The HRDC domain of *E. coli* RecQ helicase controls single-stranded DNA translocation and double-stranded DNA unwinding rates without affecting mechanoenzymatic coupling

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DNA-restructuring activities of RecQ-family helicases play key roles in genome maintenance. These activities, driven by two tandem RecA-like core domains, are thought to be controlled by accessory DNA-binding elements including the helicase-and-RnaseD-C-terminal (HRDC) domain. The HRDC domain of human Bloom's syndrome (BLM) helicase was shown to interact with the RecA core, raising the possibility that it may affect the coupling between ATP hydrolysis, translocation along single-stranded (ss)DNA and/or unwinding of double-stranded (ds)DNA. Here, we determined how these activities are affected by the abolition of the ssDNA interaction of the HRDC domain or the deletion of the entire domain in *E. coli* RecQ helicase. Our data show that the HRDC domain suppresses the rate of DNA-activated ATPase activity in parallel with those of ssDNA translocation and dsDNA unwinding, regardless of the ssDNA binding capability of this domain. The HRDC domain does not affect either the processivity of ssDNA translocation or the tight coupling between the ATPase, translocation, and unwinding activities. Thus, the mechanochemical coupling of *E. coli* RecQ appears to be independent of HRDC-ssDNA and HRDC-RecA core interactions, which may play roles in more specialized functions of the enzyme.

RecQ-family helicases, which are key players in genome maintenance in both bacteria and eukaryotes, perform ssDNA translocation and dsDNA unwinding driven by ATP hydrolysis1–3. What makes RecQ enzymes different from other helicases is their unique capability to unwind various non-canonical DNA structures that arise during DNA replication, recombination and repair1–3. *E. coli* (Ec) RecQ helicase, the prototypic family member, is required for the processing of late replication intermediates and stalled replication forks4–6, exerts both pro- and anti-recombination activities7,8 and suppresses illegitimate recombination9. Mutations in three of five human RecQ homologs (BLM, WRN (Werner syndrome helicase) and RecQ4) cause severe syndromes bearing the common feature of loss of genomic integrity, highlighting the essential role of RecQ helicases in genome maintenance1,3.

The capability of RecQ helicases to process complex DNA structures is associated with a conserved domain architecture shared by *Ec* RecQ, human BLM and WRN enzymes, as well as at least one RecQ
homolog in almost all investigated organisms (Fig. 1)\(^\text{10}\). Two conserved RecA-like domains harbor the ATPase cleft and drive 3′–5′-directed ssDNA translocation activity\(^\text{2,11}\). In most RecQ helicases, these domains are followed by a zinc-binding domain, which appears essential for the structural stability of the enzymes and contributes to DNA binding\(^\text{12–14}\). Furthermore, many RecQ helicases contain the auxiliary winged-helix (WHD) and HRDC domains\(^\text{2,11}\). The WHD mediates protein-DNA and protein-protein interactions and has been shown to facilitate DNA unwinding and contribute to substrate recognition\(^\text{10}\). The HRDC domain, which is connected to the WHD via a flexible linker, also contributes to the substrate specificity and DNA binding affinity of RecQ helicases\(^\text{16,18–21}\). For \(\text{Ec RecQ}\), atomic structures have been solved separately for the HRDC-deleted helicase core and for the isolated HRDC domain (Fig. 1b)\(^\text{16,22}\). Atomic structures containing the RecQ helicase core together with the HRDC domain have been solved only for human BLM\(^\text{23}\) (PDB codes 4O3M, 4CDG, 4CGZ). In these structures the HRDC domain folds back onto the core and interacts with both RecA domains (Fig. 1c). Deletion of the BLM HRDC domain increased the ATPase activity of the enzyme while decreasing the rate of dsDNA unwinding\(^\text{23}\). These findings led to the proposition that the HRDC-core interaction is required for efficient coupling of the ATPase activity to dsDNA unwinding\(^\text{23}\).

Previous studies by us and others showed that RecQ and BLM helicases translocate on ssDNA with moderate processivity\(^\text{24–27}\). Translocation is tightly coupled to ATP hydrolysis, and the enzymes translocate 1–2 nucleotide (nt) upon hydrolysis of one ATP molecule\(^\text{24–26}\). In addition we found that, similar to the situation in BLM, the \(\text{Ec RecQ}\) HRDC domain suppresses the ssDNA-activated ATPase activity of the enzyme\(^\text{17}\). These findings suggest a general role for HRDC domains in supporting coupling between ATPase and translocation/unwinding activities through interdomain interactions. To test this hypothesis, here we performed transient and steady-state kinetic measurements using wild-type (WT), HRDC point mutant and an HRDC deletion mutant \(\text{Ec RecQ}\) constructs to dissect the mechanism of ATPase-coupled translocation and unwinding and its modulation by HRDC domain interactions. We found that the HRDC domain slows down ssDNA translocation and dsDNA unwinding by suppressing the ATPase activity, but it does not influence the processivity of translocation and the coupling of the ATPase activity to translocation and unwinding. These results suggest that the interaction between the motor core

![Figure 1. RecQ constructs used in this study](image-url)
and the HRDC domain is conserved among RecQ helicases and this interaction moderates the rate of ATPase-driven activities, but its effect on mechanochemical coupling may vary among RecQ homologs, according to the differing physiological roles of the enzymes.

