RecQ DNA helicases are critical components of DNA replication, recombination, and repair machinery in all eukaryotes and bacteria. Eukaryotic RecQ helicases are known to associate with numerous genome maintenance proteins that modulate their cellular functions, but there is little information regarding protein complexes involving the prototypical bacterial RecQ proteins. Here we use an affinity purification scheme to identify three heterologous proteins that associate with Escherichia coli RecQ: SSB (single-stranded DNA-binding protein), exonuclease I, and RecJ exonuclease. The RecQ-SSB interaction is direct and is mediated by the RecQ winged helix subdomain and the C terminus of SSB. Interaction with SSB has important functional consequences for RecQ. SSB stimulates RecQ-mediated DNA unwinding, whereas deletion of the C-terminal RecQ-binding site from SSB produces a variant that blocks RecQ DNA binding and unwinding activities, suggesting that RecQ recognizes both the SSB C terminus and DNA in SSB-DNA nucleoprotein complexes. These findings, together with the noted interactions between human RecQ proteins and Replication Protein A, identify SSB as a broadly conserved RecQ-binding protein. These results also provide a simple model that explains RecQ integration into genome maintenance processes in *E. coli* through its association with SSB.

RecQ DNA helicases play central roles in cellular genome maintenance processes in a wide range of organisms (1–4). Bacteria and unicellular eukaryotes typically encode a single *recQ* gene, whereas multicellular eukaryotes encode several functionally distinct *recQ* genes. For example, humans have five *recQ* genes, of which individual mutation of three (*BLM, WRN, or* RecQ4) can cause profound genome instability and disease (Bloom, Werner, or Rothmund-Thomson syndromes, respectively) (5–7). RecQ proteins function in a number of diverse cellular contexts, including replication fork maintenance, DNA damage checkpoint signaling, telomere stability, and recombination regulation (1–4); as such, RecQ helicases link together several DNA metabolic activities, and the mechanisms that integrate them into these pathways are important features of cellular genome maintenance networks.

RecQ was first discovered in *Escherichia coli* as a member of the RecF pathway (8, 9). The RecF pathway contributes to the repair of gapped and ultraviolet light-damaged DNA structures, and, in *recBC* strains that are deficient for recombinational repair of double-strand DNA breaks (DSBs), can be activated to initiate repair of DSBs (10–21). In these situations, RecQ is believed to unwind aberrant DNA structures in conjunction with RecA (a 5′–3′ ssDNA3′ exonuclease), whereas other RecF pathway proteins (RecF, RecO, and RecR) facilitate RecA loading onto the ssDNA product of the RecF/RecA reaction. Activation of the RecF pathway in *E. coli* for DSB repair requires mutation of the *sbcB* gene (encoding Exonuclease I (Exol), a 3′–5′ ssDNA nuclease) (10), which is thought to be important for preserving 3′ ssDNA produced by the combined activities of RecQ and RecA at DSBs (11, 12). In addition to its roles in the RecF pathway, *E. coli* RecQ cellular activities include the suppression of illegitimate recombination (22) and SOS DNA damage signaling in response to replication fork stalling (23). Several RecQ-mediated reactions have been reconstituted *in vitro*, including ATP-dependent DNA unwinding (24), recombination initiation (25), and plasmid catenation/supercircling (26, 27). These activities either require or are stimulated by other proteins: RecQ DNA unwinding is stimulated by SSB (28, 29), RecQ-mediated recombination initiation requires SSB and RecA (25), and plasmid catenation/supercircling requires topoisomerase III and is strongly stimulated by SSB (26, 27). Although *E. coli* RecQ is involved in several activities that must be coordinated with other proteins, the molecular basis underlying this coordination is presently unclear.

Eukaryotic RecQ proteins form extensive protein interaction networks that help regulate their biochemical activities (1–3). For example, human WRN protein has been found to interact with over a dozen different DNA replication, recombination, and repair proteins (3). Some of these interactions are conserved with other RecQ proteins (*e.g.* replication protein A (RPA), the eukaryotic SSB, binds human WRN (30–32), BLM (31, 33), and RecQ1 (34) and is likely to bind to RecQ5β (35)).

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3. The abbreviations used are: ITC, isothermal titration calorimetry; ss, single-strand; Exol, exonuclease I; DSB, double-strand DNA break; RPA, replication protein A; TAP, tandem affinity purification; WH, winged helix; HRDC, helicase and RNase D C-terminal; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; wt, wild type; OB, oligonucleotide/oligosaccharide-binding.
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whereas others appear to be specific for individual RecQ paralogs. Most eukaryotic RecQ proteins are significantly larger than the prototypical *E. coli* RecQ, with extensive sequences N- or C-terminal to the conserved domains that define the RecQ family. Mapping studies have indicated that heterologous proteins can bind to these N- and C-terminal extensions and/or to the RecQ core region (1, 3). For example, RPA binds WRN through both a high affinity site located in the N-terminal extension of WRN and a lower affinity site in its winged helix (WH) subdomain; the structure of the latter WH domain is conserved in *E. coli* RecQ (31, 36). Despite the relative simplicity and well defined biochemical activities of bacterial RecQ helicases, much less is known about bacterial RecQ protein interactions than for RecQ proteins from eukaryotic systems.

