Figures and figure supplements

ParB dynamics and the critical role of the CTD in DNA condensation unveiled by combined force-fluorescence measurements

Julene Madariaga-Marcos et al
Figure 1. Possible scenarios for CTD-induced decondensation of ParB-DNA networks. (A) Model for ParB network formation and condensation via ParB-DNA and ParB-ParB interactions. ParB monomers comprise a central DNA-binding domain (CDBD) with specific and possibly non-specific DNA-binding activities and a carboxy-terminal domain (CTD) with non-specific DNA-binding capacity. The amino terminal domain (NTD) is not represented on the scheme for clarity. (B) In scenario 1, the CTD can bind dsDNA competing for the DNA-binding sites, displacing full length ParB and therefore de-condensing the DNA. (C) In scenario 2, the CTD can exchange with full length ParB forming heterodimers in free solution that are inactive so can no longer exchange with the ParB-DNA network. (D) In scenario 3, the CTD forms heterooligomers with DNA-bound ParB which retain DNA-binding activity but are not able to condense DNA because bridging interactions are ‘capped’ by the CTD. Possible scenarios for CTD-induced decondensation of ParB-DNA networks.

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Figure 2. Combined lateral MT and TIRF microscopy to study ParB-DNA interactions. (A) Cartoon of the MT-TIRF setup used to visualise ParB-DNA interactions at the single-molecule level with controlled external force. A magnet pulls laterally on the distal end of a DNA molecule which is anchored to the coverslip. Fluorescently labelled ParB<sup>AF</sup> is excited in TIRF mode using 488 nm laser light and the emitted light is collected by an EM-CCD (Madariaga-Marcos et al., 2018). (B) TIRF image showing a laterally stretched DNA molecule under a force of 1 pN and in the presence of 500 nM ParB<sup>AF</sup>. Beads showed fluorescence due to additional binding of DNA molecules, which are further labelled by non-specifically bound ParB<sup>AF</sup> proteins. (C) Intensity of several DNA molecules (n = 15) in the presence of 500 nM ParB<sup>AF</sup> as a function of time. Even though the integrated intensity changes from molecule to molecule, intensity remains constant for more than 100 s, suggesting a dynamic and fast exchange between DNA-bound ParB<sup>AF</sup> and free ParB<sup>AF</sup> in the media. (D) Kymograph from the same experimental data shown in (B) highlighting changes in intensity along the DNA molecule through the entire experiment supporting the continuous exchange of the protein. Kymographs varied from molecule to molecule. All experiments were conducted in ParB reaction buffer supplemented with 4 mM Mg<sup>2+</sup>.

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Figure 2—figure supplement 1. Purification and activity assays of ParB<sub>AF</sub> and CTD<sub>AF</sub>. (A) ParB<sub>AF</sub> and CTD<sub>AF</sub> labelling efficiency. (B) ParB<sub>AF</sub> had non-specific (in TBE) and parS specific (in TBM) DNA-binding activity equivalent to that of wild-type ParB, as measured in an EMSA, in EDTA and Mg<sup>2+</sup> containing buffers, respectively (Taylor et al., 2015; Fisher et al., 2017). (C) CTD<sub>AF</sub> had qualitatively similar DNA-binding activity to the wild-type truncated protein CTD. Right gel shows that the CTD was only capable of binding DNA in an EDTA–containing buffer.

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**Figure 2—figure supplement 2.** Control experiments showing increasing intensity due to ParB<sup>AF</sup> binding and constant intensity due to protein exchange. (A) Averaged decrease of intensity due to introduction of protein-free buffer (n = 26). (B) Representative traces of intensity decay of formaldehyde crosslinked ParB<sup>AF</sup> in the presence and absence of an oxygen scavenger system. The oxygen scavenger contributes to a longer lifetime of the ParB<sup>AF</sup> complex. From measurements on several molecules we found average half times of 28 ± 3 s and 140 ± 30 s (mean sem) in the absence and presence of the oxygen scavenger system, respectively. (C) Average intensity recovery of DNA molecules (n = 23) by the introduction of 250 nM ParB<sup>AF</sup> after photobleaching. Intensity recovery is consistent with a fast protein exchange with the media (Video 4).