Results

**Protein constructs.** To dissect the role of the HRDC domain of Ec RecQ helicase in the enzymatic mechanism, we used three protein constructs: wild-type protein (RecQ WT), a construct in which the previously characterized Y555A point mutation abolishes the ssDNA-binding ability of the HRDC domain (RecQY555A)16, and a construct lacking the entire HRDC domain (RecQ 523, comprising amino acids (a.a.) 1-523) (Fig. 1a). Circular dichroism measurements indicated proper folding of all constructs (Supplementary Fig. S1).

**ATPase suppression by the HRDC domain does not greatly affect ssDNA translocation processivity or ATPase-translocation coupling.** To monitor the ATPase activity of helicase constructs, we followed the kinetics of inorganic phosphate (P_i) generation from ATP in a stopped-flow instrument. The amount of P_i produced was quantified using a fluorescently labeled phosphate binding protein (MDCC-PBP) (Supplementary Fig. S2)24–26,28,29. In the absence of DNA, traces of P_i generation were linear for all constructs in the presence of saturating ATP concentration (shown for RecQ 523 in Fig. 2a). Steady-state DNA-free ATPase slopes for RecQ WT were consistent with previous results determined by other methods25,30. The DNA-free ATPase activity (k_{basal}) was not influenced by either the Y555A mutation or HRDC deletion (Table 1).

As shown previously, ssDNA markedly activates RecQ ATPase activity30,31. To assess the role of the HRDC domain in ssDNA-induced ATPase activation, we rapidly mixed ATP with RecQ constructs pre-mixed with increasing amounts of dT54, and monitored P_i release kinetics. Kinetic traces showed a lag followed by quasi-linear P_i generation for all constructs (shown for RecQ 523 in Fig. 2b). The onset of the linear steady-state became faster with increasing DNA concentration with no sign of saturation, suggesting that the lag originated from an enzyme-DNA binding process induced by mixing with ATP (Fig. 2b). Our earlier study showed that ATP binds very rapidly to the enzyme30, ruling out that the lag in Fig. 2b originates from ATP binding. However, we recently showed that the presence of ATP, or its analogs, markedly increases the ssDNA affinity of RecQ17. Therefore, the lag can be explained by an ATP-induced increase in the fraction of ssDNA-bound RecQ molecules occurring upon stopped-flow mixing. This leads to a concomitant increase in the rate of P_i generation until reaching the steady-state.

We determined the apparent rate constant of the ATP-induced DNA binding process (k_a) and the steady-state ATPase rate (k_{cat}) at each dT54 concentration from the P_i generation transients (Fig. 2b) using the model described in Supplementary equation (S1). Fits to the dT54 concentration dependence of k_{cat} using the Hill equation revealed ssDNA-activated k_{cat} values (i.e. maximal k_a at saturating dT54 concentration) in line with those determined previously using an NADH-coupled assay (Fig. 2c)17. The analysis indicated small positive cooperativity for the RecQ-ssDNA interaction, which was not systematically altered by the mutations (Hill coefficients were 1.7 ± 0.3, 1.0 ± 0.4 and 1.7 ± 0.3 for RecQ WT, RecQY555A and RecQ 523, respectively). In agreement with our previous results, k_{cat} was almost identical for RecQ WT and RecQY555A, but was 3 fold-higher for RecQ 523 (Table 1)17. The apparent dissociation constants for

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**Figure 2.** The HRDC domain suppresses RecQ ATPase activity (a) Kinetics of P_i generation from ATP (1 mM) by RecQ 523 (20 nM) in the absence of DNA, followed by MDCC-PBP (5 μM) fluorescence in the stopped-flow. (b) Kinetic traces of P_i release by RecQ 523 (as in panel a but in the presence of various dT54 concentrations, indicated in nM). Traces were analyzed using Supplementary equation (S1) to determine the steady-state ATPase activity values shown in (c) for RecQ WT (solid circles), RecQ Y555A (open diamonds) and RecQ 523 (open circles). Solid lines in (c) show fits using the Hill equation. Determined parameters are listed in Table 1.
Table 1. Mechanistic parameters of RecQ constructs. N.d., Not determined. aFrom P, release data. bValues in parentheses were determined from PK-LDH-coupled assays. cFrom fluorescence anisotropy titrations. dFrom oligo-dT length dependence of P, release amplitudes during single-round translocation. eFrom ref. 26. fFrom ref. 25. gFrom the initial slope of poly-dT translocation experiments. hCalculated as \( \frac{\text{ATP unwinding}}{\text{k}_{\text{off,trans}}} \). iFrom Trp fluorescence data. jFrom DsSO4 dependence of P, release amplitudes during single-round translocation on poly-dT. kCalculated as \( \frac{k_{\text{trans}}}{k_{\text{off,trans}}} \). lCalculated as \( \frac{<n_{\text{tr}}>/k_{\text{off,trans}}}{<n_{\text{tr}}>} \). mCalculated as \( \frac{<n_{\text{tr}}>/k_{\text{off,trans}}}{<n_{\text{tr}}>} + 1 \). nFrom single-turnover unwinding experiments. oFrom PK-LDH-coupled assays. pCalculated as \( \frac{k_{\text{ATP unwinding}}}{k_{\text{off,trans}}} \). qRecQWT, RecQY555A and RecQ523 compared to RecQWT (Table 1).

