Structure

Supplemental Data

"Crystal structure of *E. coli* RecE protein reveals a toroidal tetramer for processing double stranded DNA breaks"

Jinjin Zhang, Xu Xing, Andrew B. Herr, and Charles E. Bell

Supplemental Experimental Procedures

Protein Expression and Purification

DNA fragments encoding residues 564-866 and 606-866 of RecE were PCR amplified from *E. coli* genomic DNA and cloned into the NdeI and BamH1 sites of pET-14b (Novagen), which expresses the proteins with an N-terminal 6His tag and an intervening sequence for thrombin digestion. DNA sequencing of the cloned inserts revealed the presence of a P658L mutation relative to the *recE* sequence from *E. coli* K12. The Quickchange procedure (Stratagene) was used to construct plasmids for producing $RecE^{564}$ and $RecE^{606}$ with proline at amino acid position 658. Plasmids were transformed into *E. coli* BL21(AI) for expression of each protein under the control of arabinose. Cell cultures (6 × 1L) were inoculated in 2.8 L broad bottom flasks at 37 °C with shaking at 225 rpm.

For $RecE^{606}$, the temperature was lowered to 15 °C when the OD^{600} reached 0.8, and after 30 additional minutes expression was induced by addition of 0.1% arabinose followed by continued shaking for 16 hours at 15 °C. Cells were harvested by centrifugation at 10,000 x g, re-suspended in buffer containing 50 mM sodium phosphate, 300 mM sodium chloride, and 10 mM imidazole, pH 8.0. The re-suspended cells were lysed by repeated sonication on ice, and the soluble portion of the resulting lysate was clarified by centrifugation at 39,000 x g. $RecE^{606}$ protein was purified by nickel affinity chromatography (Qiagen), digested with thrombin protease (GE Healthcare), and further purified by anion exchange chromatography on Hi-Trap QHP (GE Healthcare). Purified $RecE^{606}$

protein was dialyzed into 20 mM Tris (pH 7.5), 150 mM NaCl, 10% glycerol, 1mM dithiothreitol, concentrated to 20-50 mg/ml, and stored at -80°C in small aliquots.

The selenomethionine version of $RecE^{606}$ (P658L) was expressed in *E. coli* BL21(AI) grown in minimal medium containing 50 mg/L of selenomethionine. In order to obtain this protein in a soluble form, cells were grown with shaking at 37°C to OD^{600} of 0.3, heat shocked at 42 °C for 30 minutes to induce the expression of chaperone proteins, grown an additional 15 minutes at 37 °C, chilled on ice for 15 minutes, and induced with 0.1% arabinose with continued shaking at 18 °C for 16 hours. Purification of the selenomethionine version of $RecE^{606}$ followed the same procedure as the native version, except for the inclusion of 10 mM 2-mercaptoethanol in all buffers for purification and 5 mM dithiothreitol in the final storage buffer.

$RecE^{564}$, mutant $RecE^{564}$, and λ exonuclease proteins for activity assays were expressed from pET14b in BL21(AI) cells induced with 0.1 % arabinose for four hours at 37°C. The proteins were purified by Ni^{2+} affinity chromatography followed by gel filtration on Superdex 200. The 6xHis tags were not removed by thrombin digestion. The final purified proteins were dialyzed into 20 mM Tris (pH 7.5), 1 mM DTT, concentrated to 5-20 mg/ml, and frozen at -80°C in small aliquots.

