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HELQ is a dual function DSB repair enzyme modulated by RPA and RAD51

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Summary

DNA double strand breaks (DSBs) are deleterious lesions, and their incorrect repair can drive cancer development¹. HELQ is a superfamily 2 helicase with 3' to 5' polarity, whose disruption in mice confers germ cells loss, infertility and increased predisposition to ovarian and pituitary tumours²⁻⁴. At the cellular level, defects in HELQ result in hypersensitivity to cisplatin and mitomycin C and, persistence of RAD51 foci upon DNA damage^{3,5}. Notably, HELQ binds to RPA and the RAD51 paralog BCDX2 complex but the relevance of these interactions and how HELQ functions in DSB repair remains unclear^{3,5,6}. Here, we report that HELQ helicase activity and a previously unappreciated DNA strand annealing function are differentially regulated by RPA and RAD51. Using biochemistry and single-molecule imaging (SMI), we establish that RAD51 forms a co-complex with and strongly stimulates HELQ as it translocates during DNA unwinding. Conversely, RPA inhibits DNA unwinding by HELQ but strongly stimulates DNA strand annealing.

26 **Mechanistically, we show that HELQ possesses an intrinsic ability to capture RPA-bound DNA**
27 **strands and then displace RPA to facilitate annealing of complementary strands. Finally, we show**
28 **that HELQ deficiency in cells compromises single-strand annealing (SSA) and microhomology-**
29 **mediated end joining (MMEJ) pathways and increases long-tract gene conversion tracts (LTGC)**
30 **during homologous recombination. Thus, our results implicate HELQ in multiple arms of DSB**
31 **repair by virtue of co-factor dependent modulation of intrinsic translocase and DNA strand**
32 **annealing activities.**

33

34 **Results**

35 To investigate the functions of HELQ, we purified recombinant human HELQ from insect cells (Extended
36 Data Fig.1a), which efficiently unwound substrates containing 3' overhangs or a D-loop (Fig. 1a,b,
37 Extended Data Fig. 1b-d). However, at higher concentrations of HELQ, no unwound product was
38 observed (described later; Extended Data Fig. 1e). HELQ showed no unwinding with ATP γ S, a poorly
39 hydrolysable ATP analogue, and failed to unwind dsDNA and 5' overhang substrates (Extended Data
40 Fig. 1f-h). The helicase dead mutant of HELQ, HELQ K365M lacked DNA unwinding activity but retained
41 similar ssDNA and dsDNA binding as WT protein (Extended Data Fig. 1a,i-m).

42

43 **RAD51 stimulates HELQ unwinding activity**

44 *In vivo* studies have shown that HELQ deficient cells exhibit persistent RAD51 foci upon DNA damage^{3,7}.
45 Furthermore, HELQ-1 from *C. elegans* interacts with RAD-51⁷. To investigate the interplay between
46 HELQ and RAD51, we purified human RAD51 from *E. coli* (Extended Data Fig. 2a), which interacts
47 directly with HELQ (Extended Data Fig. 2b). In unwinding assays, RAD51 strongly stimulated HELQ
48 helicase activity with all tested substrates (Fig. 1c,d, Extended Data Fig. 2c-e). Conversely, bacterial
49 RecA did not show any such stimulation, which excludes that stimulation by RAD51 is indirect through
50 sequestering of unwound product (Fig. 1c,e, Extended Data Fig. 2c-e). We also measured the kinetics
51 of DNA unwinding by HELQ in the absence and presence of RAD51. Addition of RAD51 resulted in a
52 concentration dependant increase in the HELQ DNA unwinding rate, while addition of RecA had no effect
53 (Extended Data Fig. 2f-h). In cells, the ssDNA generated during DNA processing is usually bound by
54 RPA. To mimic these conditions, we purified fluorescently tagged human RPA-mRFP1 from *E. coli*

55 (Extended Data Fig. 3a). Addition of RPA strongly inhibited DNA unwinding by HELQ especially with 3'
56 overhang substrates (Fig. 1f,g, Extended Data Fig. 3b,c). At lower concentrations, insufficient to cover
57 the entire ssDNA region, RPA still inhibited HELQ unwinding of 3'-overhang (Extended Data Fig. 3d,e).
58 Despite the inhibitory effect of RPA, RAD51 still strongly stimulated HELQ helicase in the presence of
59 RPA (Extended Data Fig. 3 f-i).

