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

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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 development\textsuperscript{1}. 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 tumours\textsuperscript{2-4}. At the cellular level, defects in HELQ result in hypersensitivity to cisplatin and mitomycin C and, persistence of RAD51 foci upon DNA damage\textsuperscript{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\textsuperscript{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.
Mechanistically, we show that HELQ possesses an intrinsic ability to capture RPA-bound DNA strands and then displace RPA to facilitate annealing of complementary strands. Finally, we show that HELQ deficiency in cells compromises single-strand annealing (SSA) and microhomology-mediated end joining (MMEJ) pathways and increases long-tract gene conversion tracts (LTGC) during homologous recombination. Thus, our results implicate HELQ in multiple arms of DSB repair by virtue of co-factor dependent modulation of intrinsic translocase and DNA strand annealing activities.

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

RAD51 stimulates HELQ unwinding activity
In vivo studies have shown that HELQ deficient cells exhibit persistent RAD51 foci upon DNA damage\(^3\). Furthermore, HELQ-1 from C. elegans interacts with RAD-51\(^7\). To investigate the interplay between HELQ and RAD51, we purified human RAD51 from E. coli (Extended Data Fig. 2a), which interacts directly with HELQ (Extended Data Fig. 2b). In unwinding assays, RAD51 strongly stimulated HELQ helicase activity with all tested substrates (Fig. 1c,d, Extended Data Fig. 2c-e). Conversely, bacterial RecA did not show any such stimulation, which excludes that stimulation by RAD51 is indirect through sequestering of unwound product (Fig. 1c,e, Extended Data Fig. 2c-e). We also measured the kinetics of DNA unwinding by HELQ in the absence and presence of RAD51. Addition of RAD51 resulted in a concentration dependant increase in the HELQ DNA unwinding rate, while addition of RecA had no effect (Extended Data Fig. 2f-h). In cells, the ssDNA generated during DNA processing is usually bound by RPA. To mimic these conditions, we purified fluorescently tagged human RPA-mRFP1 from E. coli
(Extended Data Fig. 3a). Addition of RPA strongly inhibited DNA unwinding by HELQ especially with 3’ overhang substrates (Fig. 1f,g, Extended Data Fig. 3b,c). At lower concentrations, insufficient to cover the entire ssDNA region, RPA still inhibited HELQ unwinding of 3’-overhang (Extended Data Fig. 3d,e). Despite the inhibitory effect of RPA, RAD51 still strongly stimulated HELQ helicase in the presence of RPA (Extended Data Fig. 3 f-i).

Visualization of HELQ DNA unwinding

To better understand HELQ stimulation by RAD51, we used an optical tweezers, microfluidics and confocal microscopy (C-TRAP) setup for SMI analysis. As shown in Fig 1h, a single DNA molecule (λDNA) containing a single-stranded DNA gap was tethered between two optically trapped beads and held at constant force (50 pN) to prevent reannealing of unwound DNA. On addition of HELQ, DNA unwinding was observed as an increase in distance between the beads, due to the expansion of the ssDNA region. Neither RAD51 alone nor HELQ K365M showed evidence of unwinding (Fig. 1i, Extended Data Fig. 4a). On addition of RAD51, a dramatic increase in overall DNA unwinding was observed with WT HELQ, whereas no such stimulation was observed with HELQ K365M (Fig. 1j,k, Extended Data Fig. 4b). Within unwinding traces for individual DNA molecules, rapid unwinding bursts interspersed by pauses can be distinguished (Extended Data Fig. 4c-e) and corresponded to a mean rate of 2.5 ±0.7 nm/s (S.E.M). In the presence of RAD51, two populations of unwinding can be distinguished: slow with mean rates of 4.3 ± 0.7 nm/s (S.E.M) corresponds roughly to rates measured in the absence of RAD51 and fast with mean rates of 14 ± 0.23 nm/s (S.E.M) (Extended Data Fig. 4f,g). To directly visualize RAD51 during DNA unwinding with HELQ, mutant RAD51 C319S was purified and labelled with Alexa Fluor 488 C5 maleimide dye (Alx-RAD51) (Extended Data Fig. 4h). While Alx-RAD51 alone displayed mostly static binding traces with occasional diffusing species, addition of HELQ showed unidirectional translocation traces indicating active movement of an Alx-RAD51-HELQ complex along the ssDNA backbone (Fig. 1l, Extended Data Fig. 4i). Translocation rates of this species (14 ± 5 nm/s) matches well with the fast population of unwinding bursts observed in the presence of HELQ and RAD51 (Extended Data Fig. 4j,k). Conversely, HELQ K365M retained the ability to bind RAD51 but showed no translocation with only static or diffusing traces. Together, these results indicate that RAD51 and HELQ form a complex that unwinds
DNA with approximately 3 to 5-fold faster rate than HELQ alone, which is in agreement with bulk experiments.

