Supplementary Information
E. coli RecBC Helicase has Two Translocase Activities Controlled by a Single ATPase Motor
Colin G. Wu, Christina Bradford and Timothy M. Lohman

Supplementary Methods

Buffers and Reagents. RecB and RecC storage buffer is Buffer C: 20 mM K phosphate (pH 6.8 at 25°C), 0.1 mM 2-mercaptoethanol, 0.1 mM ethylenediaminetetraacetic acid (EDTA), and 10% (v/v) glycerol. Poly-(dT) was purchased from Midland Certified Reagents Inc. (Midland, TX) and fractionated as described 1-2 to obtain a weight average length of 3.2 kb.

RecB dissociation kinetics during ssDNA translocation. RecB dissociation kinetics during ssDNA translocation was examined by monitoring the increase in RecB intrinsic tryptophan fluorescence, which is quenched when RecB is bound to ssDNA1. 50 nM RecB was pre-mixed with 20 µM poly-(dT) (nucleotides concentration) in Buffer M on ice for five minutes and the mixture was loaded into one syringe of the stopped-flow apparatus. Translocation was initiated by mixing with 10 mM ATP and 8 mg mL⁻¹ heparin. RecB tryptophan fluorescence was excited at 280 nm and fluorescence emission was monitored at 350 nm using an interference filter (Oriel Corp., Stratford, CT). RecB
dissociation time courses were fit to a single exponential function \( f(t) = Ae^{-k_{d,obs}t} \); \( A = -0.21 \pm 0.02 \) (AU), \( k_{d,obs} = 7.5 \pm 0.3 \) \( \text{s}^{-1} \)) since photobleaching was not observed within the time scale of the experiment. We note that the value of \( k_{d,obs} \) remains constant over a range constant [heparin] (up to 15 mg mL\(^{-1}\) after mixing, data not shown); therefore, heparin does not displace RecB monomers from the ssDNA during translocation.

**Analysis of ssDNA translocation and DNA unwinding kinetic time courses.** RecB monomer translocation kinetics along ssDNA was analyzed using non-linear least squares (NLLS) methods as described previously \(^1\)\(^3\). Cy3 and Oregon Green time courses were analyzed globally using **Scheme 1** and **Equation S1**, where \( f(t) \) describes the time dependent fluorescence signal resulting from RecB monomer translocating to the 5´ end of the ssDNA. \( \mathcal{L}^{-1} \) is the inverse Laplace transform operator and \( s \) is the Laplace variable. The fluorescence amplitude (\( A \)) and the number of translocation steps (\( n \)) were floated for each ssDNA length (\( L \)) while the microscopic translocation rate (\( k_t \)), the end dissociation rate constant (\( k_{end} \)), and \( r \) (which is the fraction of RecB bound to any position other than the 5´ end of the ssDNA to that of the 5´ end) were constrained to be global parameters. The average kinetic step-size for translocation, \( m_t \), was determined by replacing \( n \) in **Equation S1** with \((L - d)/m_t\), where \( d \) is the contact size of RecB in nucleotides. The value for \( k_d \) was fixed at 7.5 \( \text{s}^{-1} \) in the analysis, which was determined independently as described above.
The rates reported for RecBC translocation along ssDNA were estimated using two methods: examining the dependence of the lag phase of the translocation time courses on ssDNA extension length, or fitting the entire time courses to a kinetic scheme which describes the initial unwinding of the 24 bp duplex and subsequent ssDNA translocation. The duration of the lag phase was estimated as the intersection point of two linear fits (see Supplementary Fig. 2) which describe the lag phase and the initial increase (or decrease for fluorescein labeled substrates) in fluorescence signal. Simulated time courses have shown\(^4\) that a plot of the “time to reach the lag” as well as “peak position” versus ssDNA length always provides an accurate estimate of the macroscopic translocation rate if the translocase initiates from a unique site on the DNA as in this case. Such an analysis does not generally provide accurate estimates of the translocation rate if the translocase initiates from random sites on the DNA as is the case for RecB, UvrD, PcrA, and Rep monomer translocation on ssDNA\(^1,4-6\). In that case, the full time course must be analyzed to obtain accurate translocation rates \(^1,3\). We also analyzed the full time courses for RecBC translocation along ssDNA using Equation S2 based on the kinetic mechanism shown in Scheme 2, in which RecBC first unwinds a duplex region of length \(L_{ds}\) followed by translocation along ssDNA of length \(L_{ss}\). The DNA unwinding parameter \(L_{ds}\) was fixed at 24 bp, and average kinetic step-size and microscopic rate constant for DNA unwinding were constrained to the values determined in previous stopped-flow fluorescence experiments under the same solution conditions (\(m_U = 4.4 \pm 0.1\) bp, \(k_U = 79 \pm 11\) s\(^{-1}\); \(mk_U = 348 \pm 5\) bp s\(^{-1}\)) \(^7\). The fluorescence amplitude (\(A\)) was floated at each ssDNA length (\(L_{ss}\)) while the translocation step-size (\(m_t\)),
microscopic translocation rate \((k_t)\), and end dissociation rate constant \((k_{\text{end}})\) were constrained to be global parameters.