The apparent rate constant of ATP-induced DNA binding (\( k_{\text{app}} \), determined from lag kinetics using Supplementary equation (S1)) increased linearly with dT54 concentration (Supplementary Fig. S3). This dependence allowed another, independent means of determination of the ssDNA binding \( k_{\text{q}} \) values of RecQ constructs during ATPase cycling (Supplementary Fig. S3, Supplementary Table S1). The determined values (Supplementary Table S1) were slightly higher than those determined from \( k_{\text{q}} \) values (Table 1), but were in line with those determined previously for the RecQWT, AMPPNP (non-hydrolyzable ATP analog) complex27. The mutations caused a slight reduction in the ssDNA affinity of RecQ (Table 1, Supplementary Table S1). In summary, these results suggest that the ssDNA interaction of the HRDC domain has a minor contribution to the overall ssDNA binding affinity of RecQ, but the presence of the HRDC domain—regardless of its ssDNA binding capability—significantly suppresses the ssDNA-activated ATPase activity of the motor core.
To dissect how the HRDC domain affects ssDNA translocation, we monitored the kinetics of P_i generation from ATP hydrolysis during single-round translocation, which was previously shown to be suitable for the determination of translocation processivity and ATPase-translocation coupling (ATP hydrolyzed per nucleotide (nt) traveled)\textsuperscript{24–26,29}.

To ensure single-round translocation conditions, we used dextran sulfate (DxSO\textsubscript{4}) as a DNA-mimicking protein trap\textsuperscript{25}. First we assessed the trapping efficiency of DxSO\textsubscript{4} by rapidly mixing RecQ with a pre-mixture of ATP, poly-dT and different concentrations of DxSO\textsubscript{4} (indicated in mg/ml) in the presence of 5μM MDCC-PBP in the stopped-flow. The control trace was recorded in 6mg/ml DxSO\textsubscript{4} in the absence of poly-dT. The DxSO\textsubscript{4} concentration dependence of steady-state ATP hydrolysis rates was used to determine the trapping efficiency (based on Supplementary equation (S2)) values shown in (b). Solid lines are hyperbolic fits to determine the DxSO\textsubscript{4} concentrations required for half-maximal trapping (2.3 ± 0.1μg/ml for RecQ\textsuperscript{WT}, 1.8 ± 0.1μg/ml for RecQ\textsuperscript{Y555A}, and 62 ± 11μg/ml for RecQ\textsuperscript{523}). Symbols are as in Fig. 2c. (c) Time courses of P_i generation from ATP by RecQ\textsuperscript{523} during single-round translocation on poly-dT in the presence of 3μM MDCC-PBP. 20nM RecQ\textsuperscript{523} was pre-incubated with poly-dT (25μM, nt) and mixed with ATP (1mM) plus different concentrations of DxSO\textsubscript{4} (1–8mg/ml) in the stopped-flow apparatus. Traces consisted of an exponential and a linear phase, corresponding to ATP hydrolysis during translocation along poly-dT and in the DxSO\textsubscript{4}-bound end state, respectively. (d) DxSO\textsubscript{4} concentration dependence of the amplitude of the translocation phase for all RecQ constructs. Symbols are as in Fig. 2c. Solid lines show fits based on Supplementary equation (S3). Determined parameters are listed in Table 1.

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than did RecQ^{WT} and RecQ^{Y555A} (Fig. 3b). This result suggested that the HRDC domain, regardless of its ssDNA-binding capability, is a crucial mediator of DxsO_4 trapping of RecQ.

We characterized the ssDNA translocation processivity of RecQ constructs by determining the mean number of ATPase cycles during a single translocation run (\(<n_{\text{ATP}}\>\), Table 1). In these experiments, RecQ was preincubated with poly-dT and then rapidly mixed with ATP and different concentrations of DxsO_4 in a stopped-flow instrument. The kinetics of P_i generation during ATP hydrolysis was followed by MDCC-PBP. DxsO_4 concentrations were chosen from the regime where the trapping efficiency was above 95% (cf. Fig. 3b). P_i generation kinetic traces comprised an exponential phase (characteristic of single-round ssDNA translocation) followed by a slow steady-state that ensued upon dissociation of RecQ from ssDNA after the translocation run (Fig. 3c). Thus, the amplitude of the exponential phase (mol P/mol RecQ) equals \(<n_{\text{ATP}}\>\). This amplitude decreased with increasing DxsO_4 concentration for all RecQ constructs, indicating that DxsO_4 actively facilitates RecQ dissociation from ssDNA during translocation. In the case of RecQ^{523}, the amplitudes showed a shallower DxsO_4 concentration dependence than for RecQ^{WT} and RecQ^{Y555A} (Fig. 3d), as expected based on the experiments of Fig. 3a–b. To determine the genuine (DxsO_4-free) \(<n_{\text{ATP}}^0\>\) value, we extrapolated to zero DxsO_4 concentration using Supplementary equation (S3) 24,25,29. RecQ^{WT} and RecQ^{Y555A} showed similar \(<n_{\text{ATP}}^0\>\) values, whereas this value was slightly higher in RecQ^{523} (Table 1), reflecting that the HRDC domain does not have a profound effect on the ssDNA translocation processivity of RecQ.