To identify RecQ binding partners in *E. coli*, we used a tandem affinity purification (TAP; Ref. 37) approach to isolate protein complexes that contained a recombinantly expressed TAP-tagged RecQ (TAP-RecQ). Three proteins co-purified with TAP-RecQ: SSB, RecJ, and ExoI. We show that purified RecQ and SSB interact directly with one another to form a stoichiometric complex of modest stability. Previous studies have shown that SSB or SSB-DNA complexes can interact with RecJ (38, 39) or RecJ (40), respectively, consistent with the RecQ-ExoI and RecQ-RecJ interactions occurring by their simultaneous binding to a common SSB homotetramer. Consistent with its role as a binding site for other proteins, we find that the highly conserved C-terminus of SSB functions as its RecQ-binding site. Additionally, the RecQ WH subdomain is necessary and sufficient for interaction with SSB, indicating that *E. coli* RecQ uses a surprisingly similar mode of interaction with SSB to that used in the human WRN-RPA complex (31). *E. coli* SSB stimulates RecQ DNA unwinding reactions, whereas eukaryotic and bacteriophage SSB proteins (*Saccharomyces cerevisiae* RPA and T4 gp32) block RecQ DNA unwinding. Moreover, deletion of the RecQ-binding site from *E. coli* SSB produces a variant that strongly inhibits RecQ-mediated DNA binding and unwinding. These results establish SSB as an important feature that helps define RecQ substrates. Identification of the RecQ-SSB interaction furthermore helps explain coordination of RecQ activity with other genome maintenance enzymes through SSB-mediated complexes.

**EXPERIMENTAL PROCEDURES**

**Synthetic DNA Substrates**

Oligonucleotides o30 (5’-CTAATGACGCTTCTAGAAGCGGACGC-3’) and o100 (5’-GACGCTCGTTCGGCAGCTCTAATCAGCAAC-3’) were synthesized and purified by Integrated DNA Technologies. 3’ overhang DNA substrate was made as follows: o30 was phosphorylated by T4 polynucleotide kinase with [γ-32P]ATP, annealed to o100, resolved by 12% PAGE, and isolated by electroelution. The DNA substrate was dialyzed against 20 mM Tris, pH 8.0, 50 mM NaCl.

**Synthetic Peptides**

Peptides wt (Trp-Met-Asp-Phe-Asp-Asp-Ile-Pro-Phe), ssb113 (Trp-Met-Asp-Phe-Asp-Asp-Ile-Ser-Phe), and mixed (Trp-Asp-Phe-Met-Asp-Asp-Pro-Phe-Ile-Asp) were synthesized and purified by the University of Wisconsin Biotechnology Center. The Trp residue at the N terminus of each peptide was added for quantitation.

**Cloning of E. coli RecQ Constructs**

pTAP-RecQ encodes the *E. coli* RecQ protein with a C-terminal TAP tag. pTAP-RecQ was generated by subcloning two PCR-generated DNA fragments from *E. coli* K12 genomic DNA into pBS1539 (37). The first fragment included the 200 base pairs upstream of the start codon and the entire *E. coli* recQ open reading frame, which was subcloned for in-frame expression of RecQ with the TAP tag encoded in pBS1539. The second fragment included the 200 base pairs that follow the *E. coli* recQ stop codon, which was subcloned downstream of the stop codon of the TAP cassette. The pTAP-RecQ promoter, open reading frame, and terminator were sequenced to confirm the fidelity of the plasmid.

Genomic sequence encoding *E. coli* K12 RecQ residues 1–407 (RecQAC202, which contains the RecQ catalytic core domain but lacks the WH subdomain) or 408–523 (RecQ WH subdomain) were amplified by PCR and subcloned into pET28b, creating pET28-RecQAC202 or pET28-RecQ-WH, respectively. These constructs include sequences coding for an N-terminal hexahistidine purification tag fused to each RecQ fragment. The fidelity of the coding regions was confirmed by DNA sequencing. Similar overexpression vectors encoding full-length *E. coli* RecQ (residues 1–609), its catalytic core (residues 1–523), and HRDC domain (residues 524–609) have been described previously (41, 42).

