Segroscope assembly at the pliable 
parH centromere

Meiyi Wu¹, Massimiliano Zampini¹, Malte Bussiek², Christian Hoischen³, 
Stephan Diekmann³ and Finbarr Hayes¹,∗

¹Faculty of Life Sciences and Manchester Interdisciplinary Biocentre, The University of Manchester, 
131 Princess Street, Manchester M1 7DN, UK, ²Department of Genetics, University of Kassel, 
Heinrich-Plett-Str. 40, D-34132 Kassel and ³Department of Molecular Biology, Leibniz Institute for Age 
Research, Fritz-Lipmann-Institute, 07745 Jena, Germany

Received December 16, 2010; Revised January 28, 2011; Accepted February 14, 2011

ABSTRACT

The segroscope of multiresistance plasmid TP228 
comprises ParF, which is a member of the ParA 
ATPase superfamily, and the ParG ribbon–helix– 
helix factor that assemble jointly on the 
parH centromere. Here we demonstrate that the distinctive 
parH site (~100-bp) consists of an array of de-
generate tetramer boxes interspersed by AT-rich 
spacers. Although numerous consecutive AT-steps 
are suggestive of inherent curvature, 
parH lacks 
an intrinsic bend. Sequential deletion of 
parH tetra-
mers progressively reduced centromere function. 
Nevertheless, the variant subsites could be 
rearranged in different geometries that 
accommodated centromere activity effectively re-
vealing that the site is highly elastic in vivo. ParG 
cooperatively coated 
parH: proper centromere 
binding necessitated the protein’s N-terminal 
flexible tails which modulate the centromere 
binding affinity of ParG. Interaction of the ParG 
ribbon–helix–helix domain with major groove bases 
in the tetramer boxes likely provides direct readout 
of the centromere. In contrast, the AT-rich spacers 
may be implicated in indirect readout that mediates 
cooperativity between ParG dimers assembled on 
adjacent boxes. ParF alone does not bind 
parH but 
instead loads into the segroscope interactively with 
ParG, thereby subtly altering centromere conform-
ation. Assembly of ParF into the complex requires 
the N-terminal flexible tails in ParG that are con-
tacted by ParF.

INTRODUCTION

The transmission of genetic information from generation-
to-generation is a fundamental biological process that 
must take place with high fidelity. The molecular events 
that underpin accurate genome segregation in eucaryotes 
are comparatively well-described (1). In contrast, under-
standing of the mechanism of procaryotic DNA segrega-
tion is more rudimentary. However, the compact genetic 
modules that mediate the precise partitioning of plasmids 
are highly informative systems in which to unravel this 
process in precise detail (2).

Four distinct classes of plasmid segregation 
cassette have been defined (3). The two most well-studied types 
each comprise a pair of autoregulated genes and a 
adjacent centromere analogue. The first gene specifies an 
ATPase that either possesses Walker box motifs (ParA) 
or is an actin homologue (ParM), whereas the accom-
panying gene encodes a centromere binding factor 
(CBF) (4–8). The CBF is a site-specific DNA binding 
protein that loads onto the centromere to produce a nu-
cleoprotein complex of defined geometry (9–12). The 
ATPase does not directly contact the centromere, but 
instead interacts with the CBF to assemble the mature 
segroscope. In the case of ParM, ATP-induced 
filamentation from the segroscope propels each member 
of a plasmid pair in opposite directions to achieve segre-
gation (13). ParA homologues also polymerize in response 
to ATP binding, a process that is influenced by the CBF 
and/or by DNA (14–21). The ParA filaments emanating 
from the segroscope may drive plasmids towards the 
cell poles, or retraction of the ParA polymers may draw 
plasmids in opposite directions away from the 
cytokinetic zone (4,15). Recent in vivo studies favour the 
latter (22,23).

∗To whom correspondence should be addressed. Tel: +44 161 3068934; Fax: +44 161 3065201; Email: finbarr.hayes@manchester.ac.uk

© The Author(s) 2011. Published by Oxford University Press. 
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/ 
by-nc/2.5), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
The tri-partite segregosome of multiresistance plasmid TP228 comprises the ParA homologue, ParF, and the ParG CBF which assemble on the parH centromere (Figure 2). ATP binding promotes the polymerization of ParF into dynamic, extensive multistranded filaments that are implicated in segregation (15). The dimeric ParG protein comprises C-terminal regions that interlock into a ribbon–helix–helix (RHH) fold linked to a pair of flexible N-terminal extensions (24). The folded region harbours the major determinants for dimerization, for binding to the parH centromere and to the OF operator site, as well as for ParF interaction (18,24–26). The ParG mobile tails are also multifunctional. First, arginine fingers stabilize the transition state during nucleotide hydrolysis by their partner proteins. The ParG N-terminal tail includes an arginine finger-like motif that stimulates ATP hydrolysis by ParF (18). This stimulation may be a crucial aspect of the cycle of ParF polymerization and depolymerization during segregation. Second, ParF polymerization is stimulated by the ParG flexible tail (18). The tails either may reorganize or stabilize ParF filaments by tethering ParF monomers within a single protofilament or aligned protofilaments. Alternatively, ParG might cluster at points of polymer growth or disassembly (15,18). In this sense, ParG may play a role similar to formins and related factors that influence the elongation and disassembly of actin filaments in eucaryotes, or may be analogous to microtubule-associated proteins that modulate tubulin dynamics (19). Third, ParG binds to the OF operator during transcriptional repression of the parFG genes (25). Of comprises eight degenerate 5′-ACTC-3′ boxes arranged in a combination of direct and inverted orientation (26). Each tetramer motif recruits one ParG dimer, implying that the fully bound operator is cooperatively coated by up to eight dimers. The OF operator apparently has evolved with subsites that bind ParG dissimilarly to produce a nucleoprotein complex fine-tuned for optimal interaction with the transcription machinery (26). A transient β-strand element in the ParG mobile tail associates with the protein’s folded RHH domain thereby further modulating the binding of the protein to the operator (25). The mechanism by which this interaction between flexible and folded domains affects DNA binding is elusive.

CBFs have heterogeneous primary sequences that correlate with the diversity in plasmid centromere organization (27). The precise loading of each CBF onto its cognate centromere is a vital early step that is crucial for correct segregosome assembly and the subsequent cascade of events during partitioning. Here, the interaction of ParG with the distinctive parH centromere is dissected: parH is a complex multisite locus that nevertheless can accommodate a variety of synthetic subsite re-arrangements for accurate segregation. Both direct and indirect readout of parH potentially are required for correct coating of the centromere by ParG emphasizing that an intricate set of interactions mediate the loading of the protein onto the site. The centromere binding specificity of ParG is enhanced by the protein’s flexible N-terminal tails which also are necessary for recruitment of ParF to the mature segregosome.

