADP-Mg$_{2+}$ polymerizing bidirectionally (31, 34–37).

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