Table S1. Summary of the Crystallographic Data

	RecE^{606} P658L (Native)	Se-Met RecE^{606} P658L
X-ray Diffraction Data		
Space group	$P42_12$	$P42_12$
Unit Cell dimensions		
a = b (Å)	123.2	123.4
c (Å)	67.3	67.7
Resolution (Å)	45.4-2.8 (2.95-2.80)	36.7-3.2 (3.37-3.20)
No. unique reflections	13,187	9,072
Redundancy	23.5 (23.4)	12.5 (12.9)
Completeness (%)	99.6 (100.0)	99.9 (100.0)
I/σ	19.6 (3.3)	22.2 (6.7)
R_{merge}	0.080 (0.70)	0.099 (0.33)
Refinement Statistics		
Resolution (Å)	45.41-2.80	
No. of reflections	13,172	
R_{work}/R_{free} (%)	28.9/31.1	
No. of protein atoms	1,737	
Mean B factor (Å^2)	68.9	
R.M.S.D bond length (Å)	0.0089	
R.M.S.D bond angle (°)	1.36	
Residues in Ramachandran plot		
Most favored regions	151 (77.8%)	
Additional allowed regions	35 (18.0 %)	
Generously allowed regions	8 (4.1 %)	
Disallowed regions	0 (0 %)	

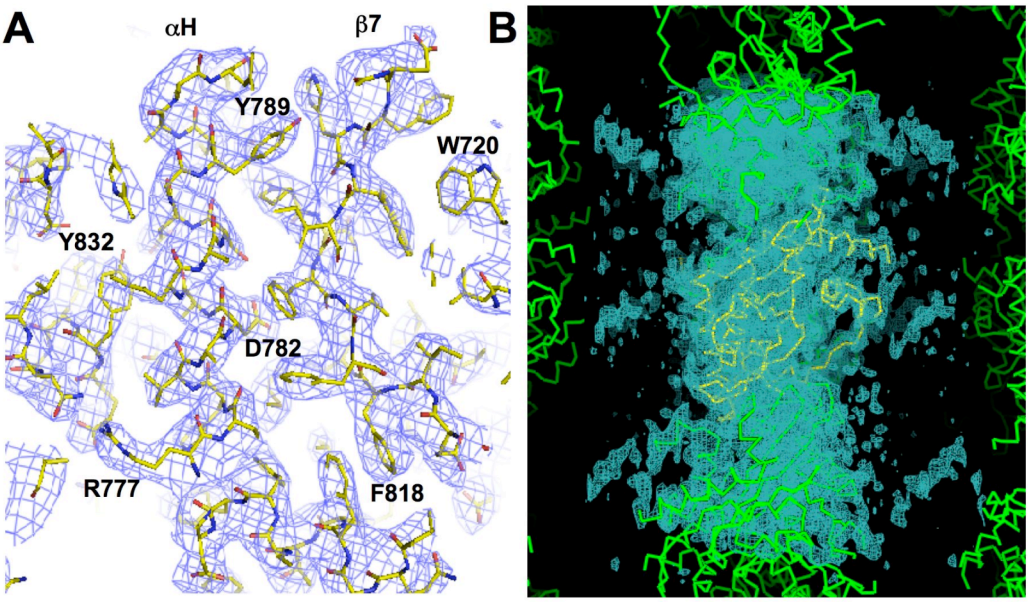

Figure S1. Electron Density for the RecE Crystal Structure.

(A) View of the experimental electron density map calculated at 3.2 Å with SAD phases after solvent flattening (72 % solvent). The map is contoured at 1 σ and superimposed on the final refined model of RecE.

(B) Packing of tetramers in the RecE crystal structure. The side view of a tetramer oriented as in Figure 5b can be seen in the center of the figure, with its front, lower subunit highlighted in yellow. The RecE tetramers pack in layers in the a-b plane of the crystal, with less extensive contacts along the c axis, which is oriented horizontally in the figure. The $2F_o$-F_c electron density map, calculated at 2.8 Å with σA-weighted model phases, is contoured at 1 σ in blue. Notice the bridging density between the layers, which corresponds to the 34-residue segment between helices D and E that is not included in the model. Although the density for this segment is not of sufficient clarity to trace the chain, it clearly shows that this segment extends out about 20 Å from the tetramer, in a position to interact with the incoming dsDNA substrate.