As described earlier, the ATPase-translocation coupling stoichiometry (\(C_{\text{trans}}\) number of ATP molecules hydrolyzed per nt traveled) can be determined from the ssDNA (poly-dT) length dependence of P_i generation amplitudes during single-round ssDNA translocation 24,25,29. In these experiments, we preincubated RecQ with oligo-dT substrates of different lengths and then rapidly mixed these premixtures with ATP plus DxsO_4 in a stopped-flow instrument. Kinetic traces showed exponential and linear phases (Fig. 4a), similar to those observed for poly-dT (Fig. 3c). As expected, the single-round (exponential) P_i generation amplitudes increased with increasing oligo-dT length, showed saturation, and their maximal value decreased with increasing DxsO_4 concentration (Fig. 4b). Fits to these data using Supplementary equation (S4) revealed a \(C_{\text{trans}}\) value of 1.0 ± 0.1 ATP/nt and an occluded site size (\(b\)) of 13 ± 1 nt for RecQ^{523} along ssDNA, both of which were independent of DxsO_4 concentration in the assessed range (2–4 mg/ml) (Table 1). Comparison of these values with those determined earlier for RecQ^{WT} under identical or similar conditions revealed that deletion of the HRDC domain does not significantly affect the ATPase-translocation coupling but it decreases the occluded site size (Table 1) 25,26.

The rate of ATP hydrolysis during translocation along poly-dT (\(k_{\text{ATP,trans}}\) determined from the initial slopes of the exponential phase in the experiments of Fig. 3c) was similar to those determined for DxsO_4-free steady-state ATP hydrolysis for each construct (Fig. 3a, Table 1).
The HRDC domain suppresses the rate of ssDNA translocation in parallel with that of ATP hydrolysis. The above results showed that the HRDC domain does not influence ATPase-translocation coupling ($k_{\text{ATP,trans}}$), but it suppresses the rate of ATP hydrolysis during translocation ($k_{\text{ATP,trans}}$). These findings imply that the rate of ssDNA translocation ($k_{\text{trans}}$ (expressed in nt/s) = $k_{\text{ATP,trans}}$/$C_{\text{trans}}$) must also be suppressed by the HRDC domain, in parallel with the ATPase rate. Furthermore, we found that $<n_{\text{ATP}}^0>$ was not greatly affected by the HRDC domain, implying that this domain neither affects the mean processive run length (nt traveled in a single run, $<n_{\text{nt}}>$ = $<n_{\text{ATP}}^0>/C_{\text{trans}}$). As $<n_{\text{nt}}>$ = $k_{\text{trans}}$/k$_{\text{off,trans}}$ where $k_{\text{off,trans}}$ is the rate constant of RecQ dissociation from ssDNA during translocation, one will expect that the latter parameter will be suppressed by the HRDC domain in parallel with the ATPase rate. Thus, the experimental determination of $k_{\text{off,trans}}$ provides an independent means of verification of the proposed mechanochemical effects.

The intrinsic (tryptophan, Trp) fluorescence intensity of each ssDNA-bound RecQ construct is markedly lower than that of the DNA-free forms. This signal is thus suitable for monitoring the kinetics of dissociation of RecQ molecules (i.e., $k_{\text{off,trans}}$) from ssDNA upon completing single-round translocation. We determined the $k_{\text{off,trans}}$ values of RecQ constructs upon rapidly mixing the RecQ-poly-dT complex with excess ATP and varying concentrations of DsSO4 (Fig. 5a). The observed rate constants ($k_{\text{obs}}$) of the transients increased linearly with DsSO4 concentration (Fig. 5b). The slopes and intercepts of the plots, reflecting the DsSO4 sensitivity of the reaction and DsSO4-free $k_{\text{off,trans}}$ value, respectively, were similar for RecQWT and RecQY555A (Fig. 5b, Table 1). Importantly, the DsSO4-free $k_{\text{off,trans}}$ of RecQ523 was 3 times higher than that of RecQWT and RecQY555A, providing independent verification of the above mechanochemical considerations.

As expected based on the experiments of Fig. 3, the ssDNA dissociation $k_{\text{obs}}$ values of RecQ523 were less sensitive to DsSO4 compared with the other constructs (Fig. 5b, Table 1). In control experiments performed in the absence of ATP, $k_{\text{obs}}$ values were several times higher than those in the presence of ATP at the corresponding DsSO4 concentration for all constructs (Fig. 5b). This finding indicates that the Trp fluorescence data obtained in the presence of ATP reliably report dissociation after single-round translocation. Calculated $<n_{\text{nt}}>$ values ($=k_{\text{trans}}$/k$_{\text{off,trans}}$) were slightly higher compared to those determined from MDCC-PBP experiments ($<n_{\text{nt}}>$ = $<n_{\text{ATP}}^0>/C_{\text{trans}}$), but were practically identical for RecQWT and RecQ523 in both cases (Table 1).

