

Bacterial centromeres and kinetochore complexes

Christian Hoischen, Malte Bussiek, Andrew Derome, Finbarr Hayes, and Stephan Diekmann

We mainly emphasize on the human kinetochore. The composition of the kinetochore is of outstanding complexity. Bacterial segregation offers a simple system for comparison. Several bacterial low-copy-number plasmids have a minimal system for active and directed DNA segregation. They use centromere- and kinetochore-like structures. These bacterial par systems encode 3 elements⁽¹⁾:

1. a centromere-like site on the plasmid DNA,
2. a protein binding to this site and
3. an actin-like ATPase (Fig. 1A).

We elucidated the structure of these kinetochore-like bacterial complexes and looked for folded structures similar to the model shown in Fig. 1B. We analysed the two par systems of the R1 plasmid of *E. coli* and of plasmid pGENT of *E. faecium* with respect to

1. the intrinsic sequence dependent curvature of the centromeric DNA,
2. the structures of the complexes (by AFM), and
3. compared it with the P1 system of *E. coli*.

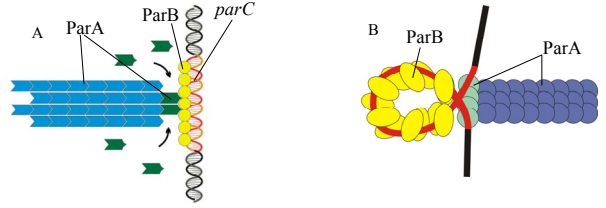


Figure 1. Bacterial kinetochores. With the DNA-binding protein (yellow), the centromeric DNA (red) forms a kinetochore-like nucleoprotein-complex. The force transmitting proteins (blue and green) bind to this complex and move the DNA. A) Simplified schematic presentation⁽²⁾. B) Our working model.

Curvature

Centromere DNA of higher eukaryotes, including human, but also centromere DNA of *S. cerevisiae* is curved⁽⁴⁾. Curved or bendable DNA supports tight winding of the DNA around the histone octamer. The centromeres of both bacterial low-copy-number plasmids are curved^(3,5): *parC* (R1) strongly and *cenE* (pGENT) moderately (Fig. 2).

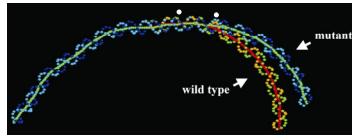


Figure 2. DNA curvature for *parC* the centromere of plasmid R1. The sequence was applied to the software CURVATURE.

The par systems of the *Escherichia coli* R1 plasmid (*parC*, ParR, ParM) and of the *Enterococcus faecium* plasmid pGENT (*cenE*, PrgO, PrgP) show strong similarities:

1. the centromeric regions are curved,
2. the centromeric regions consist of two sets of repeats,
3. the DNA binding proteins (ParR, PrgO) bind to the centromeric regions (*parC*, *cenE*) forming nucleoprotein complexes, and
4. the segregation proteins ParM and PrgP interact with their corresponding nucleoprotein complexes and form (in case of R1) dynamic actin-like filaments for active and directed plasmid partitioning (Fig. 1B)^(2,3).

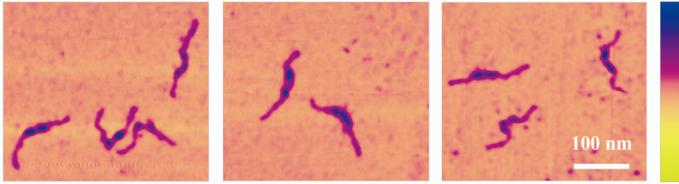


Figure 4. AFM of *cenE*-PrgO complexes scanned in air. Representative AFM images. Height scale ranges from 0 nm (yellow) to 5 nm (blue). The length scale of the right picture is valid for all pictures.

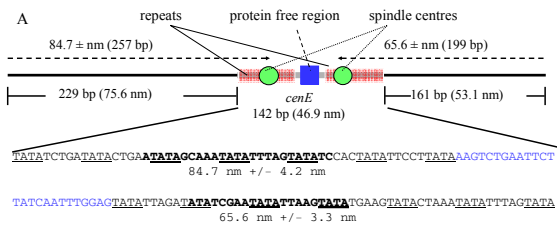


Figure 6. Localization of spindle centres. Summary of AFM analyses for A) *cenE*-PrgO and B) *parC*-ParR. Black line, DNA; grey line, centromere; dashed arrows, distances from the spindle centres to tips of nearest DNA arms. Centromere sequences are presented. Repeats are underlined, the regions between the repeats are in blue font, and the regions of spindle centres are marked in bold letters. Distances between the ends of fragments and centres of spindle complexes were measured (nm) while the lengths of flanking regions result from DNA sequences (1 bp \leftrightarrow 0.335 nm).

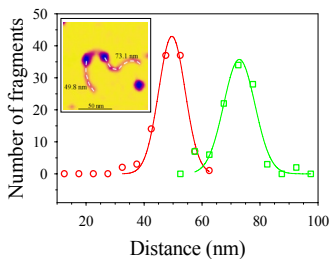


Figure 5. Distances of spindle centres to nearest DNA ends. Short DNA arm: measured distances (red circles) and Gaussian fit (red line). Long DNA arm: measured distances (green squares) and Gaussian fit (green line). Inset: *parC*/ParR complex with two ParR spindle centres. Dashed line indicates the DNA contour for distance measurements.