60

61 **Visualization of HELQ DNA unwinding**

62 To better understand HELQ stimulation by RAD51, we used an optical tweezers, microfluidics and
63 confocal microscopy (C-TRAP) setup for SMI analysis. As shown in Fig 1h, a single DNA molecule
64 (λ DNA) containing a single-stranded DNA gap⁸ was tethered between two optically trapped beads and
65 held at constant force (50 pN) to prevent reannealing of unwound DNA. On addition of HELQ, DNA
66 unwinding was observed as an increase in distance between the beads, due to the expansion of the
67 ssDNA region. Neither RAD51 alone nor HELQ K365M showed evidence of unwinding (Fig. 1i, Extended
68 Data Fig. 4a). On addition of RAD51, a dramatic increase in overall DNA unwinding was observed with
69 WT HELQ, whereas no such stimulation was observed with HELQ K365M (Fig. 1j,k, Extended Data Fig.
70 4b). Within unwinding traces for individual DNA molecules, rapid unwinding bursts interspersed by
71 pauses can be distinguished (Extended Data Fig. 4c-e) and corresponded to a mean rate of 2.5 ± 0.7
72 nm/s (S.E.M). In the presence of RAD51, two populations of unwinding can be distinguished: slow with
73 mean rates of 4.3 ± 0.7 nm/s (S.E.M) corresponds roughly to rates measured in the absence of RAD51
74 and fast with mean rates of 14 ± 0.23 nm/s (S.E.M) (Extended Data Fig. 4f,g). To directly visualize RAD51
75 during DNA unwinding with HELQ, mutant RAD51 C319S was purified and labelled with Alexa Fluor 488
76 C5 maleimide dye (Alx-RAD51) (Extended Data Fig. 4h). While Alx-RAD51 alone displayed mostly static
77 binding traces with occasional diffusing species, addition of HELQ showed unidirectional translocation
78 traces indicating active movement of an Alx-RAD51-HELQ complex along the ssDNA backbone (Fig. 1l,
79 Extended Data Fig. 4i). Translocation rates of this species (14 ± 5 nm/s) matches well with the fast
80 population of unwinding bursts observed in the presence of HELQ and RAD51 (Extended Data Fig. 4j,k).
81 Conversely, HELQ K365M retained the ability to bind RAD51 but showed no translocation with only static
82 or diffusing traces. Together, these results indicate that RAD51 and HELQ form a complex that unwinds

83 DNA with approximately 3 to 5-fold faster rate than HELQ alone, which is in agreement with bulk
84 experiments.

85

86 **HELQ possesses robust DNA strand annealing activity**

87 As shown earlier, a lack of unwound product was observed at higher concentrations of HELQ (Fig. 1a,b,
88 Extended Data Fig. 1b-e), which we considered could be due to re-annealing of the unwound product.
89 Notably, we found that reactions containing an unlabelled “cold” oligo yielded an increase in unwound
90 product with excess HELQ (Extended Data Fig. 5a, compare lanes 3 and 4 to 7 and 8). We also performed
91 kinetic analysis to monitor the fate of the unwound substrate and found that HELQ initially unwinds the
92 substrate but then reanneals it back together at later time points (Extended Data Fig. 5b). Prompted by
93 this, we directly tested HELQ for DNA strand annealing activity without and with an excess of RPA (i.e.,
94 250%) to provide full DNA coverage to ensure accurate detection of DNA annealing. We found that HELQ
95 efficiently anneals complementary DNA strands either without or with RPA (Fig. 2a,b). Interestingly, at
96 lower concentrations, RPA stimulated DNA annealing activity by HELQ ~2-fold. However, at higher
97 concentrations, HELQ showed greater DNA annealing activity in the absence of RPA. This raised the
98 possibility that RPA aids HELQ loading on ssDNA when HELQ is present in limited amounts. Titration
99 experiments further confirmed that sub-stoichiometric levels of RPA are sufficient to stimulate HELQ
100 annealing activity (Fig. 2c,d). Notably, HELQ could still anneal complementary DNA strands even in the
101 presence of excess RPA (Extended Data Fig. 5c,d).

102

103 We next tested the requirement of ATP binding and hydrolysis for DNA annealing by HELQ. Surprisingly,
104 in the presence of RPA, HELQ showed no DNA annealing without ATP whereas ATP became
105 dispensable when RPA was excluded from the reaction (Extended Data Fig. 5e-h). Even in the absence
106 of RPA, ATP stimulated the DNA annealing activity of HELQ (Extended Data Fig. 5g,h). HELQ also failed
107 to anneal DNA completely with ATPγS in the presence of RPA (Extended Data Fig. 5i). Collectively, these
108 data suggest that HELQ possesses intrinsic DNA annealing activity, which requires ATP binding and
109 hydrolysis when ssDNA is coated with RPA. We next tested the helicase inactive HELQ K365M mutant
110 for DNA annealing activity and found that HELQ K365M was defective for DNA annealing in the presence
111 of RPA (Extended Data Fig. 5j,k). However, HELQ K365M retained the ability to anneal DNA efficiently in

