Double-Strand Break Stabilisation by PARP Proteins

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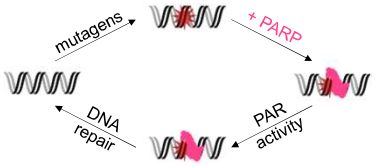
Introduction

- Poly ADP-ribosylation polymerases (PARPs) are a superfamily of proteins characterised by a conserved ADP-ribose transferase (ART) domain.
- They modulate activity by transferring ADP-ribose onto target sites.
- DNA binding PARPs sense and bind DNA breaks and are characterised by a conserved WGR domain.[1,2]
- The most abundant DNA-binding PARPs (PARP -1 and -2) are partially redundant but have varying affinities for DNA breaks[3], potentially due to PARP1 having much more extensive DNA binding domains.[2]
- PARP inhibitors (PARPi) bind PARP-DNA complexes, preventing PARP unbinding from DNA and recruitment of repair factors.
- In *BRCA*-mutant cancer cells, PARPi activity allows double strand breaks (DSBs) to accumulate and cause targeted cell death[2,4] as these cells are sensitive to DNA damage due to a dysfunctional homologous recombination repair pathway.[5]



We tested how DSB structure affects:

1. DSB stability
2. The number of distinct PARP2 binding modes
3. The stability of these PARP2-DSB complexes

Methods

Magnetic tweezer setup

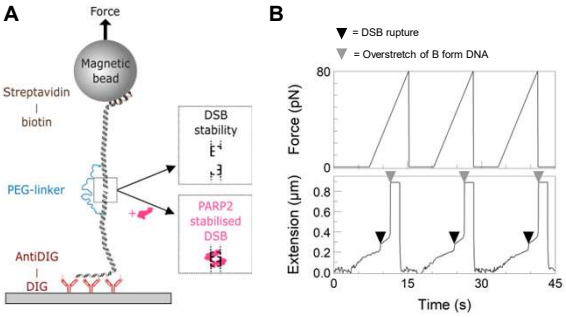


Fig. 1 Magnetic Tweezers Setup (a) A superparamagnetic bead (grey) is shown tethered via DNA to glass (white). (b) An example trace of the change in DNA extension as externally applied force increases linearly, steps indicating the coming apart of a self-complementary DSB and a DNA overstretch are labelled with black and grey arrows respectively.

Increasing tether stability

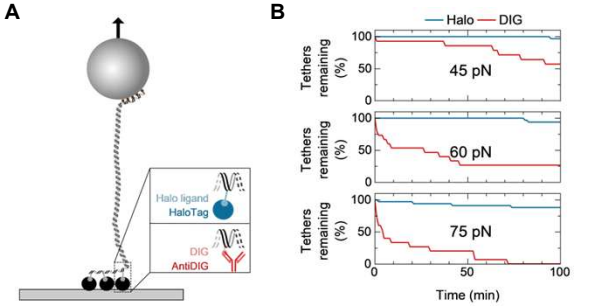


Fig 2. Lifetime comparison between DIG- and halo ligand- bound tethers. (a) Schematic of DNA tethers setup in magnetic tweezers (b) The lifetimes of tethers tested at 45-, 60- and 75-pN when bound to the surface via DIG (blue) or halo ligand (red) handles. N=14 N=15, N=15 for DIG- and N=31, N=32 and N=33 for halo -tethers respectively. Lifetimes were compared with a log-rank test, all p-values were statistically significant at values <0.005.

Results

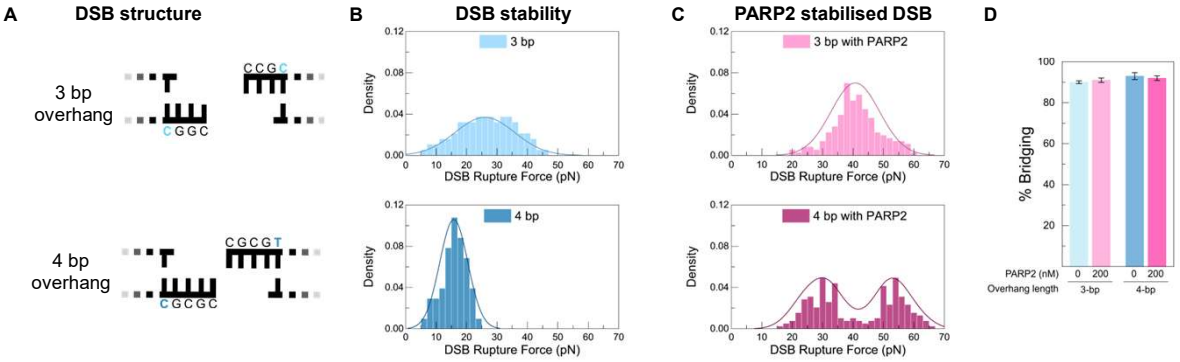


Fig. 3 Single-molecule measurements of DSB stability and PARP2-mediated stability changes (a) A schematic of DSB sequences used. Primary base stacking pairs labelled in blue bold text. (b) The distribution of forces required to rupture each DSB with the 3- and 4- bp overhangs shown in light and dark blue respectively. The curves show a Bell-Evans fit of the data. (c) The distribution of forces required to rupture each wild-type PARP2-stabilised DSB, with the 3- and 4- bp overhang DSBs shown in light and dark pink respectively. Data for fig 3b-c are taken from N = 3 beads (3 bp) and N = 10 beads (4 bp). The curves show a Kernal smooth fit of the data. (d) The percentage bridging observed in force-ramp repeats for the 3- and 4-bp overhang DSBs (0 nM and 200 nM PARP2 left to right for each). Error bars show +/- 2 SEM.

Conclusions

- The effect of base stacking on G-C base pairing stability can be measured on a single-molecule scale using magnetic tweezers.
- PARP2 has at least two distinct binding modes to DSBs.
- DSB overhang length dictates PARP2 binding mode but not affinity.

Future directions

1. Quantify the effect of other base stacking on G-C stability.
2. Investigate whether the effect of base stacking on A-T base-pairing can be detected on a single-molecule basis using magnetic tweezers.
3. Investigate how PARP2 binds to different overhang lengths and whether increasing overhang length further reveals a third binding mode.

References

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