The HRDC domain does not affect the coupling between ATP hydrolysis and dsDNA unwinding. To determine the coupling between the ATPase and dsDNA unwinding activities, we devised experiments to measure the rate of DNA unwinding and the rate of ATPase activity in the presence of a forked duplex DNA substrate comprising 33 bp dsDNA with two 21-nt arms. First we determined the binding affinity of RecQ constructs to this DNA substrate by fluorescence anisotropy titrations utilizing...
Figure 6. The HRDC domain modulates forked duplex DNA unwinding but does not affect ATPase-unwinding coupling. (a) Fluorescence anisotropy titration of 10 nM fluorescein-labeled forked duplex DNA substrate (21-nt ssDNA arms + 33-bp dsDNA) with increasing amounts of RecQ constructs. Symbols are as in Fig. 2c. Solid lines show hyperbolic fits. Determined dissociation constants ($K_{d, \text{forked duplex}}$) are listed in Table 1. (b) Electrophoretogram of a single-turnover unwinding experiment. Preincubation of fluorescein-labeled forked duplex (30 nM) with RecQ^{523} (100 nM) was followed by rapid mixing with ATP (3 mM) plus excess ssDNA trap strand (3 μM) (final post-mixing concentrations). Reactions were stopped by the addition of EDTA (40 mM) and loading dye at different time points (0–150 s; as in (c)) using a quench-flow instrument or by manual mixing. Amounts of DNA species (forked duplex and ssDNA, depicted by cartoons) labeled with fluorescein (asterisk) were detected by a fluorescence imager. “--” denotes a 150-s control reaction in which ATP was absent. (c) Single-turnover unwinding kinetics of forked DNA (as in (b)). Symbols are as in Fig. 2c. Solid lines show fits based on Supplementary equation (S5). Determined parameters are listed in Table 1. (d) Steady-state ATPase $k_{\text{cat}}$ (DNA-saturated ATPase) values of RecQ constructs (10 nM) in the presence of dT_{54}, poly-dT and forked duplex DNA, determined in a PK-LDH coupled assay. Values obtained for dT_{54} and poly-dT are in line with those obtained in MDCC-PBP-based ATPase assays (Figs 2c and 3a; Table 1).

a fluorescein label placed on the dsDNA-forming 3′-end of one strand (Fig. 6a). Compared to RecQ^{WT}, the binding affinity ($K_{d, \text{forked duplex}}$) was reduced about 4-5-fold in both RecQ^{Y555A} and RecQ^{523}, reflecting a modest contribution of the HRDC-ssDNA interaction (Table 1). To determine dsDNA unwinding rates, we performed rapid kinetic single-turnover unwinding experiments in which we rapidly mixed the RecQ-forked DNA complex with ATP and excess unlabeled ssDNA trap strand in a quenched-flow instrument, and monitored the time course of fluorescently-labeled ssDNA generation from forked duplex via gel electrophoresis of reaction products (Fig. 6b). Traces comprised a short (about 0.1-s) initial lag followed by two quasi-exponential rise phases in the case of all constructs (Fig. 6c), and were analyzed based on a previously described n-step kinetic model (Supplementary equation (S5))^{32}. This model assumes that unwinding occurs as a result of n consecutive rate limiting steps that have a uniform rate constant. The model is suitable for the calculation of the macroscopic dsDNA unwinding rate ($k_{\text{unw}}$) (Supplementary equation (S5)). The lag and the rapid unwinding phase of RecQ^{WT} was similar to that observed earlier using a Förster resonance energy transfer (FRET)-based assay^{33}. We
found that $k_{\text{unw}}$ was slightly accelerated in RecQY555A and RecQ523 compared to RecQWT, indicating that the HRDC-ssDNA interaction moderately hinders dsDNA unwinding (Fig. 6c, Table 1).

The unwinding traces of all constructs contained an additional slow exponential phase (apparent after 5 s in Fig. 6c) (Table 1). As discussed earlier for RecBCD and UvrD helicases, this phase may result from a fraction of enzyme molecules bound to DNA non-productively, necessitating a rate-limiting initiation of unwinding32,34. Alternatively, if multiple helicase molecules are initially bound to the 3′-ssDNA overhang, the slow phase may result from the action of one helicase proceeding in the trail of the leading one33,35.

To assess ATPase-dsDNA unwinding coupling, we measured the steady-state ATPase rate of RecQ constructs ($k_{\text{ATP,unw}}$) during unwinding of the same forked dsDNA substrate, using a pyruvate kinase-lactate dehydrogenase (PK-LDH) coupled assay (Fig. 6d). Compared to RecQWT, the ATPase activity of RecQY555A was slightly elevated, while RecQ523 had a significantly higher ($k_{\text{ATP,unw}}$) value (Fig. 6d, Table 1). The calculated macroscopic ATPase-unwinding coupling stoichiometry ($C_{\text{unw}}$ (expressed as ATP hydrolyzed per bp unwound) = $k_{\text{ATP,unw}}/k_{\text{unw}}$) was close to 1 ATP/bp for all constructs, suggesting that unwinding is tightly coupled to ATP hydrolysis and is not influenced by the HRDC domain (Table 1). Taken together, these results show similar trends to those found for ssDNA translocation: the HRDC domain hinders the rate of unwinding in parallel with that of the ATPase activity, without influencing the tight coupling between these processes.