112 the absence of RPA but becomes progressively impaired when RPA coverage of DNA exceeds 100%
113 (Fig. 2e,f, Extended Data Fig. 5l,m). RPA also failed to stimulate HELQ K365M (Fig. 2e,f). We also tested
114 *E. coli* SSB protein and found that it only weakly stimulates HELQ annealing activity (Extended Data Fig.
115 5n,o). The N-terminal fragment of HELQ was previously shown to displace RPA from ssDNA⁶. However,
116 full-length HELQ was not analysed for such activity. To directly visualize RPA displacement from ssDNA
117 during DNA strand annealing, we performed a “stripping” assay where the deproteination step was
118 omitted. We observed that HELQ can strip an excess of RPA from ssDNA, which occurred coincidentally
119 with the appearance of the annealed products (Fig. 2g, Extended Data Fig. 6a). Using a single-molecule
120 FRET-based assay (Extended Data Fig. 6b-i), we observed concentration dependant RPA stripping by
121 HELQ, followed by re-binding of RPA (Fig. 2h-k). The RPA re-binding is independent of HELQ
122 concentration indicating a constant transition rate from free (t_{on}) to bound (t_{off}) at various HELQ
123 concentrations (Fig. 2l). Notably, HELQ K365M did not show RPA stripping in this set up, which is likely
124 due to excess RPA binding to DNA and/or stable binding of HELQ K365M to DNA after transient RPA
125 removal (Fig. 2m). These data confirm our bulk experiments indicating that active RPA-stripping plays a
126 critical role in HELQ-mediated DNA annealing. Finally, we found that addition of RAD51 had no effect on
127 HELQ-dependent DNA annealing activity (Extended Data Fig. 6j,k).

128

129 **HELQ shows RPA-mediated sequence-independent DNA capture**

130 RAD52 also possess DNA annealing activity and plays a central role in SSA repair of DSBs⁹⁻¹³. Using an
131 optical trapping microscope, it was shown previously that RAD52 can *trans*-capture labelled oligos at
132 multiple sites along λ DNA independent of DNA sequence¹⁴. Using a similar set up, we tested the ability
133 of HELQ to capture a Cy3 labelled 80-mer oligo ssDNA in the presence of RPA-eGFP (~100% coverage;
134 Fig 3a, Extended Data Fig. 7a). HELQ facilitated the annealing of λ 4 oligo^{15,16} at multiple sites (Fig. 3b,c).
135 Notably, HELQ K365M also exhibited efficient DNA capture activity (Fig. 3b,c). We also analysed the
136 dwell times of captured oligos and found that HELQ K365M showed moderately increased dwell times
137 compared to WT (Fig. 3d,e). We also tested a 79 nucleotide T-homopolymer (dT-79) and obtained similar
138 results as with λ 4 oligo (Fig. 3c).

139

140 To understand the mechanism of oligo capture by HELQ, we force stretched the tethered ss- λ DNA. Since
141 DNA starts to melt at forces >60 pN, we reasoned that if HELQ oligo capture involves base pairing
142 interactions, short microhomologies should dissociate faster than the ones with longer homology.
143 However, to our surprise, even at very high force (90-100 pN), all oligos remained engaged with ssDNA,
144 irrespective of position (Fig. 4f). During the pulling experiment, we also observed characteristic force
145 spikes when beads are pulled apart at low-force (10-15 pN) (Extended Data Fig. 7b,c). These spikes
146 correspond to disruption of HELQ complexes capturing RPA-coated ss- λ DNA in *cis*. To dissect this
147 further, we developed a bulk capture assay, where we attempted to pull out labelled non-complementary
148 DNA (Cy3-dT79) with a biotinylated dT43 oligo with HELQ. We found that HELQ WT and the K365M
149 mutant could capture non-complementary DNA but only in the presence of RPA (Fig. 4g,h, Extended
150 Data Fig. 7d). Hence, HELQ can capture DNA strands independent of sequence likely via DNA tethering.
151 Interestingly, yeast Rad52, when bound to RPA-coated ssDNA clusters, was also shown to capture
152 additional free RPA in pre-existing Rad52-RPA-ssDNA clusters¹⁷. This activity was postulated to be
153 important for second-end capture.