Results

- Both systems *parC*-ParR (Fig 3 A – G) and *cenE*-PrgO (Fig. 4) form two separate complexes of spindle-like shape^(5, 6).
- The contour lengths of the DNA is the same in the absence and in the presence of the protein components; this holds for both systems.
- The spindles of both systems are located exactly at the two repeat regions (Figs. 5, 6A, B). The DNA between the repeats is protein free.
- ParR causes strong bending leading to a U-shaped folded complex (Fig. 3B-E), PrgO binding results in just moderate bending.
- We analyzed the *parC*-ParR complex in detail. We obtained about 10 ParR molecules per complex (1 dimer per iteron).

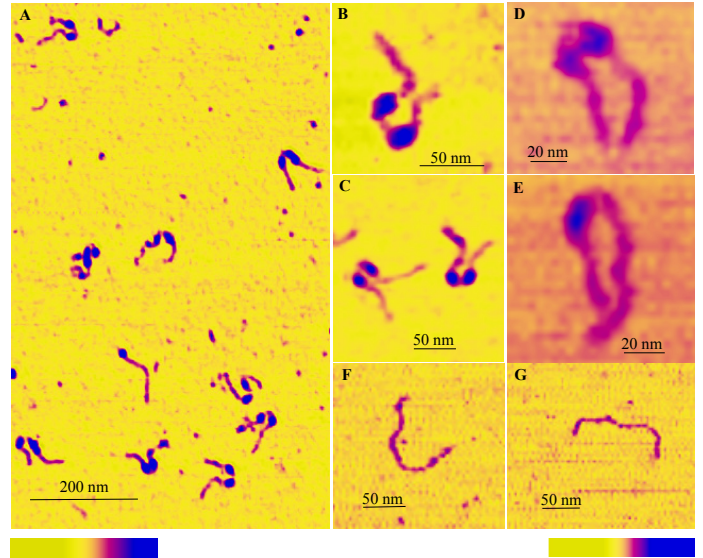


Figure 3. AFM of the *parC*-ParR complex. A) Representative overview scanned in air. B-C) Single complexes in air. Height scale for A – C: 0 nm (yellow) – 4 nm (blue). D) Single complex in fluid. E) Single complex in fluid with just one protein complex; height scale for D – E: 0 nm (yellow) – 8 nm (blue). The length scale is given for each picture by a bar with its length indicated.

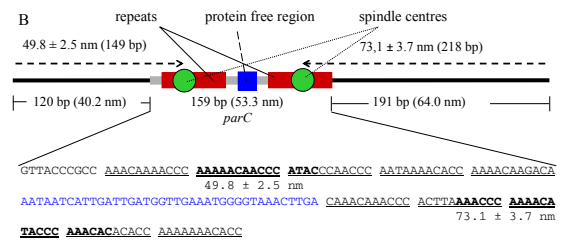


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Comparison to the eukaryotic yeast system

These bacterial segregation systems to some extent resemble the inner kinetochore complex of budding yeast (*S. cerevisiae*): (i) also the yeast centromeres contain two well separated protein binding sites (CDEI and CDEII) separated by a dA:dT-rich DNA sequence (CDEII), (ii) the yeast centromeres are curved⁽⁴⁾, and (iii) the DNA multi-protein complex forms a globular folded structure which is bound by a microtubulus⁽⁸⁾. The yeast multisubunit protein complex CBF3 binds to CDEII and bends the DNA⁽⁹⁾ as assumed for ParR binding. CDEII-binding CBF3 interacts with a CDEI-binding protein⁽¹⁰⁾, as also observed for ParB in the P1 system.

The centromeres of higher eukaryotes might fold into similar structures, since at least for *Drosophila*⁽¹¹⁾ centromeric nucleosomes seem to be tetrameric and not octameric. It remains to be seen if the resemblance of the bacterial with the yeast system is purely accidental or indicative of an evolutionary relation.

Comparison of prokaryotic segregation systems

The P1 *E. coli* plasmid partition module contains the centromeric region *parS* which is recognised by ParB^(11, 7). *parS* itself is intrinsically curved (own unpublished data) and consists of two arms that harbour ParB binding DNA motifs, separated by a central binding site for the *E. coli* integration host factor (IHF) protein (Fig. 7). IHF binding induces a pronounced bend in *parS*, thereby positioning the ParB binding sites in the two arms. Parallel to IHF function, *parC* is strongly curved, however, instead of ParB binding site bridging we observed no interaction between the two ParB binding complexes. One could speculate that, if bridging is necessary for segregation function, this scope might be performed by ParM or another, yet unknown, plasmid or host protein. Also the *cenE*/PrgO complex, shows striking similarities⁽⁵⁾, however, it remains rather straight and does not fold back on itself into a U-shaped form as *parC*/ParR and *parS*/IHF/ParB. If stabilisation of a folded back architecture would be necessary for segregation function, this might be performed by either the ParA homologue, PrgP, or another, yet unknown, plasmid or host protein. Partition complexes might have a similar overall structural organisation and common functional properties.

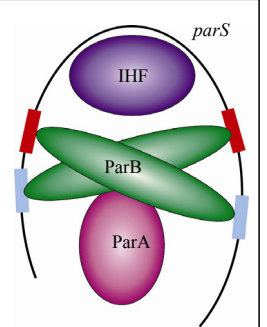


Figure 7. P1 kinetochore complex⁽¹¹⁾. Shown are centromeric DNA (*parS*; black line), IHF (purple), ParB (green), contact heptamers (red) and hexamers (blue), and contacting ParA (pink).

Literature
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