MATERIALS AND METHODS

Strains, plasmids and molecular biology procedures

Plasmids were propagated and analysed using Escherichia coli DH5α (28). Strain BL21 (Novagen) was employed for protein overproduction and plasmid partition assays were performed in the polA strain BR825 (29). Recombinant plasmids for overexpression of the parF and parG genes were described previously (30). ParG derivatives with 9, 19 or 30 amino acid deletions of the N-terminal tail were produced from plasmids constructed elsewhere (25). The partition probe vector pFH450 is a derivative of the bi-replicon plasmid pALA136 (31,32). Plasmid pFH547 comprises the parFGH region cloned in pFH450 (33). Plasmid pMW20 was constructed in two steps. First, a promoter-less parFG cassette was amplified from pFH547, digested with SacI–XbaI, and inserted between the same sites in the arabinose-inducible expression vector pBAD30 (34) to generate plasmid pMW19. The arabinose-inducible parFG cassette then was amplified from pMW19, digested with XhoI, and inserted in the same site in pFH450 to produce pMW20. Derivatives of parH possessing a full complement of 5′-ACTC-3′ boxes, but with one or more rearrangements (Figure 1), were constructed by inserting double-stranded oligonucleotides carrying the appropriate sequences and with EcoRV–NsiI compatible ends between the same sites in pMW20. Derivatives of parH bearing deletions of 5′-ACTC-3′ boxes were constructed first by amplifying the appropriate regions from pFH547, cleaving the PCR products with BamHI–XhoI, and inserting between the same sites in pFH450. The arabinose-inducible parFG cassette from pMW19 was then inserted as an XhoI fragment in the same orientation in each case. The nucleotide sequences of the inserts in all plasmid constructs were verified. DNA cloning and other molecular biology procedures followed standard protocols.

Plasmid segregation assays

Segregation assays were performed using pFH450 or pMW20 derivatives that replicate at low copy number in strain BR825 as detailed elsewhere (33). Briefly, the relevant plasmid-bearing strains were grown for 25 generations without chloramphenicol selective pressure. Plasmid retention was then determined by replica plating colonies to agar medium with and without the antibiotic. The values presented are the means of at least three independent tests with typical standard deviations (SDs) of ~10%.

Protein production and purification

The hexahistidine-tagged ParF and ParG proteins were overproduced and purified by Ni²⁺ affinity chromatography as described previously (30).

Gel retardation assays

DNA fragments for gel retardation assays were PCR products amplified from appropriate plasmid templates using one primer bearing a 5′ biotin label and a second unlabelled primer, or were generated by annealing
complementary primers one of which was 5' biotinylated. Purification of the fragments and conditions for retardation assays were outlined in detail previously (26). Briefly, biotinylated DNA (2 nM) was incubated for 20 min at 25°C in binding buffer [10 mM Tris–HCl, pH 7.5, 50 mM KCl, 1 mM dithiothreitol, 5 mM MgCl2, 0.05 mg/ml poly(dI–dC)] with the ParG concentrations shown in figure legends. Reaction mixtures were electrophoresed on 10% polyacrylamide gels in 0.5× Tris–borate–EDTA (TBE) buffer for 30–90 min at 80 V at 22°C. DNA was transferred by capillary action to positively charged nylon membranes (Roche), and the transferred DNA fragments were immobilized by UV crosslinking. The biotin end-labelled DNA was detected using the LightShift chemiluminescent EMSA kit (Pierce) (30).

DNase I footprinting
The preparation and purification of biotinylated PCR products, conditions for DNase I footprinting reactions with ParG, denaturing gel electrophoresis, and detection of biotinylated DNA followed procedures described in detail recently (26).

Atomic force microscopy and data analysis
For sample preparation in atomic force microscopy (AFM), freshly cleaved mica was functionalized with poly-l-lysine (PL) to support secure immobilization of DNA (35). The mica disc was incubated with 30 mg/ml aqueous PL solution for 30 s, subsequently washed with 4 ml of Millipore water and dried under a nitrogen stream. A DNA fragment encompassing the parH region was amplified using pFH547 as template. Protein–DNA binding reactions (20 µl) included the purified PCR fragment (2.5 nM) and ParG (1–3 µM) in binding buffer (10 mM Tris–HCl, 50 mM KCl, 1 mM EDTA, pH 7.5). After 15–30 min, the mixture was diluted 30-fold in binding buffer and 30 µl of this dilution were immediately placed on the PL-mica. After 60 s of incubation, the mica was rinsed carefully with 2 ml of Millipore water and allowed to dry under a gentle nitrogen stream. Measurements were performed with an
AFM instrument as described (36) and FESP tips (Veeco) in tapping mode. Fields of \(1 \times 1 \mu m\) were scanned at line rates of 1–2 Hz and resolution of 512 \(\times\) 512 pixels. AFM images were plane corrected with SPIP software (Image Metrology, Denmark) and saved in bitmap format for further analysis with ImageJ software (version 1.41o, NIH, USA). The images were scaled to 2048 \(\times\) 2048 pixels using bilinear pixel interpolation. The entire contours of the DNA molecules were then traced using the freehand line option and saved as \(x-y\)-coordinates. The traced contours were also marked in the images in order to pinpoint two \(x-y\)-coordinates that define the region along each contour which was occupied by ParG. To this end, the points at which the height begins to increase relative to the free DNA were identified using the height threshold tool and their \(x-y\)-coordinates were defined using the point selection tool. To compare the regions occupied by protein with the putative binding sites in the DNA fragments, the saved \(x-y\)-coordinates were characterized by their distances from the DNA terminus which is nearest the protein complex. These distances, as well as total DNA lengths, were determined by summing the distances between the successive coordinates of the entire contours using Excel.

Bending analysis

Plasmid DNA carrying \(parH\), \(O_F\) and the 5'-end of \(parF\) was digested with various restriction endonucleases producing different fragments for curvature analysis. DNA was mixed with loading dye and analysed on equilibrated native polyacrylamide gels as outlined previously (37). Gels were pre-run for \(\sim 3\) h until current and temperature remained constant. Electrophoresis was carried out in \(1 \times TBE\) at 150 V (8 mA) for \(\sim 4\) h (migration distance of bromphenol blue \(\sim 14\) cm) at \(4\) or \(23^\circ\)C. Gels were stained for 30 min in an aqueous solution of ethidium bromide (1 mg/l), followed by rinsing in water prior to documentation. The 1 kb Plus DNA ladder (Invitrogen) and the 1 kb DNA ladder (New England Biolabs) served as marker fragments together with plasmid fragments resulting from restriction digests running in the same lane as the \(parH\) fragment. Migration of all fragments was determined for each gel and calibration curves were plotted using the marker fragments (logarithm of number of base pairs versus distance migrated). The apparent sizes in the acrylamide gel relative to the calibration curve were determined.

RESULTS

Defining and dissecting the \(parH\) centromere in vivo

The \(O_F\) operator upstream of \(parFG\) comprises eight variant 5'-ACTC-3' motifs arranged in a combination of direct and inverted orientation (Figure 1). The tetramer boxes are separated regularly by 4-bp AT-rich sequences. A single ParG dimer loads onto each 5'-ACTC-3' box, suggesting that the operator is cooperatively coated by as many as eight dimers during transcriptional repression of \(parFG\) (26). Inspection of the region further upstream of \(O_F\) revealed a second cluster of 12 degenerate 5'-ACTC-3' motifs (\(parH\)), also separated by AT-rich spacers (Figure 1). One of the boxes is inverted compared to the remainder. These 12 5'-ACTC-3' boxes are embedded in a set of longer repeats that were noted previously in this region and which were originally proposed as the putative centromere locus at which segregosome assembly occurs (4,30).