154

155 **HELQ functions in SSA and Alt-EJ**

156 To extend our findings with HELQ to DSB repair *in vivo*, we first confirmed that *HELQ* depletion or deletion
157 in cells inhibits HR (Fig. 4a-c, Extended Data Fig. 8a,b). Since DNA annealing is required for SSA repair,
158 we investigated a potential role for HELQ in this process. Strikingly, HELQ depletion also impaired SSA
159 repair of an integrated SSA reporter (SA-GFP; Fig. 4d,e). This was further corroborated in *HELQ*^{-/-} cells
160 (Extended Data Fig. 8a,c). While depletion of the HR factor, BRCA2, increases SSA repair, this was
161 strongly reduced by HELQ depletion (Fig. 4f,g). Consistent with an epistatic role in SSA, co-depletion of
162 RAD52 and HELQ did not further decrease SSA repair when compared to individual depletions (Extended
163 Data Fig. 8d,e). We also assessed if HELQ functions in Alt-EJ repair, which involves an annealing step.
164 Using cells containing both EJ-RFP and DR-GFP reporter systems for simultaneous detection of Alt-EJ
165 and HR, respectively, we observed a significant reduction in both DSB repair pathways upon HELQ
166 depletion (Fig. 4 h,i). Finally, impaired DNA strand annealing during second-end capture during DSBR or
167 failure to capture the repaired strand in synthesis dependent strand annealing (SDSA) can result in a shift
168 towards long-tract gene conversions (LTGC)¹⁸⁻²². Using the same reporter system, we found that HELQ

169 deficiency results in an overall decrease in both short and long-tract GC, with the LTGC/total GC ratio
170 showing a significant increase in LTGC, which is similar to RAD52 depletion (Fig. 4j-m).

171

172 In summary, our study implicates HELQ in several distinct DSBs repair pathways, including HR, SSA and
173 Alt-EJ, which cast light on its role in genome stability and tumour avoidance. Since these repair pathways
174 each require DNA annealing steps, we propose that HELQ functions in these pathways through its
175 intrinsic ability to captured RPA-bound ssDNAs and then displace RPA to facilitate annealing of
176 complementary DNA strands. Our finding that HELQ is epistatic with RAD52 for SSA is surprising as this
177 implicates two distinct DNA strand annealing enzymes in the same repair process. Finally, the bias
178 towards long-tract gene conversion events following HELQ depletion is consistent with a role for DNA
179 annealing by HELQ during second strand capture and/or synthesis-dependent strand annealing during
180 HR.

181

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242

Figure 1

Fig. 1 RAD51 forms co-complex with and stimulate HELQ unwinding. **a**, Representative gel of DNA unwinding assay with indicated concentrations of HELQ with 3'-overhang. The asterisk (*) indicates the position of FITC (Fluorescein isothiocyanate) labelling at 5' end of oligo. The products were resolved on 10% native polyacrylamide gel. **b**, Quantification of experiments such as shown in **a** and Extended Data Fig. 1**b-d**. Only HELQ concentrations 1 to 90 nM shown. $n = 4$ independent experiments; mean $\pm$ S.E.M. **c**, Representative gel of DNA unwinding of 3'-overhang with HELQ (1 nM) and indicated concentrations of RAD51 or RecA. **d**, Quantification of experiments (with RAD51) such as shown in **c** and Extended Data Fig. 1 **c-d**. $n \geq 3$ independent experiments; mean $\pm$ S.E.M. **e**, Quantification of experiments (with RecA) such as shown in **c** and Extended Data Fig. 1 **c-d**. $n \geq 3$ independent experiments; mean $\pm$ S.E.M. **f**, Representative gel of DNA unwinding assay of 3'-overhang with indicated concentrations of HELQ in the absence and presence of RPA (20 nM). **g**, Quantification of experiments such as shown in **f**. $n = 3$ independent experiments; mean $\pm$ S.E.M. **h**, Schematics of experimental set up of optical tweezer (C-Trap) system to observe DNA unwinding. These experiments were carried out at room temperature. **i-k**, Bead center displacement measured between the traps as a function of time in indicated conditions. Traces represent individual DNA molecules ($n = 4 - 8$). **l**, Representative kymographs of single Alx-RAD51 binding events on gapped DNA in the presence/absence of 50 nM HELQ or HELQ K365M. Unidirectional movement of Alx-RAD51- indicates translocation of Alx-RAD51-HELQ complex.