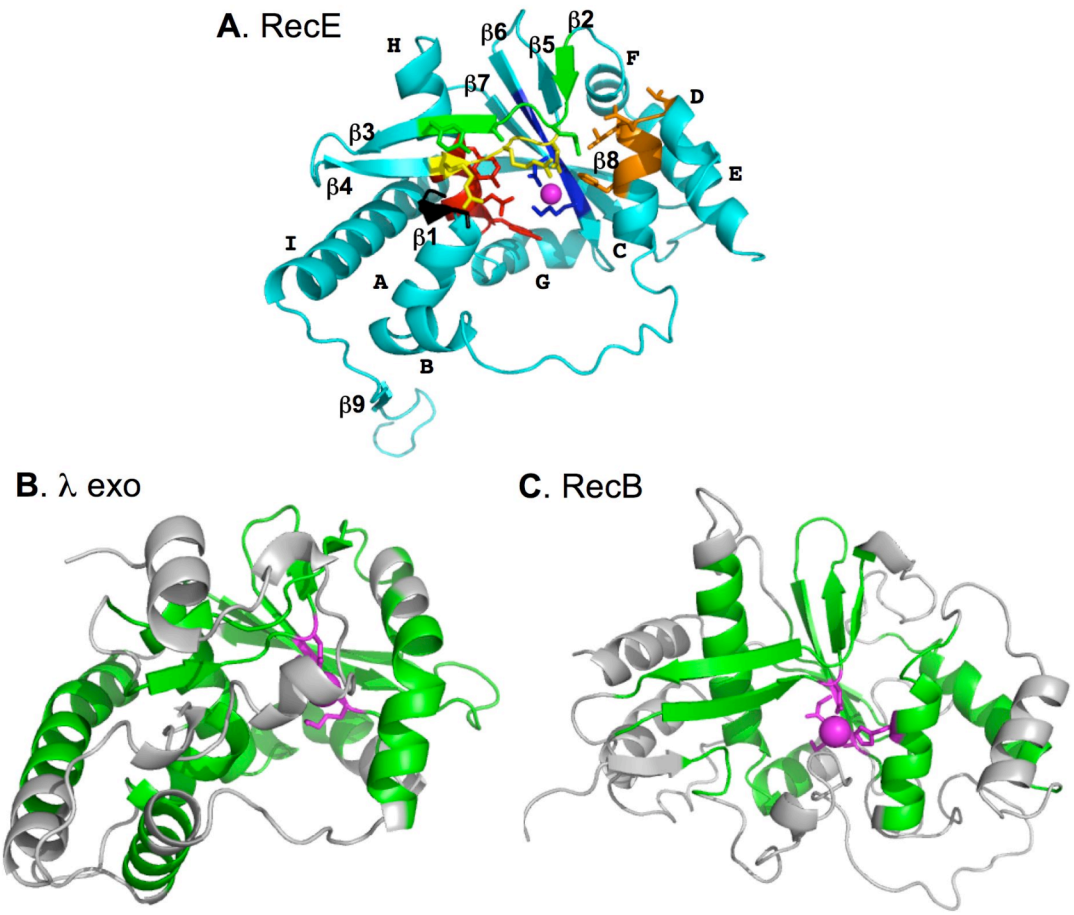

Figure S2. Structural alignment of RecE, λ exonuclease, and RecB.

(A) The RecE monomer, colored as in Figure 3.

(B) The λ exonuclease monomer, viewed in the same orientation as RecE, after structural superposition. Regions of λ exonuclease that align to within 4 Å of equivalent Cα atoms of RecE are shaded in green. Active site residues are shown in magenta.

(C) The RecB monomer, viewed in the same orientation as RecE, after structural superposition. Coloring is the same as for λ exonuclease in panel B.