As repeat motifs are characteristic of plasmid centromeres (4), attempts were made to support the partitioning of a segregationally unstable test plasmid harbouring the full complement of 20 5'-ACTC-3' boxes, i.e. \(parH\)-\(O_F\), when the ParFG proteins were provided in \(\text{trans}\) from a compatible plasmid. The proteins were produced either from genes under the control of the native \(parFG\) expression signals or from a lactose-inducible promoter. Expression of \(parFG\) from a variably inducible arabinose promoter was also tested. In addition, selected synthetic promoters with strengths from weak to high (38) were trialled. None of these approaches elicited improved segregation of the vector possessing the complete set of tetramer boxes compared to the same plasmid lacking the repeat sequences. As an alternative strategy, the \(parFG\) genes were inserted in the segregation probe vector, pFH450 (31), under the control of an arabinose-inducible promoter (PBAD; 34). This manipulation produced plasmid pMW20 that entirely lacks any of the natural regulatory sequences upstream of \(parFG\) (Figure 1). With arabinose induction, the plasmid was maintained at a frequency of 18 ± 3% during non-selective growth for approximately 25 generations in an \(E. coli\) \(polA\) mutant in which the plasmid replicates using the low copy number P1 replicon. However, insertion of the \(parH\)-\(O_F\) region 5' of the arabinose promoter improved retention to 59 ± 16%, a value close to that observed with the intact partition cassette (33). Similar retention values were obtained when the \(parH\)-\(O_F\) region was cloned elsewhere downstream of \(parFG\) in pMW20 (data not shown). Thus, the \(parH\)-\(O_F\) region exhibits ectopic, centromere-like activity when located in \(\text{cis}\) to \(parFG\). One advantage in expressing \(parFG\) from PBAD is that any contribution of \(O_F\) to centromere activity can be examined independently of its regulatory functions. The conditions required for \(\text{in trans}\) activity of the ParFG proteins at \(parH\)-\(O_F\) have yet to be defined: the appropriate intracellular protein concentrations and/or the temporal pattern of \(parFG\) expression that is required to support centromere activity in \(\text{trans}\) may be difficult to replicate artificially. Alternatively, a DNA topological requirement or a positional effect of the genes and/or centromere may influence segregation activity. These possibilities require further investigation.

As \(parH\) and \(O_F\) both comprise arrays of 5'-ACTC-3' boxes, the independent efficacies of the two regions in centromere function were tested in pMW20. The \(parH\) region displayed centromere activity that was indistinguishable from that of the complete \(parH\)-\(O_F\) region (Figure 1). The operator locus alone also was an effective centromere, albeit with slightly less activity than \(parH\); \(O_F\) may have dual roles in transcriptional repression of \(parFG\) (26) and in centromere function. As the activity of \(parH\) was not enhanced by \(O_F\), the centromeric
properties of parH alone were characterized further. Progressive deletion of pairs of 5'-ACTC-3' boxes from parH was accompanied by concomitant gradual reductions in the retention levels of the test plasmids (Figure 1). Notably, plasmids bearing only four (parH0–12) or six (parH7–12) tetramer boxes were maintained at approximately half the frequency conferred by full-length parH.

The parH and OF sites display comparable centromere activities, but the arrangements of the 5'-ACTC-3' motifs in the two loci differ markedly: parH comprises eleven direct repeats and one inverted repeat (Figure 1), whereas OF consists of three direct repeats interspersed with five inverted boxes (26). To assess further the malleability of these sequences for partition activity, synthetic parH centromeres with reconfigured 5'-ACTC-3' boxes were cloned in pMW20 and tested in segregation assays (Figure 1). A site in which all of the tetramer boxes were oriented similarly was a proficient centromere (parHDIR). Inversion of the six rightward motifs or the five leftmost boxes of parH caused only modest decreases in segregation activity (parHInv and parHInv, respectively). A site in which alternating tetramers in parH were inverted from their canonical orientation was a less effective centromere, although remained partially functional (parHALT). Insertion of one-half helical turn at the centre of parH only modestly affected centromere activity (parH+5), whereas insertion of a complete turn (parH+11) reduced centromere function more severely. Thus, a variety of natural and artificial 5'-ACTC-3' box dispositions are viable for parH centromere action, although a minimum of eight repeats is required for efficient activity. Moreover, altering the relative helical positions of the two halves of parH was well-tolerated whereas maintaining these positions, but increasing the distance between the halves, was more deleterious.

Discrete binding of ParG to parH and OF in vitro

The ParG protein binds to the OF operator to achieve transcriptional repression of the parFG genes (25,26). DNase I footprinting in vitro of the parH centromere region revealed that ParG also protects the entire set of 12 5'-ACTC-3' boxes from digestion on both DNA strands (Figure 2). The AT-rich spacers separating certain boxes were slightly less well protected, most obviously on the top strand. The DNA fragments used in these reactions harboured both parH and OF so as to ascertain whether ParG interacted continuously or discontinuously with the two loci: the zones of protection were separated by a ~5-bp window that remained fully accessible to DNase I (Figure 2). These data correlate with recent observations that delimited the extent of ParG interaction with OF (26).

As a second strategy to probe the interaction of ParG with parH, a fragment possessing the centromere (365 bp) was bound by ParG and visualized by AFM (Figure 3A). The images showed protein binding as discrete extended foci. The measured total DNA length was 117 ± 5 nm which was somewhat shorter (~5%) than the 124 nm expected for B-form DNA of this length. Shortening to this extent has been observed previously by AFM of naked DNA (35,39), so the reduced parH fragment lengths observed here do not necessarily reflect ParG-induced compaction. Therefore, the approximate DNA helical rise was 0.32 nm/bp for these surface preparations. To examine if the visible ParG binding

**Figure 2.** DNase I footprinting of ParG at the parH centromere and adjacent OF operator. The distribution and orientation of the variant 5'-ACTC-3' motifs in the parH-OF region are indicated by open arrowheads. The bent arrow indicates the putative parFG promoter (25,26). Footprinting reactions were performed as outlined in the ‘Materials and Methods’ section using PCR fragments biotinylated at the 5'-ends of either top or bottom strands. ParG concentrations (µM monomer, left to right): 0, 0.05, 0.1, 0.2, 0.6, 1.0 and 1.5. A+G, Maxam–Gilbert sequencing reactions. The relative dispositions on the top and bottom strands of the parH region that are protected from DNase I digestion are shown in the bottom panel. The 5'-ACTC-3' motifs are boxed.
corresponded to the parH region, the coordinates P1 and P2, defining the foci along digitized traces of the DNA contours, were located as described in the ‘Materials and Methods’ section. The DNA terminus that was closer to the protein focus was determined and the positions of P1 and P2 were specified as distances from this terminus. The distance distributions shown in Figure 3B comprised a correction of the tip induced lengthening of the detected protein region. For an approximation of this effect, it was assumed that the measured DNA width of 10 nm corresponded to a broadening of 8 nm relative to the known DNA diameter of 2 nm. Therefore, the points determined initially were shifted by 4 nm towards the centres of the foci. The distance maximum in the fragment containing parH (Figure 3B) was found at 102 ± 17 bp for position P1 and 182 ± 22 bp for P2. These positions roughly define the ends of the parH site which is 100-bp based on DNase I footprinting (Figure 2). In conclusion, the AFM measurements described here confirm the selective binding of ParG to parH within the probed fragment at the specified protein concentrations. Although the direction of the fragments is not defined in the AFM images, the observation of single protein foci together with the agreement between the measured distances and the parH location established previously (Figure 2) indicate that ParG binding occurred exclusively in this region.

The parH centromere lacks intrinsic curvature

The plasmid R1 parC centromere displays strong intrinsic curvature and is further distorted into a U-shaped structure by binding of its cognate CBF to the two arms of the site (11,37). In contrast, the cenE centromere of plasmid pGENT shows modest inherent curvature which is not altered significantly by the binding of its CBF on a linear DNA fragment (40). A theoretical analysis of the inherent curvature of the parS centromere of plasmid P1 suggests that the site is curved (C. Hoischen and S. Diekmann, unpublished data). Moreover, DNA bending by integration host factor (IHF) at the centre of parS allows the cognate CBF to span the two arms of the site (41,42). These different topological features may be instrumental in organizing functional segregation in different systems.