\[
f(t) = A \times L^{-1} \left( \frac{k_L/m, k_U/m_U}{(s + k_t)^L/m_U (s + k_{\text{end}})^L} \right)
\]  
(Equation S2)

**Analysis of DNA unwinding kinetics.** DNA unwinding time courses from Figure 6 were analyzed using **Equation S3** based on the kinetic mechanism shown in **Scheme 3**, which describes the initial unwinding of a proximal duplex followed by translocation along a ssDNA gap and subsequent re-initiation of the unwinding of a 40 bp distal duplex. As written in **Scheme 3**, the unwinding parameters for the proximal and distal duplexes are assumed to be the same, therefore, \(L_{\text{ds}}\) was fixed at 64 bp (24 bp proximal, 40 bp distal), and \(m_U\) and \(k_U\)

\[
f_{\text{ds}}(t) = A \times L^{-1} \left( \frac{k_L/m, k_U/m_U}{s(k_U + s)^L/m_U} \right)
\]  
(Equation S3)

were constrained to values determined previously using chemical quenched-flow under the same solution conditions \((m_U = 4.4 \pm 1.7 \text{ bp}, k_U = 90 \pm 25 \text{ s}^{-1}; m_U k_U = 396 \pm 15 \text{ bp s}^{-1})\). The extent of DNA unwinding \((A)\) was floated for each ssDNA gap length \((L_{\text{ss}})\), while \(m_t\) and \(k_t\) were constrained to be global parameters.

The RecBC translocation rates determined from “lag time” analysis as a function of [ATP] were fit to the Michaelis-Menten equation (Equation S4).

\[
m_t k_t = \frac{V_{\text{max}} [ATP]}{K_M + [ATP]}
\]  
(Equation S4)
Supplemental References

1. Fischer, C. J., Maluf, N. K. & Lohman, T. M. Mechanism of ATP-dependent translocation of E.coli UvrD monomers along single-stranded DNA. *J Mol Biol* 344, 1287-1309 (2004).

2. Tomko, E. J., Fischer, C. J., Niedziela-Majka, A. & Lohman, T. M. A Nonuniform Stepping Mechanism for E. coli UvrD Monomer Translocation along Single-Stranded DNA. *Molecular Cell* 26, 335-347 (2007).

3. Fischer, C. J. & Lohman, T. M. ATP-dependent translocation of proteins along single-stranded DNA: models and methods of analysis of pre-steady state kinetics. *J Mol Biol* 344, 1265-1286 (2004).

4. Tomko, E. J. *Transient-State Kinetic Studies of the E. coli UvrD Monomer Translocation Along Single-Stranded DNA* Ph.D thesis, Washington University School of Medicine, (2010).