**HELQ possesses robust DNA strand annealing activity**

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

We next tested the requirement of ATP binding and hydrolysis for DNA annealing by HELQ. Surprisingly, in the presence of RPA, HELQ showed no DNA annealing without ATP whereas ATP became dispensable when RPA was excluded from the reaction (Extended Data Fig. 5e-h). Even in the absence of RPA, ATP stimulated the DNA annealing activity of HELQ (Extended Data Fig. 5g,h). HELQ also failed to anneal DNA completely with ATPγS in the presence of RPA (Extended Data Fig. 5i). Collectively, these data suggest that HELQ possesses intrinsic DNA annealing activity, which requires ATP binding and hydrolysis when ssDNA is coated with RPA. We next tested the helicase inactive HELQ K365M mutant for DNA annealing activity and found that HELQ K365M was defective for DNA annealing in the presence of RPA (Extended Data Fig. 5j,k) However, HELQ K365M retained the ability to anneal DNA efficiently in
the absence of RPA but becomes progressively impaired when RPA coverage of DNA exceeds 100% (Fig. 2e,f, Extended Data Fig. 5i,m). RPA also failed to stimulate HELQ K365M (Fig. 2e,f). We also tested E. coli SSB protein and found that it only weakly stimulates HELQ annealing activity (Extended Data Fig. 5n,o). The N-terminal fragment of HELQ was previously shown to displace RPA from ssDNA. However, full-length HELQ was not analysed for such activity. To directly visualize RPA displacement from ssDNA during DNA strand annealing, we performed a “stripping” assay where the deproteinisation step was omitted. We observed that HELQ can strip an excess of RPA from ssDNA, which occurred coincidently with the appearance of the annealed products (Fig. 2g, Extended Data Fig. 6a). Using a single-molecule FRET-based assay (Extended Data Fig. 6b-i), we observed concentration dependant RPA stripping by HELQ, followed by re-binding of RPA (Fig. 2h-k). The RPA re-binding is independent of HELQ concentration indicating a constant transition rate from free ($t_{on}$) to bound ($t_{off}$) at various HELQ concentrations (Fig. 2l). Notably, HELQ K365M did not show RPA stripping in this set up, which is likely due to excess RPA binding to DNA and/or stable binding of HELQ K365M to DNA after transient RPA removal (Fig. 2m). These data confirm our bulk experiments indicating that active RPA-stripping plays a critical role in HELQ-mediated DNA annealing. Finally, we found that addition of RAD51 had no effect on HELQ-dependent DNA annealing activity (Extended Data Fig. 6j,k).

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

RAD52 also possess DNA annealing activity and plays a central role in SSA repair of DSBs. Using an optical trapping microscope, it was shown previously that RAD52 can trans-capture labelled oligos at multiple sites along λ DNA independent of DNA sequence. Using a similar set up, we tested the ability of HELQ to capture a Cy3 labelled 80-mer oligo ssDNA in the presence of RPA-eGFP (~100% coverage; Fig 3a, Extended Data Fig. 7a). HELQ facilitated the annealing of λ4 oligo at multiple sites (Fig. 3b,c). Notably, HELQ K365M also exhibited efficient DNA capture activity (Fig. 3b,c). We also analysed the dwell times of captured oligos and found that HELQ K365M showed moderately increased dwell times compared to WT (Fig. 3d,e). We also tested a 79 nucleotide T-homopolymer (dT-79) and obtained similar results as with λ4 oligo (Fig. 3c).
To understand the mechanism of oligo capture by HELQ, we force stretched the tethered ss-λDNA. Since DNA starts to melt at forces >60 pN, we reasoned that if HELQ oligo capture involves base pairing interactions, short microhomologies should dissociate faster than the ones with longer homology. However, to our surprise, even at very high force (90-100 pN), all oligos remained engaged with ssDNA, irrespective of position (Fig. 4f). During the pulling experiment, we also observed characteristic force spikes when beads are pulled apart at low-force (10-15 pN) (Extended Data Fig. 7b,c). These spikes correspond to disruption of HELQ complexes capturing RPA-coated ss-λDNA in cis. To dissect this further, we developed a bulk capture assay, where we attempted to pull out labelled non-complementary DNA (Cy3-dT79) with a biotinylated dT43 oligo with HELQ. We found that HELQ WT and the K365M mutant could capture non-complementary DNA but only in the presence of RPA (Fig. 4g,h, Extended Data Fig. 7d). Hence, HELQ can capture DNA strands independent of sequence likely via DNA tethering. Interestingly, yeast Rad52, when bound to RPA-coated ssDNA clusters, was also shown to capture additional free RPA in pre-existing Rad52-RPA-ssDNA clusters. This activity was postulated to be important for second-end capture.

