Figures and figure supplements

Poly(ADP-ribose) polymerase 1 searches DNA via a ‘monkey bar’ mechanism

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Figure 1. Domain organization of PARP1 and structural details of how PARP1 binds to a DSB. (A) Schematic of the domains of PARP1; (B) DNA-binding domains (Zn1, green, Zn3 gray, and WGR, blue) of PARP1 engaging a DNA DSB (red). Residues R34 and F44 of the phosphate backbone grip and the base stacking loop in the Zn1 domain are shown in yellow and W589 in the WGR domain is shown in light blue. Coordinates were taken from 1dqy. DOI: https://doi.org/10.7554/eLife.37818.003
Figure 1—figure supplement 1. SDS-PAGE showing purified PARP1, its deletion constructs and the W589A point mutant. Each construct was also assayed for activity in the presence (+) of NAD+ (200 μM) and one equivalent of p18mer DNA. Higher molecular weight smears are seen for WT and ΔZn2, indicating auto-PARylation.

DOI: https://doi.org/10.7554/eLife.37818.004
Figure 2. PARP1 association with DNA as monitored by fluorescence anisotropy. (A) Representative measurement of PARP1 association with DNA as monitored by fluorescence anisotropy. Shown are the data in the absence of PARP1 (blue) and in the presence of 83 nM PARP1 (red). The black line shows a first-order exponential fit to the data.

Figure 2 continued on next page
Figure 2 continued

data and the residuals ($r$) from this fit are shown above. The inset shows a replot of $k_{obs}$ vs. varying concentrations of PARP1. (B) Global fitting of three representative concentrations of PARP1 using the mechanism in Scheme 1: 50 nM in blue, 83 nM in green and 133 nM in red. Residuals ($r$) for the three concentrations are shown in the corresponding colors above.

DOI: https://doi.org/10.7554/eLife.37818.005
Figure 2—figure supplement 1. Association of ΔZn1 with p18mer*. Global fits of the kinetic model in Scheme 1 for three representative protein concentrations: 42 nM in blue, 72 nM in green, 120 nM in red. Residuals for the three concentrations are shown overlaid in the corresponding colors above.

DOI: https://doi.org/10.7554/eLife.37818.006
Figure 2—figure supplement 2. Association of ΔZn2 with p18mer*. Global fits of the kinetic model in Scheme 1 for three representative protein concentrations: 35 nM in blue, 52 nM in green, 95 nM in red. Residuals for the three concentrations are shown overlaid in the corresponding colors above.

DOI: https://doi.org/10.7554/eLife.37818.008
Figure 2—figure supplement 3. Association of ΔZn3 with p18mer*. Global fits of the kinetic model in Scheme 1 for three representative protein concentrations 50 nM in blue, 83 nM in green, 133 nM in red. Residuals for the three concentrations are shown overlaid in the corresponding colors above.
DOI: https://doi.org/10.7554/eLife.37818.010
Figure 2—figure supplement 4. Association of ΔWGR with p18mer*. Global fits of the kinetic model in Scheme 1 for three representative protein concentrations: 37 nM in blue, 74 nM in green, 129 nM in red. Residuals for the three concentrations are shown overlaid in the corresponding colors above.

DOI: https://doi.org/10.7554/eLife.37818.012
Figure 2—figure supplement 5. Association of W589A with p18mer*. A global fit of the kinetic model in Scheme 1 to three representative protein concentrations is shown (38 nM in blue, 51 nM in green, and 63 nM in red).

DOI: https://doi.org/10.7554/eLife.37818.014
Scheme 1. Kinetic model for association of PARP1 with DNA.
DOI: https://doi.org/10.7554/eLife.37818.018
**Scheme 2.** Kinetic model for dissociation of PARP1 from labeled dDNA in the presence of competing unlabeled DNA.

DOI: https://doi.org/10.7554/eLife.37818.021
Figure 3. Representative measurement of PARP1 dissociation from DNA as monitored by fluorescence anisotropy. Shown are the data in the absence of competitor DNA (in blue) and in the presence of 2.2 μM (green) and 4 μM DNA (red). The black line shows a first-order exponential fit to the data and the residuals from these fits are shown in the corresponding colors above. The inset shows a replot of $k_{obs}$ vs. varying concentrations of competitor DNA. DOI: https://doi.org/10.7554/eLife.37818.022
Figure 3—figure supplement 1. Label-swap experiment demonstrating that monitoring p18mer* and p18mer release are kinetically identical. The pseudo-residuals generated by addition of the two signals are centered at the total concentration of the probe DNA (25 nM).

DOI: https://doi.org/10.7554/eLife.37818.023
Scheme 3. Kinetic model for the dissociation of PARP1 from labeled DNA that depends on formation of a ternary complex with the unlabeled DNA.

DOI: https://doi.org/10.7554/eLife.37818.026
Figure 4. PARP1 dissociation from DNA as monitored by fluorescence anisotropy. Global fitting of six representative concentrations of competitor p18mer DNA using the mechanism in Scheme 3. 76 nM (light green), 149 nM (blue), 225 nM (red), 398 nM (violet), 1 µM (dark green), and 4 µM (brown). Residuals for the seven concentrations are shown overlaid in the corresponding colors above.