### Discussion

Although the HRDC domain has generally been considered as an auxiliary ssDNA-binding element, recent crystal structures of human BLM constructs showed that the HRDC domain can fold back onto and interact with both RecA domains, both in the presence and absence of DNA (pdb ids.: 4CGZ, 4CGD, 4O3M) (Fig. 1c)23. These interdomain interactions were proposed to influence the ATPase activity and the coupled ssDNA translocation and dsDNA unwinding by BLM23. Indeed, suppression of DNA-activated ATPase activity by the HRDC domain was observed for various RecQ helicases (Table 2)14,17,23,36. Moreover, in the case of Ec RecQ, ATPase suppression is independent of the ssDNA-binding ability of the HRDC domain, further indicating interdomain interactions (RecQY555A data in Table 1)17.

Importantly, however, the present study shows that the coupling of the RecQ ATPase activity to ssDNA translocation is unaffected by either the HRDC-ssDNA or the HRDC-motor core interactions. Moreover, the processivity of translocation is also unaffected by HRDC deletion in RecQ (Table 1), similar to the lack of effect of WHD-HRDC deletion on ATPase-translocation coupling and processivity in human BLM14.

We also found that the rate of forked duplex DNA unwinding, determined explicitly in transient kinetic experiments (Fig. 6c), is suppressed by the RecQ HRDC domain in parallel with the ATPase activity, indicating that coupling between these processes is not affected by the HRDC domain (Table 1). Interestingly, the DNA unwinding rate of RecQY555A fell between those of RecQWT and RecQ523, indicating

### Table 2. Comparison of effects of HRDC domain mutation/deletion in RecQ helicases.

| Construct | ATPase activity (relative to WT) | Unwinding efficiency (relative to WT) |
|-----------|----------------------------------|-------------------------------------|
| Ec RecQ (RecQWT) | 1 | 1 |
| Y555A (RecQY555A) | 1.2 | 1.5a |
| dHRDC (RecQ523) | 1.9 | 1.9a |
| BLM (BLM536–1298, BLM486–1298)p | 1 | 1 |
| dHRDC (BLM536–1298)p | 3.7 | 0.11a |
| dWHD-dHRDC (BLM536–1077)pb | 1.5 | 0.19a |
| Dr RecQ (WT containing 3 tandem HRDC domains (HRDC1–3), DrRecQ1–410) | 1 | 1 |
| dHRDC1 (Dr RecQ1–728) | 1.8b | 1p |
| dHRDC2–3 (Dr RecQ3–410) | 2.1b | −5f |
| dHRDC1–3 (Dr RecQ1–519) | >2.5b | ~5f |

Data were obtained using forked duplex DNA if not otherwise indicated. Ec RecQ data are from this study. “dWHD” and “dHRDC” refer to winged-helix domain and HRDC domain deletion, respectively. *Based on unwinding rates determined in single-turnover experiments. †From ref. 14. ‡From ref. 23. Monomeric constructs considered as WT, comprising all domains present in Ec RecQ. §Based on steady-state unwinding rates. ∞Based on enzyme concentration required for half-maximal unwinding efficiency. From ref. 36. hT28 data. 3′-tailed dsDNA substrate.
that HRDC-ssDNA interactions slightly suppress the rate of unwinding, unlike that of ssDNA translocation (Table 1).

Explicit kinetic rates of dsDNA unwinding have not been measured for HRDC-deletion constructs of other RecQ helicase homologs. Available steady-state unwinding kinetic23 and end-point measurements14,16 are not directly informative of possible changes in mechanochemical coupling, as they can be influenced by more complex features (unwinding processivity, unproductive initiation, reversal during unwinding etc.). Nevertheless, such data reflect the relative unwinding efficiencies of helicase constructs and indicate that the HRDC domain may affect ATPase-driven unwinding of forked duplex DNA by BLM in a way that is different from that in Ec and Deinococcus radiodurans (Dr) RecQ enzymes (Table 2)25,26. BLM constructs lacking the HRDC25 or both the WHD and HRDC domains14 showed decreased efficiency of forked duplex DNA unwinding despite increased ATPase activities (Table 2). Further studies are needed to clarify whether and how the varying properties of HRDC domains of different RecQ homologs contribute to mechanochemical coupling and/or more complex dynamic processes during dsDNA unwinding (see below).