Figure 2

Fig. 2 HELQ strips RPA from and anneals ssDNA. **a**, Representative gel of DNA annealing assay with indicated concentrations of HELQ in the absence and presence of RPA (40 nM). The black and blue colours of substrate represent complementary DNA strands. The asterisk (*) indicates the position of FITC labelling at 5' end of oligo. The products were resolved with 10% native polyacrylamide gel. **b**, Quantification of experiments such as shown in **a**. $n = 6$ independent experiments; mean $\pm$ S.E.M. **c**, Representative gel of DNA annealing assay with HELQ (10 nM) and indicated concentrations of RPA. **d**, Quantification of experiments such as shown in **c**. $n = 3$ independent experiments; mean $\pm$ S.E.M. **e**, Representative gel of DNA annealing assay with HELQ K365M (10 nM) and indicated concentrations of RPA. **f**, Quantification of experiments such as shown in **e**. $n = 4$ independent experiments; mean $\pm$ S.E.M. **g**, Native gel (longer 6% polyacrylamide) showing RPA stripping assay with HELQ (10 nM) alone and with indicated concentrations of RPA. **h**, Schematic of the experimental set up of single-molecule FRET-based RPA stripping assay. DNA dual-labelled with the FRET pair Cy3 and Cy5 is immobilised on the microscope slide. In the absence of RPA, a short 6 nt sequence of homology causes the DNA to fold into a high FRET state. Upon RPA binding, the DNA unfolds resulting in a low FRET state. Addition of HELQ results in cycling between the low (bound) and high (free) FRET states as RPA is bound and removed respectively. **i**, Example of single-molecule fluorescence trajectory (top, Cy3 in blue, Cy5 in red) and corresponding FRET (bottom) showing the transition from low FRET (bound) to high FRET (free). **j**, Representative FRET trajectory of DNA in the presence of 1 nM RPA and 200 nM HELQ, spikes of high FRET correspond to RPA removal events. **k**, Plot of dwell time of RPA bound (t_{off}), low FRET, state with increasing HELQ concentration. **l**, Plot of dwell time of free state (t_{on}), high FRET, on RPA removal with increasing HELQ concentration. **m**, Representative FRET trajectory of DNA in the presence of 1 nM RPA and 200 nM HELQ KM.

Figure 3

Fig. 3 HELQ exhibits RPA-mediated sequence-independent DNA capture activity.

a, Schematics of experimental optical tweezer set up for observing capture of Cy3-labelled DNA oligos. **b**, Top, kymographs showing the capture of targeted Cy3 labelled-80-mer oligo (λ 4) in *trans* with HELQ and HELQ K365M in the presence of RPA-eGFP at multiple positions of RPA-eGFP coated ss- λ DNA. Bottom, kymographs showing the capture of non-targeted Cy3 labelled-dT80 homopolymer in *trans* with HELQ in the presence of RPA-eGFP at multiple positions of RPA-eGFP coated ss- λ DNA. **c**, Quantification of the experiments shown in **b**. Each datapoint represents single DNA molecule. Mean values are indicated. Error bars represent S.D. **d**, Histograms of dwell times of captured λ 4 by HELQ in presence of RPA-eGFP. (n = 61, black line represents exponential fit, Tau = 136). **e**, Histograms of dwell times of captured λ 4 by HELQ K365 in presence of RPA-eGFP. (n = 16, black line represents exponential fit, Tau = 202). **f**, Kymograph showing the Cy3- λ 4 oligo captured on RPA-eGFP coated ss- λ DNA in the presence of HELQ and RPA-eGFP upon stretching of tethered ss- λ DNA by gradual increase of force. **g**, Schematic representation of bulk capture assay. **h**, Native gel showing the capture assay with indicated concentrations of HELQ and RPA (82 nM). **i**, Native gel showing the capture assay with indicated concentration of HELQ in the absence of RPA.

Figure 4

Fig. 4 HELQ functions in DSB, SSA and MMEJ

a, Schematic representation of DR-GFP reporter assay for measuring DSB repair by HR. **b**, HELQ protein levels 72 hours post siRNA transfection confirmed by HELQ-immunoprecipitation and western blot. **c**, I-SceI-induced HR frequency in U2OS-DR cells treated with the indicated siRNA. $n \geq 3$ independent experiments $\pm$ S.E.M, compared to non-target siRNA control. statistical analysis; two-tailed paired t-test. $*p < 0.05$; $****p < 0.0001$. **d**, Schematic representation of SA-GFP reporter assay for measuring SSA repair of DSB. **e**, I-SceI-induced SSA frequency in U2OS-SA cells treated with the indicated siRNA. $n \geq 3$ independent experiments $\pm$ S.E.M, compared to non-target siRNA control. statistical analysis; two-tailed paired t-test. $****p < 0.0001$. **f**, BRCA2 protein levels 72 hours post siRNA transfection confirmed by western blot with anti-BRCA2. **g**, I-SceI-induced SSA frequency in U2OS-SA cells treated with the indicated siRNA. $n \geq 3$ independent experiments $\pm$ S.E.M, compared to non-target siRNA control. Statistical analysis, two-tailed paired t-test. $**p < 0.01$. **h**, Schematic representation of EJ-RFP reporter assay for measuring mutagenic end-joining repair events. **i**, I-SceI-induced mutagenic end-joining (EJ-RFP) and HR (DR-GFP) frequencies in U2OS-EJDR cells treated with the indicated siRNA. $n \geq 3$ independent experiments $\pm$ S.E.M, compared to non-target siRNA control. Statistical analysis, two-tailed paired t-test. $*p < 0.05$; $**p < 0.01$, $***p < 0.001$. **j**, Schematic representation of RFP-SCR reporter assay for measuring long and short tract gene conversion in HR. **k**, I-SceI-induced STGC frequency in U2OS-RFP-SCR cells treated with the indicated siRNA. $n \geq 3$ independent experiments $\pm$ S.E.M, compared to non-target siRNA control. statistical analysis; two-tailed paired t-test. $**p < 0.01$. **l**, I-SceI-induced LTGC frequency in experiments from panel g. $n \geq 3$ independent experiments $\pm$ S.E.M, compared to non-target siRNA control. statistical analysis; two-tailed paired t-test. $**p < 0.01$. **m**, Ratio of LTGC/total gene conversion from experiments in **k,l**. $n \geq 3$ independent experiments $\pm$ S.E.M, compared to non-target siRNA control. statistical analysis; two-tailed paired t-test. $**p < 0.01$, $***p < 0.001$.