The sequence of a HindIII–EcoRI fragment encompassing parH, O_F and the 5'-end of parF (Figure 4) is AT rich (61.2%) and contains blocks of AT nucleotides. These mostly comprise four or five consecutive AT steps suggesting that this region might be curved and show anomalous gel migration. Inherent DNA curvature has clearly identifiable properties. First, curvature can be measured as retarded migration in polyacrylamide gel electrophoresis. Second, curvature is most evident at moderate temperatures and decreases at elevated temperatures (43–45). The quotient apparent sequence length divided by known sequence length is termed the k-factor with an experimental error in k-factor measurement of ±0.03. Variation of curvature values in normal DNA sequences is between k-factor values 0.98 and 1.05. Applying this experimental strategy, we recently detected DNA curvature with large k-factors for yeast centromeres [k-values up to 1.20; (46)] and the plasmid R1 parC centromere [k > 2.0; (37)]. The parH locus and flanking regions were inserted in pUC18 and a set of overlapping fragments were generated by
restriction enzyme digestion (Figure 4). Fragment migration relative to marker fragments in different lanes, as well as to plasmid fragments in the same lane, was analysed in 6% polyacrylamide gels under different temperature conditions, and in agarose gels. Apparent fragment sizes were determined relative to marker fragments with normal migration. Most of the test fragments from the region surrounding \( \text{parH} \) did not show any appreciable gel retardation under standard conditions (23°C, TBE buffer) (Figure 4). Only rather weak retardation of a 520-bp HindIII–EcoRI fragment (\( k \)-factor = 1.08), a 267-bp AgeI–MunI fragment (\( k \)-factor = 1.06), and a 219-bp MunI–EcoRI fragment (\( k \)-factor = 1.06) was measured. The \( k \)-factors of all tested fragments slightly increased when the temperature was reduced to 4°C. However, a weak moderation in the \( k \)-factor was evident for the AgeI–MunI fragment at the lower temperature. Although this analysis suggests some secondary structure, perhaps DNA curvature, resulting in a slightly anomalous gel migration, it reveals that the DNA sequence that includes \( \text{parH} \)-OF is not substantially curved when tested in a linear fragment. This conclusion was supported by the analysis of permuted fragments of identical size and sequence context produced from cloning the \( \text{parH} \)-OF region in a bending vector: these fragments did not display intrinsic curvature in gel electrophoresis (data not shown).

### The clustered 5′-ACTC-3′ motifs in \( \text{parH} \) provide a high affinity docking site for ParG

To parallel the \textit{in vivo} deletion analysis of \( \text{parH} \) (Figure 1), DNA fragments with decreasing numbers of 5′-ACTC-3′ boxes (Figure 1) were tested for ParG binding \textit{in vitro}. Full-length \( \text{parH} \) was assembled into a single complex at the lowest ParG concentration that was examined in gel retardation assays (Figure 5A). In contrast, ParG incompletely bound a substrate lacking two 5′-ACTC-3′ boxes even at the highest protein concentration (\( \text{parH}_3\text{–}12 \)). Multiple nucleoprotein complexes with intermediate migrations were also detectable, suggestive of partial occupancy of the motifs by ParG. Target fragments possessing only eight, six or four tetramer boxes each were bound progressively less avidly by the protein (\( \text{parH}_{5\text{–}12}, \text{parH}_{7\text{–}12} \) and \( \text{parH}_{9\text{–}12} \), respectively), and a single pair of 5′-ACTC-3′ motifs was sufficient only for very weak and limited binding (\( \text{parH}_{11\text{–}12} \)) (Figure 5A). DNase I footprinting of fragments possessing different numbers of motifs confirmed a positive correlation between ParG binding and increasing motif number (Figure 5B). Footprinting also demonstrated that ParG maintained specificity in protecting the regions harbouring the 5′-ACTC-3′ boxes from digestion even when presented with as few as four boxes.

The variable tetramer motifs in \( \text{parH} \) comprise either canonical 5′-ACTC-3′ boxes or derivatives of this sequence (Figure 2). A single 5′-ACTC-3′ tetramer is insufficient for ParG binding \textit{in vitro} (26). Thus, to assess whether ParG recognizes the assorted tetramer motifs with different avidities, the centromere was subdivided experimentally into pairs of motifs flanked by their natural AT-rich spacers (\( \text{parH}_{1\text{–}2} \) to \( \text{parH}_{11\text{–}12} \)) (Figure 6A). Double-stranded oligonucleotides bearing these sequences were tested in gel retardation assays with titrations of ParG. The subsites displayed distinct binding patterns

![Figure 4](http://nar.oxfordjournals.org/)

**Figure 4.** Testing for intrinsic curvature in the \( \text{parH}\)-OF region. A partial restriction map of the region cloned in pUC18 is illustrated at the top. The cloned fragment spans \( \text{parH} \) and OF (shown as shaded boxes) and the 5′-end of \( \text{parF} \) (filled box). Vector sequences are shown by the hatched box. A set of subfragments that contain \( \text{parH}\)-OF at different locations relative to the fragment ends were used in bending analysis. A, AgeI; E, EcoRI; H, HindIII; M, MunI; N, NdeI; P, PstI. The \( k \)-factors derived from analysis of these restriction fragments under standard electrophoretic conditions (6% polyacrylamide in TBE buffer at 4 or 23°C) are shown.
with ParG: parH1-2 and parH5-6 were recognized most strongly, whereas ParG bound the parH8-10 fragment least well (Figure 6A). Other motif pairs showed intermediate binding properties.

To investigate further the differences in ParG affinities for different parH subsites, ParG binding to the biotinylated parH1-2 oligonucleotide was challenged with increasing concentrations of each of the unlabelled subsites (Figure 6B). The parH1-2 and parH5-6 fragments competed most efficiently, whereas parH7-8 and parH9-10 were weak competitors. Thus, gel retardation assays and competition data revealed that ParG recognizes different centromere subsites with different affinities (parH1-2 ≈ parH5-6 > parH11-12 ≈ parH7-8 ≈ parH5-4 > parH9-10). These differences likely arise both from dissimilarities in the tetramer box sequences as well as from variations in the intervening AT-rich spacers in parH, both of which contribute significantly to DNA binding by ParG (26).

The influence of the mobile N-terminus of ParG on centromere interaction

The flexible N-terminal tail of ParG is multifunctional, harbouring an arginine finger-like motif that stimulates
nucleotide hydrolysis by the partner ParF ATPase, as well as possessing sequences that promote ParF polymerization (18). Moreover, the interaction of ParG with the OF operator is modulated by transient associations between the flexible N-terminal and folded C-terminal domains in complex with the target DNA (25). To examine whether the tail also influences interaction with the parH centromere, derivatives lacking 9, 19 or 30 amino acids from the ParG N-terminus (25) were tested for centromere assembly (Figure 7). This progressive truncation of the mobile tail was accompanied by increasingly stronger interactions of the ParG deletion derivatives with the centromere both in gel retardation assays and DNase I footprinting. Notably, at protein concentrations at which ParG only weakly shifted parH into a series of intermediate nucleoprotein complexes in retardation assays, Δ30ParG assembled the DNA into a major retarded complex (Figure 7A). In addition, Δ30ParG fully protected the parH site from DNase I digestion at protein concentrations that were insufficient for protection by the full-length protein (Figure 7B).