DOI: https://doi.org/10.7554/eLife.37818.027
Figure 4—figure supplement 1. Thermodynamic parameters for DNA binding to PARP1. All values (mean and standard deviation) were derived from the kinetic parameters in Table 1.

DOI: https://doi.org/10.7554/eLife.37818.028
Figure 4—figure supplement 2. PARP1 dissociation from DNA as monitored by fluorescence anisotropy. Global fitting of six representative concentrations of competitor DNA using the mechanism in Scheme 2: 76 nM (light green), 149 nM (blue), 225 nM (red), 398 nM (violet), 1 μM (dark green), and 4 μM (brown). Residuals for the seven concentrations are shown overlaid in the corresponding colors above.

DOI: https://doi.org/10.7554/eLife.37818.030

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<td>ΔZn3</td>
<td>&lt; 1.8</td>
<td>2600 ± 1300</td>
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<tr>
<td>ΔWGR</td>
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<tr>
<td>W589A</td>
<td>4.9 ± 1.1</td>
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Figure 4—figure supplement 3. Dissociation of \( \Delta Zn1 \) from p18mer*. Global fits of five representative concentrations of competitor DNA are shown: 0.15 \( \mu \)M (blue), 0.4 \( \mu \)M (light green), 1 \( \mu \)M (violet), 2 \( \mu \)M (dark green), and 4 \( \mu \)M (red) to the kinetic mechanism in Scheme 3. Residuals for the five concentrations are shown overlaid in the corresponding colors above each plot.

DOI: https://doi.org/10.7554/eLife.37818.032
Figure 4—figure supplement 4. Dissociation of ΔZn2 from p18mer*. Global fits of five representative concentrations of competitor DNA are shown: 0.15 µM (blue), 0.4 µM (light green), 1 µM (violet), 2 µM (dark green), and 4 µM (red) to the kinetic mechanism in Scheme 3. Residuals for the five concentrations are shown overlaid in the corresponding colors above each plot.
DOI: https://doi.org/10.7554/eLife.37818.034
Figure 4—figure supplement 5. Dissociation of –Zn3 from p18mer*. Global fits of five representative concentrations of competitor DNA are shown: 0.15 μM (blue), 0.4 μM (light green), 1 μM (violet), 2 μM (dark green), and 4 μM (red) to the kinetic mechanism in Scheme 3. Residuals for the five concentrations are shown overlaid in the corresponding colors above each plot.

DOI: https://doi.org/10.7554/eLife.37818.036
Figure 5. PARP1 dissociation from DNA as monitored by fluorescence anisotropy. Global fitting of three representative concentrations of competitor p18mer DNA using the mechanism in Scheme 2: 0.4 μM in blue, 1.3 μM in green, and 4 μM in red for (A) ΔWGR and (B) W589A. Residuals for the three concentrations are shown overlaid in the corresponding colors above.

DOI: https://doi.org/10.7554/eLife.37818.039
Figure 6. PARP1 dissociation from DNA as monitored by fluorescence anisotropy. (A) The observed rate of dissociation triggered by 5 nM of intact or variably cut plasmid is compared to 5 nM and 1 μM p18mer. (B) Global fitting of three representative concentrations of competitor intact plasmid DNA using the mechanism in Scheme 3: 0.7 nM in blue, 2.0 nM in green, and 5.8 μM in red. Residuals for the three concentrations are shown overlaid in the corresponding colors above.
DOI: https://doi.org/10.7554/eLife.37818.042
Figure 6—figure supplement 1. DNA 1% agarose gel demonstrating the various forms of plasmid used to demonstrate that undamaged DNA can effectively promote the monkey-bar mechanism for PARP1 to move from one segment of DNA to another. Note the characteristic faster migration of supercoiled DNA compared to singly-cut plasmid. Also note that digest with EcoRV yields the parent plasmid and 12 copies of the 147 bp inserts.

DOI: https://doi.org/10.7554/eLife.37818.043
Figure 7. PARP1 dissociation from DNA as monitored by fluorescence anisotropy in the presence of various inhibitors of PARP1. (A) Apparent $k_{\text{obs}}$ at 1 μM competitor DNA using four different inhibitors (50 nM) (ola = olaparib, veli = veliparib, nira = niraparib, tala = talazoparib). (B) Apparent $k_{\text{obs}}$ at 1–4000 nM competitor DNA for PARP1 alone (red) and PARP1 the presence of 50 nM talazoparib (blue).

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Figure 7—figure supplement 1. Smear assay of PARP1 demonstrating the effectiveness of four different inhibitors in blocking the activity of PARP1. Each inhibitor was added at 1 μM final concentration in the presence of 0.5 μM PARP1, 1 μM p18mer, and 500 μM NAD⁺, and the reactions were allowed to proceed for 5 min at room temperature.
DOI: https://doi.org/10.7554/eLife.37818.051
Figure 8. Model for the monkey bar mechanism for PARP1 depicting the proposed role of the WGR domain in capturing the second DNA strand prior to release of the originally bound DNA strand and subsequent re-arrangement around the second DNA.

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