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

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

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

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Figure 1

(a) HELQ (nM) vs Unwound substrate (%)

(b) HELQ (nM) vs Unwound substrate (%)

(c) RADS1 (nM) vs Unwound substrate (%)

(d) RAD51 (nM) vs Unwound substrate (%)

(e) HELQ (nM) vs Unwound substrate (%)

(f) HELQ (nM) vs Unwound substrate (%)

(g) HELQ (nM) vs Unwound substrate (%)

(h) HELQ (nM) vs Unwound substrate (%)

(i) 25 nM RAD51 position vs Time (s)

(j) 50 nM HELQ position vs Time (s)

(k) 50 nM HELQ + 25 nM RAD51 position vs Time (s)
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 (Fluorescin 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. 1b-d. Only HELQ concentrations 1 to 90 nM shown. n= 4 independent experiments; mean ± 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. 1c-d. n ≥ 3 independent experiments; mean ± S.E.M. **e**, Quantification of experiments (with RecA) such as shown in **c** and Extended Data Fig. 1c-d. n ≥ 3 independent experiments; mean ± 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 ± 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

(a) gels showing HELQ and RPA binding at different concentrations.

(b) Graph showing HELQ binding with and without ATP.

(c) Gel images with RPA binding at different concentrations.

(d) Bar graph showing HELQ binding at different RPA concentrations.

(e) Gel images with HELQ K365M binding at different RPA concentrations.

(f) Bar graph showing HELQ K365M binding at different RPA concentrations.

(g) Gel images with DNA-protein complex and free-RPA at different RPA concentrations.

(h) Diagram showing DNA-protein complex and free-RPA at different RPA concentrations.

(i) FRET intensity over time with bound and free DNA-protein complex.

(j) Graph showing FRET intensity over time with 200 nM HELQ.

(k) Graph showing HELQ off-time with 200 nM HELQ.

(l) Graph showing FRET intensity over time with HELQ.

(m) Graph showing HELQ KM with FRET intensity over time.
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 ± 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 ± 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 ± 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 striping 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 (λ4) 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 λ4 by HELQ in presence of RPA-eGFP. (n = 61, black line represents exponential fit, Tau = 136).
e, Histograms of dwell times of captured λ4 by HELQ K365 in presence of RPA-eGFP. (n = 16, black line represents exponential fit, Tau = 202). KM, HELQ K365M.
f, Kymograph showing the Cy3-λ4 oligo captured on RPA-eGFP coated ss-λ DNA in the presence of HELQ and RPA-eGFP upon stretching of tethered ss-λDNA 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

(a) DR-GFP

(b) DR-GFP

(c) DR-GFP

(d) DR-GFP

(e) SA-GFP

(f) anti-BRAC2

(g) SA-GFP

(h) SA-GFP

(i) SA-GFP

(j) SJ-GFP

(k) STGC

(l) LTGC

(m) LTGC/Total GC
**Fig. 4 HELQ functions in DSBR, 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-Scel-induced HR frequency in U2OS-DR cells treated with the indicated siRNA. n ≥ 3 independent experiments ± 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-Scel-induced SSA frequency in U2OS-SA cells treated with the indicated siRNA. n ≥ 3 independent experiments ± 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-Scel-induced SSA frequency in U2OS-SA cells treated with the indicated siRNA. n ≥ 3 independent experiments ± 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-Scel-induced mutagenic end-joining (EJ-RFP) and HR (DR-GFP) frequencies in U2OS-EJDR cells treated with the indicated siRNA. n ≥ 3 independent experiments ± 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-Scel-induced STGC frequency in U2OS-RFP-SCR cells treated with the indicated siRNA. n ≥ 3 independent experiments ± S.E.M, compared to non-target siRNA control. statistical analysis; two-tailed paired t-test. **p<0.01. **l**. I-Scel-induced LTGC frequency in experiments from panel g. n ≥ 3 independent experiments ± 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 ≥ 3 independent experiments ± S.E.M, compared to non-target siRNA control. statistical analysis; two-tailed paired t-test. **p<0.01, ***p<0.001.
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 ± 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 ATPγS (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