Figure 6. ParG binding to adjacent pairs of 5′-ACTC-3′ boxes from the parH centromere. (A) Gel retardation assays of ParG with fragments possessing pairs of tetramer boxes from parH. The fragments were generated by second-strand synthesis of 49-nt single-strand oligonucleotides bearing the relevant boxes at the centres of the fragments. The single-stranded oligonucleotides each included a common priming site for second-strand synthesis by a 5′-biotinylated primer. Thus, the fragments contained the relevant boxes and immediate flanking regions, but no other parH sequences. ParG concentrations (μM monomer, left to right): 0, 0.1, 0.2, 0.5, 1.0, 2.0, 4.0 and 6.0. Open and filled arrows indicate unbound DNA and ParG–DNA complexes, respectively. (B) Competition gel retardation assays of ParG binding to the parH1–2 fragment. Binding reactions containing ParG (4 μM), a biotinylated fragment (2.5 nM) harbouring the parH1–2 pair of 5′-ACTC-3′ boxes that are strongly bound by the protein (panel A), and increasing amounts (up to 200 nM) of unlabelled 19-bp competitor oligonucleotides were incubated at 22°C for 20 min. Reactions were analysed further as described in the ‘Materials and Methods’ section. Unlabelled competitor DNAs contained the same sequences used in panel A.
The preceding observation that deletion of the flexible tail increases parH binding by ParG apparently contradicts previous findings that Δ30ParG interacts more weakly at the OF locus in both gel retardation and surface plasmon resonance assays (25). However, the OF site subsequently was found to be more extensive than originally described, comprising eight variant 5'-ACTC-3' boxes instead of the five motifs used in previous experiments (26). The observation that Δ30ParG recognized a substrate containing three tetramer boxes less avidly than did the full-length protein (25) is also intriguing as it suggests that the flexible tail affects DNA binding differently dependent on the number of 5'-ACTC-3' boxes present in the target site. Therefore, the interaction of the ParG deletion derivatives with pairs of 5'-ACTC-3' motifs flanked by their natural AT-rich spacers (parH1–2 to parH11–12) (Figure 6A) was examined (Figure 8A). Unlike the stronger binding that the truncated derivatives displayed to the complete parH locus (Figure 7), binding of full-length and deletion versions of ParG was broadly similar to the parH1–2, parH3–4, parH5–6 and parH7–8 fragments. However, Δ19ParG generally interacted more weakly with the subsites than either full-length ParG,
Δ9ParG or Δ30ParG. Strikingly, the parH9–10 and parH11–12 oligonucleotides were also bound less well by Δ30ParG than by the wild-type protein. Thus, complete truncation of the N-terminal tail elicited improved binding of ParG to the full-length centromere, but caused weaker binding when certain subsites of parH that contain fewer 5'-ACTC-3' boxes were tested.

As the variant entirely lacking the mobile tail binds parH at a lower protein concentration than does full-length ParG, relative centromere binding by ParG and Δ30ParG was assessed by challenging a preformed ParG:parH complex with increasing concentrations of Δ30ParG (Figure 8B, left). The complex formed by ParG with parH migrates more slowly in gel retardation assays than does the Δ30ParG:parH complex allowing ready discrimination between the two species. At a 1:1 ratio, the ParG:parH complex was entirely disassembled by Δ30ParG and only nucleoprotein complexes with

Figure 8. Binding of ParG deletion derivatives to parH subsites and competition assays with full-length ParG. (A) Gel retardation comparison of ParG, Δ9ParG, Δ19ParG and Δ30ParG binding to adjacent pairs of 5'-ACTC-3' boxes from the parH centromere. The DNA fragments consisted of biotinylated, double-stranded oligonucleotides possessing pairs of tetramer boxes (Figure 6A). Protein concentrations (μM monomer, left to right): 0, 0.5 and 2.0. Arrows and boxes indicate unbound DNA and protein–DNA complexes, respectively. (B) Left: competition assays in which a preformed ParG:parH complex was challenged with increasing concentrations of Δ30ParG. Where indicated, ParG was present at 0.4 μM. In binding reactions that contained only Δ30ParG, the protein was also included at 0.4 μM. In reactions containing both proteins, Δ30ParG was present at 0.4, 0.8, 2.0, 4.0, or 8.0 μM (left–right). Right, competition assays in which a preformed Δ30ParG:parH complex was challenged with increasing concentrations of ParG. Where indicated, Δ30ParG was present at 0.4 μM. In binding reactions that contained only ParG, the protein was also included at 0.4 μM. In reactions containing both proteins, ParG was present at 0.4, 0.8, 2.0, 4.0 or 8.0 μM (left–right). Open and filled arrows indicate unbound DNA and protein–DNA complexes, respectively. (C) Deletion of the ParG N-terminal tail increases non-specific DNA binding. ParG and Δ30ParG were incubated with a biotinylated 60-bp fragment bearing the Epstein–Barr virus nuclear antigen binding site. This fragment lacks the 5'-ACTC-3' boxes recognized by ParG. Protein concentrations (μM monomer, left to right): 0, 2, 2.5, 3, 3.5, 4, 4.5 and 5.0. Arrows and boxes indicate unbound DNA and protein–DNA complexes, respectively.
intermediate migration were observed. This likely reflects the formation of complexes that include both protein species: either ParG and Δ30ParG or the formation of complexes that include both protein intermediate migration were observed. This likely reflects that the deletion protein cannot fully displace ParG from the centromere. The converse experiment was also performed. When a preformed Δ30ParG:parH complex was challenged with the full-length protein, mixed complexes with migrations intermediate between those produced with ParG or Δ30ParG only were again evident (Figure 8B, right). However, at a high ParG:Δ30ParG ratio, parH was assembled entirely into a complex that comigrated with the complex observed only with ParG. Thus, ParG can actively dislodge Δ30ParG from the centromere more effectively than the reverse.

In summary, truncation of the ParG flexible tail permits binding to the full-length parH centromere at lower protein concentrations than with the native protein (Figure 7). In contrast, binding of the deletion proteins to subsites possessing only two 5'-ACTC-3' motifs is only as strong, and in some instances weaker, than binding by full-length ParG (Figure 8A). Moreover, ParG not only competes better than Δ30ParG for the parH site, but can displace the deletion protein from the centromere more effectively than Δ30ParG can dislodge ParG (Figure 8B and C). The combined results suggest that the mobile tail modulates the association rate of ParG for the centromere, but that the stability of the ParG-parH complex that lacks the tail may be perturbed.

Like all DNA binding proteins, ParG binds to DNA non-specifically in vitro when the protein concentration is sufficiently high (24). To ascertain whether the ParG mobile tail contributes to its binding specificity, full-length ParG and Δ30ParG proteins were tested at concentrations up to 5 μM with a biotinylated 60-bp fragment bearing the Epstein–Barr virus nuclear antigen binding site. This fragment lacks the characteristic 5'-ACTC-3' motifs recognized by ParG and is not bound by the protein at ≤3.5 μM, a concentration that is sufficient for full binding of parH (Figure 8C, left). The non-specific DNA is shifted into a series of complexes with progressively slower migration at elevated ParG concentrations. In contrast, the non-specific DNA is fully bound in the presence of Δ30ParG at a concentration of 3 μM (Figure 8C, right). Equivalent results were obtained with a second fragment of non-specific DNA (data not shown). Thus, removal of the flexible N-terminal tail from ParG also causes the protein to bind non-specific DNA more avidly.