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Figure 3. ParB binding and unbinding kinetics. (A) Scheme of the multilaminar flow system employed to fast-exchange of buffers. The fluid cell contains two inlets and a single outlet. Switching the velocities of both channels shifts the boundary of laminar flows resulting in the fast exchange of buffers. (B) Normalised integrated fluorescence intensity for a representative DNA molecule in a laminar-flow experiment with 250 nM ParB<sup>AF</sup> as a function of time (red) and for an equivalent background area (gray). Buffer injection (shadowed area) is correlated with decreasing intensity. Best fit curves to obtain $k_{\text{off}}$ (Equation 3) and $k_{\text{obs}}$ (Equation 2) are shown. Boundary exchange with fluorescein is also shown for comparative purposes (Figure 3—figure supplement 1D). (C) Unbinding rate $k_{\text{off}}$ measured at different initial ParB concentrations, obtained from fitting individual curves as shown in B, and then averaged at different concentrations. The values obtained are in the order of values published before (Song et al., 2016). (D) Observed binding rate $k_{\text{obs}}$ measured at different ParB concentrations, calculated as $k_{\text{off}}$. Errors are SD. (n ~ 15-30 molecules). All experiments were conducted in ParB reaction buffer supplemented with 4 mM Mg<sup>2+</sup>.

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Figure 3—figure supplement 1. Correct performance of the syringes demonstrated by tracking DNA tethers and boundary shift fluorescence measurements. (A) Vertical Magnetic Tweezers. (B) Lateral Magnetic Tweezers. Peaks in Y and Z (vertical) and X and Y (lateral) correlate with changes in flow, showing a correct performance by computer controlled syringes. Peaks are asymmetric due to differences in syringe volumes (10 ml and 1 ml). (C) Cycles of buffer/fluorophore exchange at three different flow rates showing that exchange rates range from 3 to 6.5 s (90% total intensity). (D) Data were well fitted to the Taylor-Aris model consistent with a change of boundary as described in Niman et al. (2013).
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Figure 3—figure supplement 2. Measuring $k_{\text{off}}$ and $k_{\text{obs}}$ for different ParB$^{\text{AF}}$ concentrations. (A) Representative integrated fluorescence intensity for a DNA molecule in a laminar-flow experiment, as a function of time, coated with 125, 250 or 500 nM ParB$^{\text{AF}}$. Protein injection correlates with increasing fluorescence intensity along the DNA tether, while protein unbinding correlates with decreasing intensity (shadowed area). Best-fit curves to obtain $k_{\text{off}}$ (Equation 3) and $k_{\text{obs}}$ (Equation 2) are shown. (B) Plot of intensities obtained at equilibrium (plateaus in Figure 3—figure supplement 2) for different ParB concentrations. The Hill plot using $k_d$ values from Taylor et al. (2015) is shown as reference.

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Figure 4. ParB binding kinetics in the presence and absence of Mg$^{2+}$ and the effect of the free CTD. (A) Unbinding rate $k_{\text{off}}$ and observed binding rate $k_{\text{obs}}$ values for 250 nM ParB$^{\text{AF}}$. We observed a reduction in $k_{\text{off}}$ (slower unbinding) in the case of EDTA but no significant difference was observed in $k_{\text{obs}}$ values. (B) Unbinding rate $k_{\text{off}}$ and observed binding rate $k_{\text{obs}}$ values for 250 nM ParB$^{\text{AF}}$ in the presence (red) and absence (gray) of 2.5 μM CTD, in Mg$^{2+}$- or EDTA-containing buffer. We report faster unbinding in the presence of the CTD in EDTA. No effect of the CTD in the unbinding rate was observed in Mg$^{2+}$ buffer, according to Student’s t-test. No significant difference was observed in $k_{\text{obs}}$ values in the presence or absence of the CTD in both Mg$^{2+}$ and EDTA conditions. All errors are SD. (n ~ 12-28 molecules). Data were accounted for statistical differences following a Student’s t-test (see Table 1 for $k_{\text{off}}$ p-values and Table 2 for $k_{\text{obs}}$ p-values).