**Purification of E. coli RecQ and SSB Proteins**

*Tandem Affinity Purification—* *E. coli* K12 strain MG1655 (American Type Culture Collection) transformed with pTAP-RecQ was grown at 37 °C in Luria-Bertani medium (43) supplemented with 50 μg/ml ampicillin. An 8-liter culture was grown to an A600nm of ~0.8, harvested by centrifugation, suspended in 40 ml of Nonidet P-40 buffer (6 mM dibasic sodium phosphate, 4 mM monobasic sodium phosphate, 150 mM NaCl, 2 mM EDTA, 50 mM sodium fluoride, 4 mg/liter leupeptin, 100 μM sodium vanadate, 19.5 mg/liter benzamidine, 8.7 mg/liter phenylmethylsulfonyl fluoride, 1% Nonidet P-40 substitute) with an EDTA-free protease inhibitor tablet (Roche Applied Science), and lysed by French press. Soluble lysate was incubated for 1 h at 4 °C with IgG-Sepharose beads (pre-equilibrated in 10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% Nonidet P-40); the beads were then washed with 3 volumes of equilibration buffer. TEV cleavage buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% Nonidet P-40) with 15 μl of 15 μM TEV protease was incubated with the lysate/bead mixture with shaking for 1.5 h at 16 °C. The eluent was incubated with 300 μl of calmodulin affinity resin (Stratagene) and 3 μl of 1 M calcium chloride in calmodulin binding buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% Nonidet P-40, 10 mM magnesium acetate, 1 mM imidazole, 10 mM β-mercaptoethanol) for 1 h with shaking. Resin was washed with calmodulin binding buffer, and TAP-RecQ was eluted using EGTA elution buffer (10 mM Tris-HCl, pH 8.0, 150 mM...
NaCl, 0.02% Nonidet P-40, 1 mM magnesium acetate, 10 mM imidazole, 10 mM β-mercaptoethanol, 200 mM EGTA). Eluted protein was then precipitated with trichloroacetic acid (25% w/v) on ice for 30 min, pelleted by centrifugation, washed twice with ice-cold acetone, and suspended in 200-μl gel buffer components. After SDS-PAGE, individual bands or interband regions were excised, digested with trypsin, and subjected to MALDI-TOF mass spectrometry for identification of peptides (University of Wisconsin Mass Spectrometry Facility).

Recombinant RecQ Fragment Purification—Purification schemes for full-length E. coli RecQ, the RecQ catalytic core domain, and RecQ HRDC domain have been described previously (41, 42). BL21 DE3 cells transformed with pLysS (Novagen) and either pET28-RecQC202 or pET28-RecQ-WH were grown at 37 °C in Luria-Bertani medium supplemented with 50 μg/ml kanamycin and 50 μg/ml chloramphenicol. The cells at an A_{600 nm} of ~0.7 were induced to overexpress protein with 1 mM isopropyl β-D-thiogalactopyranoside for an additional 2.5 h of growth and were harvested by centrifugation. The cells were suspended in lysis buffer (20 mM Tris-HCl, pH 8.0, 20 mM imidazole, 0.3 M NaCl, 1 mM β-mercaptoethanol, 10% (v/v) glycerol, 100 mM glucose) and lysed by sonication on ice. Soluble lysate was loaded onto nickel-nitrilotriacetic acid resin (Qiagen) and washed with lysis buffer until protein was undetectable in the eluent. Protein was eluted by the addition of 20 mM Tris-HCl, pH 8.0, 0.1 M imidazole, 0.3 M NaCl, 1 mM β-mercaptoethanol, 10% (v/v) glycerol. The eluent was dialyzed against lysis buffer with 1 mM EDTA. The protein solution was diluted with 20 mM Tris-HCl, pH 8.0, 10% (v/v) glycerol until the concentration of NaCl was ~50 mM, further purified by ion exchange on a Q-Fastflow column (GE Healthcare), concentrated, and passed over a Sephacryl S-300 column. Fractions containing pure protein were concentrated to ~1–5 g/liter and dialyzed against 20 mM Tris-HCl, pH 8.0, 0.3 M NaCl, 1 mM β-mercaptoethanol, 10% (v/v) glycerol. All of the protein concentrations were determined by measuring their A_{280} in 6.0 M guanidine HCl (44).

SSB, SSBΔC8, RPA, T4 gp32—Plasmids for overexpressing E. coli SSB and SSBΔC8 were gifts from Michael Cox and were purified as in Lohman et al. (45). S. cerevisiae RPA was a gift from Michael Cox. T4 gp32 was purchased from New England Biolabs.