Loading of ParF into the segrosome

The influence of ParF on the binding of ParG to the region upstream of parFG was examined previously, both in the absence and presence of ATP (30). However, this analysis was performed before the boundaries of the parH and O_F loci were delineated accurately [Figure 1; (26)], and before the dynamic effects of ATP on ParF behaviour had been investigated (15,18). In view of this, the assembly of ParF into the segrosome was re-assessed using parH centromeric DNA defined here. First, the ParF protein does not bind the parH centromere in gel retardation assays either in the absence (30) or presence of ATP (data not shown). Second, the ParG:parH complex was supershifted progressively into a more slowly migrating complex that remained in the gel loading well as ParF was titrated into the reactions. At a ParF:ParG ratio of 32:1, the centromere was supershifted entirely (Figure 9A, left). Third, ParF slightly modified the DNase I footprinting patterns that ParG produced at parH (Figure 9B). ParG was included in footprinting reactions at three concentrations: 0.1 (low), 0.25 (medium) and 0.5 (high) μM. In each case, ParF was omitted or was included at 4 or 20 μM. At low ParG concentration, no protection of the centromere was observable on top or bottom DNA strands either in the absence or presence of ParF. Incomplete protection of the site was apparent at medium ParG concentration. ParF modestly enhanced this protection. At high ParG concentration, ParG fully protected both the centromere and O_F operator from DNase I digestion. The two loci were separated by the distinctive window of DNase I accessibility noted earlier (Figure 2). However, DNase I access to this zone was hindered by the presence of ParF which also slightly ameliorated protection at the other boundary of parH. The alterations in DNase I protection patterns induced by ParF were subtle, but reproducible, and demonstrate that centromeric DNA assembled into the segrosome may be organized differently than when bound only by ParG.

ParG binds progressively more weakly to parH subsites that comprise decreasing numbers of 5'-ACTC-3' boxes (Figure 5A). The capacity of ParF to co-assemble with ParG into these subsites was tested. Supershifted complexes were weakly detectable with as few as six tetramer motifs derived from the centromere (parH7-12). In contrast, supershifted species were not evident with four (parH9-12) or two (parH11-12) 5'-ACTC-3' boxes (data not shown). Indeed, the ParG-only complexes disappeared at the highest ParF concentrations used in these experiments indicating that the complexes are sufficiently unstable that ParF can strip weakly bound ParG from the DNA via protein–protein interactions (30).

The N-terminal tail of ParG is required for segrosome assembly

The ParFGH segrosome was detectable in gel retardation assays when increasing concentrations of ParF were incubated with ParG and a parH fragment (Figure 9A, left). To examine whether segrosome assembly necessitated the N-terminal mobile tails of ParG, deletion versions of the protein were employed in retardation assays with ParF and the parH site. A biotinylated fragment (25 nM) bearing the parH locus was incubated with 0.5 μM of Δ9ParG, Δ19ParG or Δ30ParG (Figure 7A) and titrated with ParF (0.2–20 μM). Segrosome formation was not detectable when Δ9ParG (Figure 9A, right), Δ19ParG or Δ30ParG were present.
Thus, removal of the flexible tail abolishes segrosome assembly in gel retardation assays.

**DISCUSSION**

Plasmid centromeres are diverse. They typically comprise tandem repeats whose numbers, lengths, sequences, orientations and spacing are highly variable from replicon-to-replicon (4,5). Although characteristically positioned either upstream or downstream of the corresponding segregation genes, atypical centromeres that are situated more remotely have been described (47,48). Moreover, there are examples in which more than one plasmid locus functions as an independent centromere, or in which sites may act synergistically (49,50), suggesting that cross-talk between segrosomes assembled at discrete positions may contribute to partitioning in certain cases. Among the most well-studied examples, the ParB protein uses separate domains to associate with two different types of motif in the parS centromere of the P1 plasmid, as well as to bridge sites located on different molecules. ParB binding is facilitated by the DNA bending protein IHF, producing nucleoprotein complexes with distinctive topologies (9,51). In the case of the R1 plasmid, the interaction of the cognate CBF with the parC centromere generates ring-like, superhelical complexes (10–12). These characteristic protein–DNA superstructures are recognized in turn by the polymerizing ATPase (52) to form the mature segrosome. The parH centromere of plasmid TP228 was proposed to comprise a short set of /C2420-bp iterated sequences based on bioinformatics analysis (30). However, in light of recent work that defined the core binding site of the ParG protein as a...
5′-ACTC-3′ box flanked by AT-rich sequences (26), we have reappraised the organization of the parH locus here (Figure 1). The region 5′ of the parFG genes harbours 20 degenerate 5′-ACTC-3′ motifs separated by AT-rich spacers. The complete array of tetramer boxes, a subset of eight motifs that comprise the Oβ operator (26), and the 12 distal boxes (parH) each were proficient centromeres, although the independent activity of Oβ was modestly weaker than that of parH alone. Remarkably, synthetic parH sites in which multiple tetramer boxes were inverted relative to their normal orientation also were effective centromeres, albeit none were as competent as the wild-type locus. This observation parallels previous findings that inversion of tetramer boxes did not diminish ParG binding at the Oβ locus (26). ParG dimers may be able to load onto the native tetramer boxes in the artificial parH sites and subsequently tether additional dimers non-specifically to neighbouring, mutated boxes through protein–protein interactions. This may produce a complex that mimics the wild-type configuration sufficiently that the ParF factor can still be recruited to assemble a functional segrosome. Thus, segregation may require only that a sufficient number of ParG dimers assemble on the centromere, permitting ParF to interact effectively with ParG displayed on a variety of promiscuous tetramer box arrangements. The absence of intrinsic curvature in parH fits with this concept as it reveals that the centromere is not locked into a single topology. Accordingly, insertion of a half-helical turn at the centre of the site was more well-tolerated than insertion of a full turn indicating that distance constraints between the halves of the site may be more crucial than their spatial positioning relative to helical phase. Thus, the site may be a tractable element that potentially can be flexed, if required, when complexed with ParG and ParF in the segrosome. Analogously, the ParB protein of the P1 plasmid can interact with multiple binding motifs in the parS centromere to generate a variety of nucleoprotein configurations (51). How the proposed plasticity of the ParG-parH interaction integrates with loading of ParF and the subsequent segregation process that requires directional plasmid movement remains to be resolved.

The antiparallel β-strands within RHH factors such as ParG comprise their principal DNA binding determinants, directing the protein dimers to the major groove (53). However, the binding of ParG at parH also is influenced profoundly by the N-terminal flexible tail (~30 amino acids): derivatives with increasing truncations of the ParG tail shifted the centromere progressively more efficiently in gel retardation assays, as well as more readily protecting the full-length site from DNase I digestion (Figure 7). Nevertheless, subsites of parH are bound equally well by ParG and its deletion derivatives, or are bound less proficiently by the latter in certain instances (Figure 8A). Moreover, a version of ParG that entirely lacks the mobile tail competes less well for centromeric DNA when challenged with the full-length protein. Previous chemical shift mapping of ParG-Oβ interactions highlighted the improved discrimination between the operator and non-specific DNA that the ParG mobile tail confers (25). Correspondingly, deletion of the tail increases non-specific DNA binding by ParG (Figure 8C). Bases in the 5′-ACTC-3′ boxes in parH are likely contacted by residues in the anti-parallel β-strands of ParG, whereas the mobile tail may provide phosphate backbone contacts that enhance the strength of the interaction. In addition, a transient β-strand previously detected in the mobile tail (25) may be implicated in enhancing the specificity of the interaction with centromeric DNA by a mechanism that has yet to be fully revealed. It is noteworthy that other RHH factors similarly possess disordered extensions that modulate their DNA binding properties (54–58) indicating that this may be a widespread feature among this class of proteins. The weaker interaction of ParG deletion derivatives with certain parH subsites also may reflect compromised dimer–dimer interactions in these shorter substrates compared to the full-length sequence on which an array of dimers can cooperatively assemble.