5. Brendza, K. M. *et al.* Autoinhibition of *Escherichia coli* Rep monomer helicase activity by its 2B subdomain. *Proc Natl Acad Sci U S A* 102, 10076-10081 (2005).

6. Niedziela-Majka, A., Chesnik, M. A., Tomko, E. J. & Lohman, T. M. Bacillus stearothermophilus PcrA Monomer Is a Single-stranded DNA Translocase but Not a Processive Helicase in Viro. *J. Biol. Chem.* 282, 27076-27085, (2007).

7. Wu, C. G. & Lohman, T. M. Influence of DNA end structure on the mechanism of initiation of DNA unwinding by the Escherichia coli RecBCD and RecBC helicases. *J Mol Biol* 382, 312-326 (2008).
**Supplementary Figure 1. RecB monomer dissociation kinetics.** RecB monomer dissociation kinetics during 3’ to 5’ ssDNA translocation was examined by monitoring the increase in intrinsic tryptophan fluorescence of RecB, which is quenched when bound to ssDNA. The dissociation time course was fit to a single exponential function \( f(t) = Ae^{(-k_{d,obs}t)} \); \( A = -0.21 \pm 0.02 \) (AU), \( k_{d,obs} = 7.5 \pm 0.3 \) s\(^{-1}\) as indicated by the smooth red curve. This value of \( k_{d,obs} \) was independent of [heparin] (up to 15 mg mL\(^{-1}\) after mixing) and was constrained in the analysis of RecB monomer translocation kinetics (see Fig. 2a–2b).
Supplementary Figure 2. “Lag time” analysis of a RecBC translocation time course. Data from Figure 3a is plotted ($L = 60$ nucleotides). The duration of the lag was estimated as the intersection point of two linear fits which describe the lag phase and the initial increase (or decrease with fluorescein labeled substrates) in fluorescence intensity. This value was determined for each $L$ (see Fig. 3e) and the dependence of the lag time on $L$ was used to estimate the translocation rate.
Supplementary Figure 3. Comparison of RecBC primary and secondary translocase time courses. (a–e). Data from Figure 3a and 3c are overlaid for \( L = 15, 30, 45, 60, \) and \( 75 \) nucleotides. Closed squares (■) indicate primary translocase time courses shown in Figure 3a and opened squares (□) denote secondary translocase kinetics shown in Figure 3c. Although the duration of the lag phase and initial increase of fluorescence intensity are similar for each \( L \), the translocation kinetics differ in the peak positions of fluorescence as well as the dissociation rates from the ssDNA ends. (f). Overlay of the raw fluorescence intensity for the data shown in Figure 5a (\( L = 30 \)). The two time courses are obtained from DNA substrates possessing two \((dT)_30\) ssDNA extensions and are labeled either on the 3’-end (○) or 5’-end with Cy3 (●). Although the normalized translocation kinetics are identical for the two substrates (see Fig. 5a), the total extent of fluorescence increase is ~15% larger for the 5’-end labeled DNA substrate.