**a**

![Image](a.png)

**b**

![Image](b.png)

**c**

![Image](c.png)

**d**

![Image](d.png)

**e**

![Image](e.png)

**f**

![Image](f.png)

**g**

![Image](g.png)

**h**

![Image](h.png)
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 ≥ 3 independent experiments; shaded area represents mean ± 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 ± S.E.M.; black lines represent exponential or linear fits.
Extended data figure 3

**a**
![Western Blot Image]

**b**
![Western Blot Image]

**c**
![Graph Image]

**d**
![Western Blot Image]

**e**
![Graph Image]

**f**
![Western Blot Image]

**g**
![Graph Image]

**h**
![Western Blot Image]

**i**
![Graph Image]
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 ± 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 ± 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 ± 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 ± S.E.M.
Extended data figure 4

(a) 50 nM HELQ KM

(b) 50 nM HELQ KM + 25 nM RAD51

(c) 50 nM HELQ KM

(d) 1st derivative of 50 nM HELQ

(e) 1st derivative of 50 nM HELQ + 25 nM RAD51

(f) Relative frequency (fractions)

(g) Relative frequency (fractions)

(h) [Image of Western Blot]

(i) Unwinding rate (nm/s)

(j) Displacement (µm)

(k) MSD (µm²)

(l) Lag time (s)
**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

a) "Cold oligo"

b) Graph showing relative unwinding over time with different concentrations of HELQ.

c) Graph showing relative unwinding with different RPA concentrations.

d) Graph showing annealed substrate (%) with different RPA concentrations.

e) Graph showing annealed substrate (%) with different HELQ concentrations.

f) Graph showing annealed substrate (%) with different HELQ concentrations.

g) Graph showing annealed substrate (%) with different HELQ concentrations.

h) Graph showing annealed substrate (%) with different HELQ concentrations.

i) Graph showing annealed substrate (%) with different HELQ concentrations.

j) Graph showing annealed substrate (%) with different HELQ concentrations.

k) Graph showing annealed substrate (%) with different HELQ concentrations.

l) Graph showing annealed substrate (%) with different HELQ concentrations.

m) Graph showing annealed substrate (%) with different HELQ concentrations.

n) Graph showing annealed substrate (%) with different HELQ concentrations.

o) Graph showing annealed substrate (%) with different HELQ concentrations.
Extended Data Fig. 5 ATP is important for HELQ annealing activity in presence of RPA. 

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 ± 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 ± 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 ± 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 ± S.E.M. 

i, Native gel showing DNA annealing assay with indicated concentrations of HELQ and RPA (40 nM) in the presence of ATPγS. 

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 ± 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 ± 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 ± S.E.M.
Extended data figure 6

a

b

DNA only

DNA + 1 nM RPA

f

DNA-protein complex

RPA (40 nM)

HELQ (nM)

3 10 30 3 10 30

RPA (40 nM)

HELQ (3 nM)

0 3 10 30 90

0 3 10 30 90

RAD51 (nM)

RAD51 (nM)

Annealed substrate (%)

Extended data figure 6

j

k
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 ± S.E.M.
Extended data figure 7

(a) Gel electrophoresis showing marker bands and protein bands.

(b) Diagram illustrating the interaction between proteins and ssDNA.

(c) Force-distance curves comparing different conditions:

- No protein
- HELQ (5 nM)

(d) Gel electrophoresis with bands corresponding to RPA (82 nM), No RPA, HELQ K365M (nM), Cy3-dT79 (10 nM), B-dT43 (10 nM), and Cy3-dT79.
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 (~4.8 μm diameter) was collapsed by bringing beads at <5 μm distance. Beads were subsequently pulled apart at constant speed (step size = 0.2 μm, 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 ± 0.5 μm, 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 ≥ 3 independent experiments ± 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 ≥ 3 independent experiments ± 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 ≥ 3 independent experiments ± S.E.M, compared to non-target siRNA control. statistical analysis; two-tailed paired t-test. ns; p>0.05, not significant. 
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Methods.pdf