Direct readout of sequences by DNA binding proteins involves interaction with specific bases in the binding site. In contrast, indirect sequence readout entails modulation of protein–DNA complex formation by bases that are not specifically contacted by the protein. These non-contacted bases may contribute, for example, to distortion of the DNA thereby promoting stable nucleoprotein complex formation by assisting in the precise alignment of amino acid residues and bases contacted during direct readout (59). We speculate that the 5′-ACTC-3′ boxes in parH are read out directly by ParG and that the intervening AT-rich spacers participate indirectly and that these combined contributions explain the dissimilar binding patterns of ParG to different parH subsites. For example, the parH-1 and parH-7,8 subsites both possess identical pairs of 5′-ACTC-3′ boxes, but different spacer and flanking sequences whose indirect read out by ParG elicits different affinities for the subsites in gel retardation assays (Figure 6). Although the parH site does not exhibit global curvature (Figure 4), the AT-rich flanking regions may display localized flexibility that promotes the association of ParG dimers assembled on adjacent tetramer boxes. Accordingly, increasing the AT content of the spacer in a natural binding site ameliorates the interaction with ParG, whereas this interaction is disimproved by other mutations (26). Thus, direct and indirect readout, combined with cross-talk between protein dimers loaded onto neighbouring tetramer boxes, may cooperatively promote coating of the parH site with an array of interacting ParG dimers. The flexible tails of ParG provide another layer of complexity to the centromere interaction: the tails modulate the DNA binding affinity of ParG. This modulation may be via a direct interaction with the the parH site, or by cooperative ParG dimer–dimer interactions mediated through the N-terminal tails. Elucidation of the tertiary structures of ParG complexed with the centromere will clarify further the nucleoprotein interactions that underpin segrosome assembly.

Like ParG, the ParR and ω proteins encoded by plasmids R1/pSK41 and pSM19035, respectively, are RHH factors that bind their cognate centromeres.
ACKNOWLEDGEMENTS

We thank Daniela Barillà and Marisa Caccamo for their critical reading of the manuscript. AFM studies were performed at the University of Twente, The Netherlands.

FUNDING

Biotechnology and Biological Sciences Research Council (grant G003114 to F.H.). Overseas Research Student Awards Scheme, scholarship (to M.W., partial). Funding for open access charge: Biotechnology and Biological Sciences Research Council.

Conflict of interest statement. None declared.

REFERENCES

1. Bloom,K. and Joglekar,A. (2010) Towards building a chromosome segregation machine. Nature, 463, 446–456.
2. Hayes,F. and Barillà,D. (2010) Extrachromosomal components of the nucleoid: recent developments in deciphering the molecular basis of plasmid segregation. In Dorman,C.J. and Dame,R.T. (eds), Bacterial Chromatin. Springer Publishing, Dordrecht, The Netherlands, pp. 49–70.
3. Schumacher,M.A. (2008) Structural biology of plasmid partition: uncovering the molecular mechanisms of DNA segregation. Biochem. J., 412, 1–18.
4. Hayes,F. and Barillà,D. (2006) The bacterial segregase: a dynamic nucleoprotein machine for DNA trafficking and segregation. Nature Rev. Microbiol., 4, 133–143.
5. Hayes,F. and Barillà,D. (2006) Assembling the bacterial segregase. Trends Biochem. Sci., 31, 247–250.
6. Schumacher,M.A. (2007) Structural biology of plasmid segregation proteins. Curr. Opin. Struct. Biol., 17, 103–109.
7. Salje,J. (2010) Plasmid segregation: how to survive as an extra piece of DNA. Crit. Rev. Biochem. Mol. Biol., 45, 296–317.
8. Gerdes,K., Howard,M. and Szardenings,F. (2010) Pushing and pulling in prokaryotic DNA segregation. Cell, 141, 927–942.
9. Schumacher,M.A. and Funnell,B.E. (2005) Structures of ParB bound to DNA reveal mechanism of partition complex formation. Nature, 438, 516–519.
10. Schumacher,M.A., Glover,T.C., Brzoska,A.J., Jensen,S.O., Dunham,T.D., Skurray,R.A. and Firth,N. (2007) Segrosome structure revealed by a complex of ParR with centromere DNA. Nature, 450, 1268–1271.
11. Hoischen,C., Busiek,M., Langowski,J. and Diekmann,S. (2008) Escherichia coli low-copy number plasmid R1 centromere parC forms a U-shaped complex with its binding protein ParR. Nucleic Acids Res., 36, 607–615.
12. Moller-Jensen,J., Ringgaard,S., Mercogliiano,C.P., Gerdes,K. and Löwe,J. (2007) Structural analysis of the ParR/parC plasmid partition complex. EMBO J., 26, 4413–4422.
13. Garner,E.C., Campbell,C.S., Weibel,D.B. and Mullins,R.D. (2007) Reconstitution of DNA segregation driven by assembly of a prokaryotic actin homolog. Science, 315, 1270–1274.
14. Eberbach,G. and Gerdes,K. (2004) Bacterial mitosis: partitioning protein ParA oscillates in spiral-shaped structures and positions plasmids at mid-cell. Mol. Microbiol., 52, 385–398.
15. Barillà,D., Rosenberg,M.F., Nobbmann,U. and Hayes,F. (2005) Bacterial DNA segregation dynamics mediated by the polymerizing protein ParF. EMBO J., 24, 1453–1464.
16. Lim,G.E., Derman,A.I. and Pogliano,J. (2005) Bacterial DNA segregation by dynamic SopA polymers. Proc. Natl Acad. Sci. USA, 102, 17658–17663.
17. Adachi,S., Hori,K. and Hiraga,S. (2006) Subcellular positioning of F plasmid mediated by dynamic localization of SopA and SopB. J. Mol. Biol., 356, 850–863.
18. Barillà,D., Carmelo,E. and Hayes,F. (2007) The tail of the ParG DNA segregation protein remodels ParF polymers and enhances
ATP hydrolysis via an arginine finger-like motif. Proc. Natl Acad. Sci. USA, 104, 1811–1816.

19. Machón,C., Fothergill,T.J.G., Barilla,D. and Hayes,F. (2007) Promiscuous stimulation of ParF protein polymerization by heterogeneous centromere binding factors. J. Mol. Biol., 374, 1–8.

20. Bouet,J.Y., Ah-Seng,Y., Benmerad,N. and Lane,D. (2007) Polymerization of SopA partition ATPase: regulation by DNA binding and SopB. Mol. Microbiol., 63, 468–481.

21. Pratt,F., Cleeke,A., Weihofen,W.A., Lurz,R., Saenger,W. and Alonso,J.C. (2008) Streptococcus pyogenes pSM19035 requires dynamic assembly of ATP-bound ParA and ParB on parS DNA during plasmid segregation. Nucleic Acids Res., 36, 3676–3689.

22. Fogel,M.A. and Waldor,M.K. (2006) A dynamic, mitotic-like mechanism for bacterial chromosome segregation. Genes Dev., 20, 3269–3282.

23. Ringaard,S., van Zon,J., Howard,M. and Gerdes,K. (2009) Movement and equipping of plasmids by ParF filament disassembly. Proc. Natl Acad. Sci. USA, 106, 19369–19374.