Extended data figure 1

Extended Data Fig. 1 HELQ specifically unwinds substrates with 3'-overhang and D-loop. **a**, SDS-PAGE gel (4-12% polyacrylamide) showing purified recombinant human HELQ WT and HELQ K365M from insect cells. The gel was stained with Coomassie brilliant blue (CBB). **b-d**, Representative native gels (10% polyacrylamide) of DNA unwinding assay of D-loop, Y-structure and lagging strand fork with indicated concentrations of HELQ. The asterisk (*) indicates the position of FITC labelling at 5' end of oligo. **e**, Quantification of experiments such as shown in **b-d** and Fig. **1a** with HELQ concentration ranging from 1-270 nM. n= 4 independent experiments; mean $\pm$ S.E.M. **f-g**, Representative native gels of DNA unwinding assay of dsDNA and 5'-overhang with indicated concentrations of HELQ. **h**, Native gel showing the DNA unwinding assay of 3'-overhang by HELQ in the presence of ATP (2 mM) and ATP γ S (2 mM), a poorly hydrolysable ATP analogue. **i**, Native gel showing DNA unwinding assay of 3'-overhang with indicated concentrations of HELQ K365M. **j**, Electrophoretic mobility shift assay (EMSA) with ssDNA and indicated concentrations of HELQ and HELQ K365M. The final products were resolved with native 6% polyacrylamide gels. **k**, Quantification of experiments such as shown in **j**. The line represents average; n = 2 independent experiments. **l**, EMSA with dsDNA and indicated concentrations of HELQ and HELQ K365M. **m**, Quantification of experiments such as shown in **l**. The line represents average; n = 2 independent experiments.

Extended data figure 2

Extended Data Fig. 2 RAD51 interacts directly to HELQ and promotes its helicase activity. **a**, SDS-PAGE gel (4-12%) showing purified recombinant human RAD51 from *E. coli*. The gel was stained with CBB. **b**, Protein interaction analysis of MBP-HELQ-FLAG and RAD51 using both amylose and FLAG pull-down assay. The final eluates were run on SDS-PAGE gel (4-12%) and stained with CBB. **c-e**, Representative native gels of DNA unwinding of D-loop, Y-structure and lagging strand fork with HELQ (1 nM) and indicated concentrations of either RAD51 or RecA. **f**, Schematics representation of quenching-based kinetic DNA unwinding assay of 3'-overhang. Initially, oligo F (49-mer), labelled at 5' end with fluorescein (F), is annealed with oligo R, which is labelled with rhodamine (R) at 3' end. Due to close proximity, FRET from fluorescein is quenched by rhodamine constitutively resulting in low FRET signal. Upon DNA unwinding, DNA strands are separated and thus rhodamine no longer able to quench fluorescein, results in higher FRET signal. **g**, Relative unwinding of 3'-overhang with HELQ and indicated concentrations of RAD51 as determined by quenching-based kinetic assay. $n \geq 3$ independent experiments; shaded area represents mean $\pm$ S.E.M.; black lines represent exponential or linear fits. **h**, Relative unwinding of 3'-overhang with HELQ and indicated concentrations of RecA as determined by quenching-based kinetic assay. $n = 3$ independent experiments; shaded area represents mean $\pm$ S.E.M.; black lines represent exponential or linear fits.