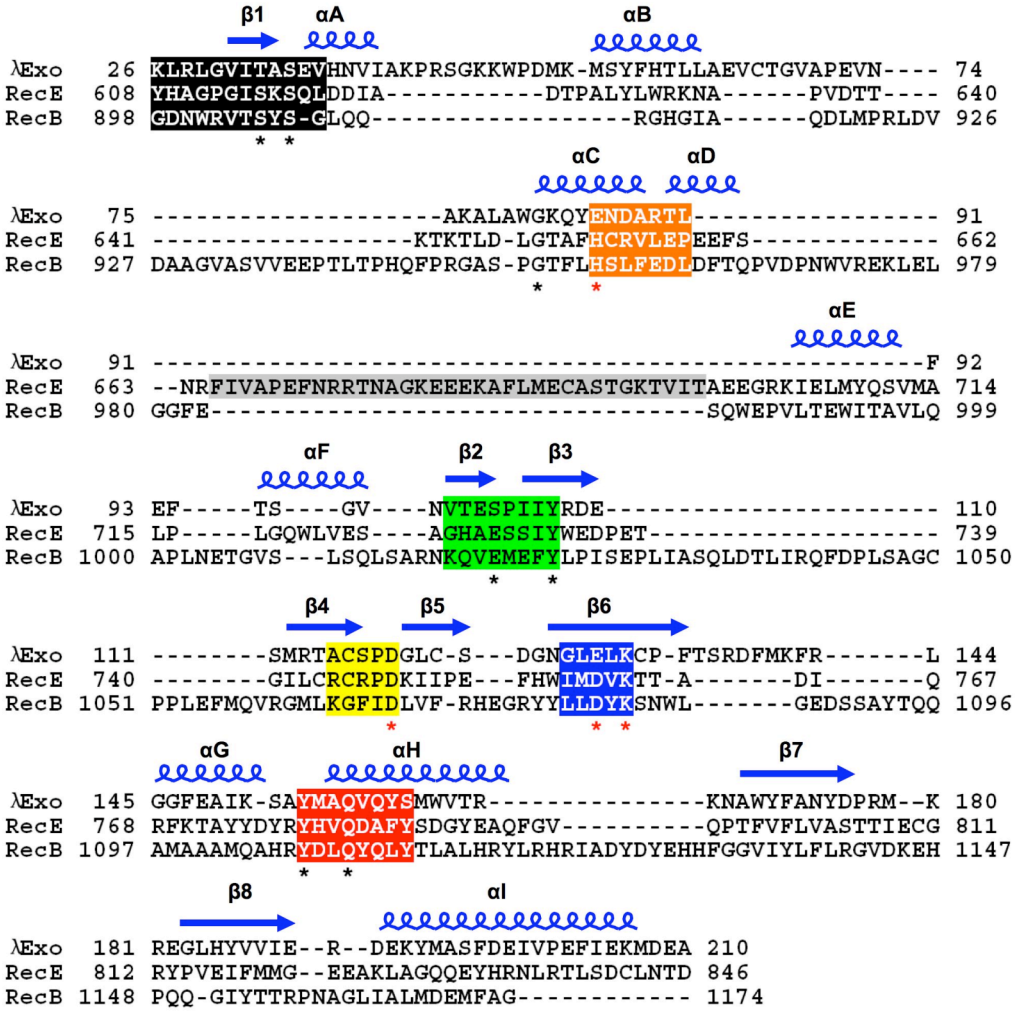

Figure S3. Structure-based sequence alignment of RecE, RecB and λ exonuclease.

The structures of λ exonuclease and RecB were superimposed with the structure of RecE and the resulting sequence alignments are shown above and below the sequence of RecE, respectively. The conserved sequence motifs of RecE are colored onto the sequences, as in Figure 3 (Motif I, green, Motif II, yellow; Motif III, blue; Motif IV, red; Motif V, orange; Motif VI, black). The positions of secondary structures in RecE are indicated above the sequence alignment. Residues that are conserved in the three proteins are indicated below the sequence. Residues that coordinate the Mg^{2+} ion are shown with these symbols in red. The 34-residue insertion in RecE that forms an extended loop that is poorly resolved in the structure is shaded in gray.