RecQ helicases are thought to translocate along ssDNA and unwind dsDNA via ATP-driven inchworm-type stepping24–26. Thus, the interactions and the relative positions of the two RecA-like domains are likely to undergo coordinated changes during the ATP hydrolytic cycle, as reported for various other helicases harboring a similar motor core27,28. Based on the lack of direct polar interactions between the two RecA domains of BLM, it was proposed that the interactions of the HRDC domain with both RecA domains contribute to mechanochemical coupling25. HRDC deletion will therefore increase the flexibility of the RecA core, thereby accelerating the ATPase cycle but possibly decreasing ATPase-unwinding coupling efficiency25. However, other crystal structures (PDB codes 4CDG, 4CGZ) show polar interactions between the RecA domains of ADP-bound BLM, both in the DNA-free and the DNA-bound form. Interestingly, crystal structures of ADP- and DNA-bound ternary complexes of BLM show two different conformations (PDB codes 4CGZ and 4OM1)23. These two structures possibly represent different snapshots of the ATPase cycle, suggesting transient interactions between the two RecA domains and/or between the bound nucleotide and the C-core RecA domain. Similarly, the crystal structure of an Ec RecQ construct lacking the HRDC domain25 shows no direct polar interactions between the RecA domains in the absence of substrates, but such interactions are present in the ATP·S (pre-hydrolytic ATP analog) -bound form of the same construct.

Crystal structures of human RECQ1, a RecQ homolog naturally lacking the HRDC domain, also suggest inter-RecA communication via transient polar interactions39. RECQ1 is present in its bound to a dsDNA substrate with a 5-nt 3′-ssDNA overhang exhibits no direct polar inter-RecA interactions, while the C-core RecA domain interacts with DNA39. On the other hand, RECQ1 bound to dsDNA with a 4-nt 3′-ssDNA overhang contains an inter-RecA salt bridge, but shows no C-core RecA-DNA interaction (PDB code 4U7D). These findings reflect that inter-RecA coordination can be modulated by interactions with the DNA substrate. Interestingly, the two RecA domains form numerous direct as well as water-mediated polar contacts in ADP-bound DNA-free RECQ139. Together with the markedly (about 10 times) lower DNA-activated ATPase activity of RECQ140 compared to Ec RecQ and human BLM14,41, this finding provides further indication for the inverse relationship between inter-RecA rigidity and ATPase kinetics. Thus, the HRDC-induced suppression of the ATPase activity of various HRDC-containing RecQ helicases (Table 2) is likely brought about by HRDC-mediated coordination of the RecA domains.

Our previous work showed that the similar macroscopic mechanochemical properties of Ec RecQ and human BLM result from different underlying kinetic mechanisms24,30. The steady-state rate of DNA-activated ATP hydrolysis by Ec RecQ is limited by the ATP cleavage step, whereas in BLM a transition between two ADP bound states is rate-limiting24,30. ATP hydrolysis by RecQ-type ATPases is thought to be triggered by the so-called “arginine finger” residue that interacts with the γ-phosphate of the bound nucleotide in SF1 helicases23–25. The putative arginine finger located in the C-core RecA domain of different RecQ helicases shows variation in its nucleotide interactions, suggesting its possible role in kinetic tuning. The arginine finger does not interact with the bound nucleotide in DNA-free Ec RecQ·ATP·S22,44 and human RECQ1·ADP complexes39. In contrast, the arginine finger of DNA-bound BLM45 interacts with both phosphate groups of ADP (PDB code 4CGZ). These differences may indicate that the dynamic interaction of the arginine finger with the bound nucleotide during the hydrolytic cycle, which can in turn be influenced by HRDC-RecA core interactions, contributes to limiting the rate of enzymatic activity.

The HRDC domain of RecQ helicases is connected to the rest of the protein through a long and flexible loop, raising the possibility of dynamic interactions with the RecA core of the protein and/or the ssDNA regions of the DNA substrate. Amino acids involved in ssDNA binding by isolated HRDC domain of BLM18 are buried in crystal structures of HRDC-containing BLM constructs23 due to the interaction with the RecA core, suggesting that the HRDC-ssDNA and HRDC-RecA core interactions are mutually exclusive.

Synthesis of our current findings on Ec RecQ with earlier data on BLM reveals that in both enzymes, depending on the structure of the DNA substrate encountered, the HRDC domain is prone to interact with ssDNA regions outside the DNA segment tracked by the RecA core. We found that the unwinding of forked DNA substrates by Ec RecQ is noticeably slowed by HRDC-ssDNA interactions (cf. RecQWT vs. RecQ3555A profiles in Fig. 6c, Table 1), but not by HRDC-RecA core interactions (cf. quasi-identical profiles
of RecQ<sup>Y555A</sup> and RecQ<sup>S23</sup> in the same experiment). Consistent with this observation, we found that the deletion of the HRDC domain drastically reduced the DsSO<sub>4</sub> sensitivity of ssDNA translocation kinetics by Ec RecQ (Figs 3d and 5b), suggesting that the HRDC domain greatly assists ssDNA-RecQ-DsSO<sub>4</sub> ternary complex formation. In parallel with these observations, the HRDC domain of BLM was suggested to bind to ssDNA regions outside the one tracked by the RecA core during unwinding of G4-containing DNA substrates<sup>48</sup>, and the HRDC domains of Dr RecQ and BLM were found to greatly contribute to recognition and processing of Holliday junction structures<sup>36,47</sup>.

On the other hand, in the presence of simple ssDNA substrates and absence of trap, the HRDC domain appears to dominantly interact with the RecA core, as inferred from the very small effects of the Y555A point mutation but marked effects of HRDC deletion under these conditions (Fig. 2c; and trap-free dissociation rate constants in Fig. 5b; Table 1). Taken together, available data suggest that the HRDC domain may dynamically switch between ssDNA- and RecA core-interacting modes, thereby fine-tuning DNA-restructuring processes.