Extended data figure 3

Extended Data Fig. 3 RPA inhibits HELQ DNA unwinding activity. **a**, SDS-PAGE gel (4-12%) showing purified recombinant human RPA-mRFP1 from *E. coli*. The gel was stained with CBB. **b**, Representative gel of DNA unwinding assay of D-loop with HELQ in the absence and presence of RPA (20 nM). **c**, Quantification of experiments such as shown in **b**. $n = 3$ independent experiments; mean $\pm$ S.E.M. **d**, Representative gel of DNA unwinding assay of 3'-overhang with HELQ (1 nM) and indicated concentrations of RPA. **e**, Quantification of experiments such as shown in **d**. $n = 3$ independent experiments; mean $\pm$ S.E.M. **f**, Representative gel of DNA unwinding assay of 3'-overhang with HELQ (1 nM) and indicated concentrations of RAD51, in the absence and presence of RPA (20 nM). **g**, Quantification of experiments such as shown in **f**. $n = 5$ independent experiments; mean $\pm$ S.E.M. **h**, Representative gel of DNA unwinding assay of D-loop with HELQ (3 nM) and indicated concentrations of RAD51, in absence and presence of RPA (20 nM). **i**, Quantification of experiments such as shown in **h**. $n = 5$ independent experiments; mean $\pm$ S.E.M.

Extended data figure 4

Extended Data Fig. 4 RAD51-HELQ complex translocates along ssDNA backbone.

a-b. Bead center displacement measured between the traps as a function of time in indicated conditions. Traces represent individual DNA molecules ($n = 3 - 4$). **c.** Example of two real-time bead displacement traces for indicated conditions. Unwinding bursts of linearly increasing bead distance are interspersed by pauses where no distance change is observed.

d-e. First derivative with applied smoothening of traces shown in **c**. Height of individual peaks corresponds to the rate of individual unwinding bursts. **f-g.** Histogram showing HELQ unwinding burst rate distribution in the absence ($n = 41$, mean = 2.5 ± 0.7 nm/s (S.E.M)) or presence ($n = 45$, mean 1 = 4.3 ± 0.7 nm/s (S.E.M), mean 2 = 14 ± 0.23 nm/s (S.E.M)) of RAD51. Black line represents single or double Gaussian fits. **h.** Chemical labelling of RAD51. RAD-51 C319S was labelled in pH 7.0 using maleimide esters of Alexa Fluor 488. After reaction termination and purification of labelled species, labelling efficiency was assessed, and free dye component was evaluated using SDS-PAGE (4-12%) and subsequent fluorescent imaging. 1:1 labelling stoichiometry was achieved as measured spectrophotometrically. Proteins were labelled typically with 80-100% labelling efficiency.

i, Displacement of proteins bound to individual DNA tethers. The representative trajectories illustrate either unidirectional movement (red), 1D diffusion (green) and static binding with occasional diffusion (blue). The positions of molecules in time were measured by fitting a moving window of three kymograph frames with Gaussian function. **j,** Total displacement of translocating HELQ+RAD51 complexes (red) used to calculate the translocation rate. Two populations of translocating molecules can be distinguished: faster with the mean rate of 14 ± 5 nm/s and slower with the mean rate of 4 ± 1 nm/s. The total displacement of a representative RAD51 filament (green) includes all frame-to-frame displacements, regardless of directionality. The stationary molecule apparent displacement (blue) of 2 ± 1 nm/s results from thermal fluctuations of the tethered DNA. **k,** Mean Square Displacement calculated from the trajectories shown in panel **i**, plotted as a function of time interval for a period up to 35 sec. A quadratic dependence of MSD (red) indicates directed motion of HELQ+RAD51 complexes, whereas linear MSD which asymptotically approaches a threshold value.

Extended data figure 5

Extended Data Fig. 5 ATP is important for HELQ annealing activity in presence of RPA. a,

Representative gel of DNA unwinding assay of 3'-overhang with HELQ, in the presence and absence of "cold oligo" (25 nM) i.e., unlabelled oligo with identical DNA sequence as FITC-labelled oligo. **b,** Relative unwinding of 3'-overhang with indicated concentrations of HELQ as determined by quenching-based kinetic assay. $n = 3$ independent experiments; shaded area represents mean $\pm$ S.E.M.; black lines represent exponential or linear fits. **c,** Representative gel of DNA annealing assay with HELQ (10 nM) and indicated concentrations of RPA. The black and blue colours of substrate represent complementary DNA strands. The asterisk (*) indicates the position of FITC labelling at 5' end. The products were resolved on 10% native polyacrylamide gel. **d,** Quantification of experiments such as shown in **c**. $n = 5$ independent experiments; mean $\pm$ S.E.M. **e,** Representative gel of DNA annealing assay with indicated concentrations of HELQ and RPA (40 nM), in the absence and presence of ATP. **f,** Quantification of experiments such as shown in **e**. $n = 3$ independent experiments; mean $\pm$ S.E.M. **g,** Representative gel of DNA annealing assay with indicated concentrations of HELQ in the absence and presence of ATP. **h,** Quantification of experiments such as shown in **g**. $n = 3$ independent experiments; mean $\pm$ S.E.M. **i,** Native gel showing DNA annealing assay with indicated concentrations of HELQ and RPA (40 nM) in the presence of ATP γ S. **j,** Representative gel of DNA annealing assay with indicated concentrations of HELQ K365M in the absence and presence of RPA (40 nM). **k,** Quantification of experiments such as shown in **j**. $n = 4$ independent experiments; mean $\pm$ S.E.M. **l,** Representative gel of DNA annealing assay with HELQ K365M (60 nM) and indicated concentrations of RPA. **m,** Quantification of experiments such as shown in **l**. $n = 3$ independent experiments; mean $\pm$ S.E.M. **n,** Representative gel of DNA annealing assay with HELQ (10 nM) and various concentrations of SSB. **o,** Quantification of experiments such as shown in **n**. $n = 4$ independent experiments; mean $\pm$ S.E.M.