Supplementary Figure 4. Translocation rates of RecBC primary and secondary activities as a function of [ATP] determined from “lag time” analyses. The translocation rates determined from this analysis are plotted versus [ATP] in Figure 3f and fit to the Michaelis-Menton Equation (Eq. S4) (see Fig. 3f). (●) 3´ to 5´ primary translocase data; (O) 5´ to 3´ secondary translocase data. (a). 10 µM ATP (after mixing). Smooth curves indicate linear fits: “time” = 0.0232L + 0.0111 (primary), “time” = 0.0132L + 0.014 (secondary). (b). 37.5 µM ATP (after mixing). Smooth curves indicate linear fits: “time” = 0.0074L + 0.0109 (primary), “time” = 0.0043L + 0.0068 (secondary). (c). 75 µM ATP (after mixing). Smooth curves indicate linear fits: “time” = 0.0039L + 0.0171 (primary), “time” = 0.0024L + 0.0146 (secondary). (d). 120 µM ATP (after mixing). Smooth curves indicate linear fits: “time” = 0.0028L + 0.0187 (primary), “time” = 0.0019L + 0.0092 (secondary).
Supplementary Figure 5. RecBC primary translocase is sensitive to ssDNA backbone polarity but the secondary translocase is not. (a). Overlay of the time courses shown in Figure 5a and 5d, which were normalized to the maximum fluorescence intensity. DNA III, as shown in Figure 5d, contains a 3′–3′ phosphodiester linkage (“X”) that reverses the backbone polarity on the 5′-terminating strand. The ssDNA backbone polarity is unchanged in DNA V (L = 75 in Figure 5a). The translocation time courses obtained for both DNA substrates are identical indicating that the secondary translocase in not sensitive to the ssDNA backbone polarity. (b). The backbone polarity of both 5′-terminating and 3′-terminating strands are reversed (“X”) after the initial 24 bp duplex. DNA VI is labeled with Cy3 at the end of the 5′-terminating strand while DNA VII is labeled with Cy3 at the end of the 3′-terminating strand. The primary translocase is blocked by the reverse polarity backbone (see Fig. 5c) while the secondary translocase is unaffected.
Supplementary Figure 6. [NaCl] dependence on RecBC primary and secondary translocase activities. (a). Primary translocase activity was examined using the DNA substrates shown in Figure 3a at 30, 100, 250, and 400 mM NaCl. (b). Secondary translocase activity was examined using the DNA substrates shown in Figure 3c at 30, 100, 250, and 400 mM NaCl. (c). lag time analysis of the primary translocase at different [NaCl]. (●) 30 mM NaCl: “time” = 0.0011L + 0.0064; (●) 100 mM NaCl: “time”= 0.0011L + 0.0117; (●) 250 mM NaCl: “time”= 0.0011L + 0.0133; (●) 400 mM NaCl: “time”= 0.0011L + 0.0133. (d). lag time analysis of the secondary translocase at different [NaCl]. (●) 30 mM NaCl: “time” = 0.0007L + 0.0275; (●) 100 mM NaCl: “time”= 0.0008L + 0.0247; (●) 250 mM NaCl: “time”= 0.0009L + 0.021; (●) 400 mM NaCl: “time”= 0.0007L + 0.0272.
DNA substrates I-VIII are fluorescently labeled on the 5’ end (designated as “X”) with either Cy3 or Oregon Green, and are used to monitor RecB monomer translocation along ssDNA in the 3’ to 5’ direction (Fig. 2a-2b). DNA IX is fluorescently labeled on the 3’ end with Cy3 and is used to test whether RecB can translocate along ssDNA with 5’ to 3’ directionality (Fig. 2c).