Materials and Methods

Reagents. All reagents were from Sigma-Aldrich unless otherwise stated. ATP was from Roche Applied Science. Inorganic phosphate (P<sub>i</sub>) standard was from Merck. MDCC (7-diethylamino-3-(((2-

Fluorescently labeled P<sub>i</sub> binding protein (MDCC-PBP) was prepared as described in ref. 28. Multi-Mode Microplate Reader (BioTek).

ing concentrations of RecQ constructs. Fluorescence anisotropy was measured in a Synergy H4 Hybrid

utes in Buffer H (30 mM Tris-HCl pH 7.5, 100 mM KCl, 1 mM DTT, 50

300 instrument. Post-mixing concentrations are stated. Trp fluorescence was detected through a 320-nm

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MDCC-PBP . The setup for MDCC-PBP calibration and single-round translocation experiments were as

described earlier<sup>24,25,30</sup>.

PK-LDH-coupled ATPase measurements. Steady-state ATPase experiments were carried out in

SF50 buffer by using a pyruvate kinase-lactate dehydrogenase (PK-LDH) coupled assay (14 U/mL PK, 20

μM NADH). Time courses of NADH absorbance (ε<sub>340</sub> = 6220 M<sup>−1</sup> cm<sup>−1</sup>) were followed in a Shimadzu UV-2101PC spectrophotometer.

Stopped-flow measurements. Stopped-flow measurements were carried out in a BioLogic SFM

300 instrument. Post-mixing concentrations are stated. Trp fluorescence was detected through a 320-nm
cutoff filter at 280-nm excitation with a 4-nm bandwidth. P<sub>i</sub> generation from ATP was followed using

MDCC-PBP. The setup for MDCC-PBP calibration and single-round translocation experiments were as

described earlier<sup>24,25,30</sup>.

Fluorescence anisotropy titrations. 10 nM of forkedduplex substrate was titrated with increasing

concentrations of RecQ constructs. Fluorescence anisotropy was measured in a Synergy H4 Hybrid

Multi-Mode Microplate Reader (BioTek).

DNA unwinding experiments. In single-turnover unwinding experiments DNA substrates (30 nM; final reaction concentrations stated) were incubated with excess enzyme (100 nM) at 25 °C for 5 minutes in Buffer H (30 mM Tris-HCl pH 7.5, 100 mM KCl, 1 mM DTT, 50 μg/ml BSA, 20 mM creatine phosphate, 20 μg/ml creatine kinase). Reactions were started at 25 °C by mixing the DNA-enzyme complex with Buffer H containing ATP (3 mM), MgCl<sub>2</sub> (3 mM) and ssDNA trap strand (3 μM) to provide single-turnover conditions. Reactions were stopped after pre-set reaction times by the addition of EDTA (40 mM final, pH 8.1) and loading dye (10 mM Tris-HCl pH 7.5, 40 mM EDTA, 60% v/v glycerol, 0.075% w/v Orange G, 0.83% w/v SDS). Reaction times shorter than 5 seconds were achieved by using a rapid quench-flow instrument (RQF-3, KinTek). Longer reactions were started and stopped manually.
Post-reaction mixtures were incubated at 25°C for additional 3 min and were held on ice until further processing. Samples were then loaded on 12% non-denaturing polyacrylamide gels in TBE buffer (89 mM Tris–HCl pH 7.5, 89 mM boric acid, 20 mM EDTA). Electrophoresis was carried out at 4°C. Fluorescently-labeled DNA was detected by using a Typhoon TRIO+ Variable Mode Imager (Amersham Biosciences). The intensities of bands corresponding to the DNA substrate and unwinding products were quantified by densitometry (GelQuant Pro software (DNR Bio Imaging Ltd.)).

**Data analysis.** Means ± SEM values (n = 3) are reported in the paper, unless otherwise specified. Data analysis was performed using OriginLab 8.0 (Microcal corp.).

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Acknowledgments
This work was supported by the Human Frontier Science Program (RGY0072/2010 to M.K. and K.C.N.), the “Momentum” Program of the Hungarian Academy of Sciences (LP2011-006/2011 to M.K.) and ELTE KMOP-4.2.1/B-10-2011-0002 Grant to M.K. This work was supported in part by the Intramural Research Program of the National Heart, Lung, and Blood Institute, National Institutes of Health (HL001056-07 to K.C.N.). We are grateful to Dr. Tünde Juhász for assistance with CD measurements and to Dr. Yeonhee Seol for discussions.

Author Contributions
G.M.H., K.C.N. and M.K. conceived and designed the work. G.M.H., N.T.N. and M.M. performed experiments. G.M.H. and M.K. analyzed data. G.M.H., K.C.N. and M.K. wrote the paper.

Additional Information
Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Harami, G.M. et al. The HRDC domain of E. coli RecQ helicase controls single-stranded DNA translocation and double-stranded DNA unwinding rates without affecting mechanoenzymatic coupling. Sci. Rep. 5, 11091; doi: 10.1038/srep11091 (2015).

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