24. Golovanov,A.P., Barilla,D., Golovanova,M., Hayes,F. and Lian,L.Y. (2003) ParG, a protein required for active partition of bacterial plasmids, has a dimeric ribbon-helix-helix structure. Mol. Microbiol., 50, 1141–1153.

25. Carmelo,E., Barilla,D., Golovanov,A.P., Lian,L.Y., Derome,A. and Hayes,F. (2005) The unstructured N-terminal tail of ParG modulates assembly of a quaternary nucleoprotein complex in transcription repression. J. Biol. Chem., 280, 28683–28691.

26. Zamponi,M., Derome,A., Bailey,S.E.S., Barilla,D. and Hayes,F. (2009) Recruitment of the ParG segregation protein to different affinity DNA sites. J. Bacteriol., 191, 3832–3841.

27. Fothergill,T.J.G., Barilla,D. and Hayes,F. (2005) Protein diversity confers specificity in plasmid segregation. J. Bacteriol., 187, 2651–2661.

28. Woodcock,D.M., Crowther,P.J., Doherty,J., Jefferson,S., DeCruz,E., Noyer-Weidner,M., Smith,S.S., Michael,M.Z. and Graham,M.W. (1989) Quantitative evaluation of Instrument Compact stand-alone atomic force microscope. Nature, 339, 528–541.

29. Ludtke,D.N., Eichorn,B.G. and Austen,S.J. (1989) Plasmid-partition functions of the P7 prophage. J. Mol. Biol., 209, 393–406.

30. Barilla,D. and Hayes,F. (2003) Architecture of the ParF-ParG protein complex involved in procaryotic DNA segregation. Mol. Microbiol., 49, 487–499.

31. Hayes,F. (1998) A family of stability determinants in pathogenic bacteria. J. Bacteriol., 180, 6415–6418.

32. Martin,K.A., Friedman,S.A. and Austin,S.J. (1987) Partition site of the P1 plasmid. Proc. Natl Acad. Sci. USA, 84, 8544–8547.

33. Hayes,F. (2000) The partition system of multidrug resistance plasmid TP228 includes a novel protein that epitomizes an protein complex involved in procaryotic DNA segregation. J. Mol. Biol., 302, 3469–3478.

34. Ludtke,D.N., Eichorn,B.G. and Austen,S.J. (1989) Plasmid-partition functions of the P7 prophage. J. Mol. Biol., 209, 393–406.

35. Belmont,E., Davis,R.W. and McLaughlin,R. (1990) Intrinsically bent DNA. J. Mol. Biol., 265, 7093–7096.

36. Bechert,T., Heck,S., Fleig,U., Diekmann,S. and Hegemann,J.H. (1999) All 16 centreomere DNAs from Saccharomyces cerevisiae show DNA curvature. Nucleic Acids Res., 27, 1444–1449.

37. Williams,D.R., Macartney,D.P. and Thomas,C.M. (1998) The partitioning activity of the RK2 control region requires only incC, korB and KorB-binding site Op3 but other KorB-binding sites form destabilizing complexes in the absence of Op3. Microbiology, 144, 3369–3378.

38. Grigoriev,P.S. and Lobocka,M.B. (2001) Determinants of segregational stability of the linear plasmid-prophage N15 of Escherichia coli. Mol. Microbiol., 42, 355–368.

39. Schumacher,M. and Almstrom,T. (2001) The parS locus of virulence factor pB171: DNA segregation is correlated with oscillation of Par. Proc. Natl Acad. Sci. USA, 98, 15078–15083.

40. DeCruz,E., Noyer-Weidner,M., Smith,S.S., Michael,M.Z. and Graham,M.W. (1989) Quantitative evaluation of Instrument Compact stand-alone atomic force microscope. Nature, 339, 528–541.

41. Funnell,B.E. (1991) The P1 plasmid partition complex at 2.8Å resolution reveals DNA recognition by the 6.4 shape of the CRD2 central control region requires only incC, korB and KorB-binding site Op3 but other KorB-binding sites form destabilizing complexes in the absence of Op3. Microbiology, 144, 3369–3378.

42. Schumacher,M.A. and Funnell,B.E. (2007) P1 partition complex assembly involves several modes of protein-DNA recognition. J. Biol. Chem., 282, 10944–10952.

43. Pratto,F., Suzuki,Y., Takeyasu,K. and Alonso,J.C. (2009) The double par locus of virulence factor pB171: DNA segregation is correlated with oscillation of Par. Proc. Natl Acad. Sci. USA, 98, 15078–15083.

44. Somers,W.S. and Phillips,S.E. (1992) Crystal structure of the Met repressor-operator complex at 2.8Å resolution reveals DNA recognition by β-strands. Nature, 359, 387–393.

45. Vecchiarelli,A.G., Schumacher,M.A. and Funnell,B.E. (2007) P1 partition complex assembly involves several modes of protein-DNA recognition. J. Biol. Chem., 282, 10944–10952.

46. Praatto,F., Suzuki,Y., Takeyasu,K. and Alonso,J.C. (2009) Single-molecule analysis of protein-DNA complexes formed during partition of newly replicated plasmid molecules in Streptococcus pyogenes. J. Bacteriol., 284, 30298–30306.

47. Schreiter,E.R. and Drennan,C.L. (2007) Ribbon-helix-helix transcription factors: variations on a theme. Nature Rev. Microbiol., 5, 710–720.

48. Somers,W.S. and Phillips,S.E. (1992) Crystal structure of the Met repressor-operator complex at 2.8Å resolution reveals DNA recognition by β-strands. Nature, 359, 387–393.

49. Raumann,B.E., Rould,M.A., Pabo,C.O. and Sauer,R.T. (1994) Determinants of sequence-dependent intrinsic and extrinsic forces. Nature Rev. Genes, 5, 81–90.

50. KorB-binding site OB3 but other KorB-binding sites form destabilizing complexes in the absence of Op3. Microbiology, 144, 3369–3378.

51. Vecchiarelli,A.G., Schumacher,M.A. and Funnell,B.E. (2007) P1 partition complex assembly involves several modes of protein-DNA recognition. J. Biol. Chem., 282, 10944–10952.

52. Phillips,K. and Phillips,S.E.V. (1994) Electrostatic activation of Escherichia coli metionine repressor. Structure, 2, 309–316.

53. Schumacher,M.A. and Funnell,B.E. (2007) P1 partition complex assembly involves several modes of protein-DNA recognition. J. Biol. Chem., 282, 10944–10952.

54. Benati,E.L. and Chivers,P.T. (2007) The N-terminal arm of the Helicobacter pylori NifD-dependent transcription factor NifRK is required for specific DNA binding. J. Biol. Chem., 282, 20365–20375.

55. Schreiter,E.R. and Drennan,C.L. (2007) Ribbon-helix-helix transcription factors: variations on a theme. Nature Rev. Microbiol., 5, 710–720.

56. Somers,W.S. and Phillips,S.E. (1992) Crystal structure of the Met represor-operator complex at 2.8Å resolution reveals DNA recognition by β-strands. Nature, 359, 387–393.

57. Raumann,B.E., Rould,M.A., Pabo,C.O. and Sauer,R.T. (1994) Determinants of sequence-dependent intrinsic and extrinsic forces. Nature Rev. Genes, 5, 81–90.

58. Schreiter,E.R. and Drennan,C.L. (2007) Ribbon-helix-helix transcription factors: variations on a theme. Nature Rev. Microbiol., 5, 710–720.

59. KorB-binding site OB3 but other KorB-binding sites form destabilizing complexes in the absence of Op3. Microbiology, 144, 3369–3378.