| DNA | Length (nt) | DNA sequence               |
|-----|-------------|----------------------------|
| I   | 54          | 5’-X (dT)_{54}-3’          |
| II  | 64          | 5’-X (dT)_{64}-3’          |
| III | 79          | 5’-X (dT)_{79}-3’          |
| IV  | 84          | 5’-X (dT)_{84}-3’          |
| V   | 97          | 5’-X (dT)_{97}-3’          |
| VI  | 101         | 5’-X (dT)_{101}-3’         |
| VII | 104         | 5’-X (dT)_{104}-3’         |
| VIII| 114         | 5’-X (dT)_{114}-3’         |
| IX  | 54          | 5’-(dT)_{54} (Cy3)T-3’     |
### Supplementary Table 2. DNA substrate sequences used for examining RecBC translocation along ssDNA

| DNA | Length (nt) | DNA sequence |
|-----|-------------|--------------|
| A   | 30          | 5'-(dT)₆ CCA TGG CTC CTG AGC TAG CTG CAG (ZT)-3' |
| Ia  | 45          | 5'-(Y)(dT)₁₅ CTG CAG CTA GCT CAG GAG CCA TGG (dT)₆-3' |
| IIa | 60          | 5'-(Y)(dT)₃₀ CTG CAG CTA GCT CAG GAG CCA TGG (dT)₆-3' |
| IIIa| 75          | 5'-(Y)(dT)₄₅ CTG CAG CTA GCT CAG GAG CCA TGG (dT)₆-3' |
| IVa | 90          | 5'-(Y)(dT)₆₀ CTG CAG CTA GCT CAG GAG CCA TGG (dT)₆-3' |
| Va  | 105         | 5'-(Y)(dT)₇₅ CTG CAG CTA GCT CAG GAG CCA TGG (dT)₆-3' |
| B   | 30          | 5'-(Z) CTG CAG CTA GCT CAG GAG CCA TGG (dT)₆-3' |
| Ib  | 45          | 5'-(dT)₆ CCA TGG CTC CTG AGC TAG CTG CAG (dT)₁₅ (YT)-3' |
| IIb | 60          | 5'-(dT)₆ CCA TGG CTC CTG AGC TAG CTG CAG (dT)₃₀ (YT)-3' |
| IIIb| 75          | 5'-(dT)₆ CCA TGG CTC CTG AGC TAG CTG CAG (dT)₄₅ (YT)-3' |
| IVb | 90          | 5'-(dT)₆ CCA TGG CTC CTG AGC TAG CTG CAG (dT)₆₀ (YT)-3' |
| Vb  | 105         | 5'-(dT)₆ CCA TGG CTC CTG AGC TAG CTG CAG (dT)₇₅ (YT)-3' |

DNA substrates from **Figure 3a-3b** are formed by annealing DNA strand A with DNA strands Ia-Va, which are fluorescently labeled on the 5’ end with Cy3 or fluorescein (denoted as “Y” where appropriate). This forms a 24 bp duplex with a twin (dT)₆ high affinity loading site for RecBC on one end of the duplex, and a series of (dT)₇₅ ssDNA extensions (L = 15, 30, 45, 60, 75 nucleotides) on the other end of the 3’-terminating DNA strand, which allows us to examine the primary 3’ to 5’ translocase activity of RecBC. DNA substrates from **Figure 3c-3d** are formed by annealing DNA strand B with DNA strands Ib-Vb, which are fluorescently labeled on the 3’ end with Cy3 or fluorescein (denoted as “Y” where appropriate). These substrates also form a 24 bp duplex with a high affinity RecBC loading site, but the (dT)₇₅ extensions now reside on the 5’-terminating DNA strand and are used to examine the secondary 5’ to 3’ translocase activity of RecBC.

DNA substrates from **Figure 4a-4b** are formed by annealing DNA strand B with IVb and DNA strand A with IVa as described above but now the A and B strands are labeled with Cy5 (denoted as “Z”).

DNA substrates from **Figure 5a-5b**, which are used to examine RecBC translocation along the two ssDNA extensions simultaneously, are formed by annealing DNA strands Ia-Va with Ib-Vb such that either the a or b strands are labeled with Cy3 (designated as “Y”). Substrates with reverse polarity linkages on the DNA backbone have the same sequences but 5’-5’ or 3’-3’ linkages are introduced to the DNA backbone as depicted in **Figure 5c-5d** (“X”). For **Figure 5e-5f**, DNA strand IVb (Y=Cy3) was annealed with IVa (Y=Cy5).
DNA substrates from Figure 6a, which possess 3’ to 5’ ssDNA gaps of dT$_2$, dT$_{20}$, and dT$_{41}$ are formed by annealing DNA strands IIa, IIb, and IV-VIc. This forms a 24 bp proximal duplex with a high affinity loading site for RecBC on one end and a gap region on the other end, followed by a distal duplex of 40 bp. Similarly, DNA substrates from Figure 6b are formed by annealing DNA strands Ia, Ib, and I-IIIc. These DNA possess 5’ to 3’ ssDNA gaps of dT$_2$, dT$_{20}$, and dT$_{41}$ in between the proximal and distal duplexes.