Extended data figure 6

Extended Data Fig. 6 HELQ strips RPA from ssDNA. **a**, Native gel (longer 6% polyacrylamide) showing RPA stripping assay with indicated concentrations of HELQ in the absence and presence of RPA (40 nM). **b**, Schematic of immobilised dual labelled (Cy3 and Cy5) DNA in the absence of RPA. **c-d**, Representative intensity trajectory (top) and corresponding FRET trajectory (bottom) of dual labelled DNA in the absence of RPA.

e, Time-binned FRET histogram of DNA only, fit with gaussian. **f**, Schematic of immobilised dual labelled DNA in the presence of RPA. **g-h**, Representative intensity trajectory (top) and corresponding FRET trajectory (bottom) of dual labelled DNA in the presence of RPA. Stable high FRET is observed. **i**, Time-binned FRET histogram of DNA in the presence of RPA. Fit with gaussian. **j**, Representative gel of DNA annealing assay with HELQ (3 nM), RPA (40 nM) and indicated concentrations of RAD51. **k**, Quantification of experiments such as shown in **j**. $n = 3$ independent experiments; mean $\pm$ S.E.M.

Extended data figure 7

Extended Data Fig. 7 HELQ can capture non-complementary DNA strands in presence of RPA.

a, SDS-PAGE gel (4-12%) showing purified recombinant human RPA-eGFP from *E. coli*. The gel was stained with CBB. **b**, A schematic of DNA pulling process. RPA-eGFP-coated ss- λ DNA tethered between the two streptavidin beads ($\sim 4.8 \mu\text{m}$ diameter) was collapsed by bringing beads at $< 5 \mu\text{m}$ distance. Beads were subsequently pulled apart at constant speed (step size = $0.2 \mu\text{m}$, frequency = 500 Hz). Force-extension curves were then recorded. **c**, Force-distance curves of individual eGFP-RPA-coated ss- λ DNA molecules recorded in the absence or presence of HELQ (left and right panels, respectively). Sawtooth-like patterns in the FD curves (red) indicate disruption of ssDNA loops held together by HELQ. The average loop sizes ($1.5 \pm 0.5 \mu\text{m}$, $N = 10$) were estimated from the differences in the fitted contour lengths between the disruption events (dashed lines). **d**, Native gel showing the capture assay in the absence and presence of RPA (82 nM) with indicated concentrations of HELQ K365M.

Extended data figure 8

Extended Data Fig. 8 HELQ is epistatic with RAD52 for SSA. **a**, HELQ protein levels in U2OS wild type, U2OS siHELQ_M treated, U2OS-DR *HELQ*^{-/-}, and U2OS-SA *HELQ*^{-/-} cells confirmed by HELQ-immunoprecipitation and western blot. **b**, I-SceI-induced HR frequency in U2OS-DR wild type and *HELQ*^{-/-} cells. $n \geq 3$ independent experiments $\pm$ S.E.M. Statistical analysis; two-tailed paired t-test. *** $p < 0.001$. **c**, I-SceI-induced SSA frequency in U2OS-SA wild type and *HELQ*^{-/-} cells. $n \geq 3$ independent experiments $\pm$ S.E.M. Statistical analysis; two-tailed paired t-test. **** $p < 0.0001$. **d**, RAD52 protein levels 72 hours post siRNA transfection confirmed by western blot. **e**-SceI-induced SSA frequency in U2OS-SA cells treated with the indicated siRNA. $n \geq 3$ independent experiments $\pm$ S.E.M, compared to non-target siRNA control. statistical analysis; two-tailed paired t-test. ns; $p > 0.05$, not significant.

Supplementary Files

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