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Figure 4—figure supplement 1. The CTD is capable of binding DNA. (A) This DNA molecule is visible due to binding by 10 μM CTD^AF in buffer supplemented with EDTA, but not with Mg^2+. ParB^AF complexes are also visible in buffer supplemented with EDTA. (B) Fluorescence intensity traces of CTD^AF showing protein exchange, similar to the full-length ParB protein. Differences in bead mobility affect the measured fluorescence signal. (C) Comparison of FRAP experiments. At such high concentrations ($\geq 1\, \mu\text{M}$), there is no noticeable difference between ParB^AF and CTD^AF. (D) Dissociation experiments of ParB^AF and CTD^AF flowing protein-free EDTA buffer. Both ParB^AF and CTD^AF dissociate from DNA, but CTD^AF unbinds ~2.3 fold faster than ParB (i.e., has a higher $k_{off}$).

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Figure 4—figure supplement 2. Measuring the effect of the CTD on the dissociation rate in EDTA and Mg$^{2+}$ buffer conditions. Representative traces of normalised fluorescence intensity as a function of time for $k_{off}$ data shown in Figure 4B. Protein binding and unbinding were cyclically measured as described before (Figure 2B) and unbinding traces were fit to Equation 3 to obtain $k_{off}$ values. The different decay trend under EDTA and Mg$^{2+}$ conditions, and both in the presence or absence of the CTD, can be appreciated.

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Figure 5. Competition of the CTD in ParB binding and unbinding kinetics, and effects in condensation. (A) Visualisation of condensation of a single DNA molecule induced by 250 nM ParB<sup>AF</sup> binding. The volume of the bead causes the DNA to be slightly tilted with respect to the surface such that emission of fluorescence is limited by the penetration depth of the excitation field. The DNA does not condense while held at non-permissive force of 1 pN. However, as in conventional magnetic tweezers experiments, at a permissive force of 0.2 pN a progressive reduction of the DNA length is observed. (B) Condensation experiment as in Figure 5A but in the presence of 2.5 μM non-fluorescent CTD showing that at a constant force of 0.2 pN, ParB is not able to condense to the inhibition of the CTD. Co-incubation with the CTD does not inhibit ParB binding to DNA, as indicated by clearly visible fluorescence filaments. (C) Condensation experiment as in Figure 5B. The CTD inhibits ParB-dependent DNA condensation, even at forces as low as 0.07 pN. Note that some residual condensation was observed at very low forces, visible at the anchoring point (arrow) as shown by the bright dot. (D) Percentage of DNA molecules that were condensed (more than 90% extension reduction), partially condensed or not condensed by ParB<sup>AF</sup>.
(less than 10% extension reduction), in the presence (n = 47) or absence of an excess of the CTD (n = 36). (E) Integrated fluorescence intensity in a laminar flow experiment. Intensity remains constant throughout the cycles of ParB\textsuperscript{AF} and ParB\textsuperscript{AF} + CTD (shadowed area), indicating that the excess of CTD is not competing for the binding sites on the DNA, but rather ‘capping’ the DNA-bound ParB\textsuperscript{AF}. Intensity changes when alternating ParB\textsuperscript{AF} with a combination of ParB\textsuperscript{AF} and unlabelled ParB (shadowed areas). (F) Integrated intensity of ParB\textsuperscript{AF} plotted versus the integrated intensity of ParB\textsuperscript{AF} + CTD or ParB\textsuperscript{AF} + ParB. ParB\textsuperscript{AF} + CTD shows a slope of 1.00 ± 0.02 corresponding to unchanged intensity, while ParB\textsuperscript{AF} + ParB shows a slope of 1.23 ± 0.02. (n = 10–16 molecules). All experiments were conducted in ParB reaction buffer supplemented with 4 mM Mg\textsuperscript{2+}.

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