Ammonium Sulfate Co-precipitation

Co-precipitation experiments were performed essentially as described earlier (39). Briefly, E. coli RecQ or a RecQ variant (20 μM monomers) was incubated with E. coli SSB or SSBΔC8 (20 μM tetramers) in 20-μl reactions in co-precipitation buffer (10 mM Tris-HCl, pH 7.2, 150 mM NaCl, 10% (v/v) glycerol) on ice for 15 min. The reactions were adjusted to 150 g/liter ammonium sulfate (final reaction volume, 30 μl), incubated on ice for an additional 15 min, and then centrifuged for 1 min at 13,000 RPM. Supernatant was removed, and pellets were washed three times with 50 μl of co-precipitation buffer plus 150 g/liter ammonium sulfate. Pellet fractions were suspended in 45 μl of Laemmli loading buffer (43). 15 μl of Laemmli loading buffer were added to supernatant fractions. 9 μl of each fraction were subjected to SDS-PAGE on 4–15% or 8–16% polyacrylamide gradient gels.

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Isothermal Titration Calorimetry

Experiments were carried out using a VP-ITC microcalorimeter with VPViewer software employed for instrument control and data collection (MicroCal). RecQ (10 μM), RecQ-WH (20 μM), and SSB (200 μM) were dialyzed extensively against 20 mM Tris, pH 8.0, 150 mM NaCl, 10% glycerol, 1 mM EDTA, 1 mM β-mercaptoethanol prior to binding experiments. Lyophilized peptides were resuspended in the dialysis buffer used above. RecQ or RecQ-WH was thermostatted at 25 °C in a stirred (300 rpm) sample cell (1.4 ml), and the titrations were carried out as follows: 1) for RecQ with SSB or peptide titrants, 1 injection (1 μl for 2 s), 8 injections (4 μl for 8 s each), and then 21 injections (8 μl for 16 s each) were measured with 130-s equilibration between injections; 2) for RecQ-WH with wt peptide titrant, 1 injection (1 μl for 2 s), 8 injections (4 μl for 8 s each), and then 21 injections (8 μl for 16 s each) were measured with 130-s equilibration between injections; and 3) for RecQ-WH with ssb113 or mixed peptide titrants, 1 injection (1 μl for 2 s), 18 injections (4 μl for 8 s each), and then 21 injections (8 μl for 16 s each) were measured with 130-s equilibration between injections. The calorimetric data were processed using the MicroCal ORIGIN software package, and binding isotherms were fit by a nonlinear iterative least squares algorithm to a model containing a single set of independent sites. Binding parameters were determined from the fits.

Helicase Assays

Twenty nM SSB or SSBΔC8 (tetramers), RPA (trimer), or gp32 was incubated with ~1 nM 3’ overhang substrate at room temperature in 20 mM Tris, pH 8.0, 50 mM NaCl, 1 mM β-mercaptoethanol, 1 mM MgCl2, 1 mM ATP, 0.1 g/liter bovine serum albumin, and 5.5% (v/v) glycerol. RecQ was added to initiate the unwinding reaction at indicated concentrations and incubated for 20 min at 25 °C. The reactions were terminated by adding 9.6 μg of proteinase K, 0.25% SDS, 30 mM EDTA, 0.75 ng of unlabeled o30 and incubating at 37 °C for 30 min. The products were resolved by 12% native PAGE, dried, and imaged using a Molecular Dynamics Storm 820 phosphorimager.

Electrophoretic Mobility Shift Assays

Two nM SSB or SSBΔC8 (tetramers) or the appropriate buffer control was incubated with ~1 nM 3’ overhang substrate at room temperature in 20 mM Tris, pH 8.0, 50 mM NaCl, 1 mM β-mercaptoethanol, 1 mM MgCl2, 0.1 g/liter bovine serum albumin, and 5.5% (v/v) glycerol with 0–500 nM of E. coli RecQ. The products were resolved by 10% native PAGE, dried, and imaged using a Molecular Dynamics Storm 820 phosphorimager.

RESULTS

TAP-RecQ Co-purifies with SSB, RecJ, and ExoI—TAP allows efficient isolation of protein complexes through two purification steps that take advantage of protein A and calmodulin peptide binding moieties attached to a target protein. We used the TAP process to identify candidate RecQ binding partners in E. coli K12 strain MG1655. TAP-RecQ was expressed from a multicopy plasmid (pTAP-RecQ) into which the recQ promoter and open reading frame (46) were fused in frame with a sequence encoding a C-terminal TAP tag. The recQ gene ter-
Interaction between E. coli RecQ and SSB

FIGURE 1. TAP purification of RecQ-containing protein complexes from E. coli. A, SDS-PAGE of TAP-RecQ and co-purifying proteins. Lane 1 is a protein molecular mass marker with masses of each band indicated to the left in kDa. Lane 2 is the TAP-RecQ purification product; each discrete band is labeled to the right (Bands 1–8). B, schematic diagram of peptide fragments identified by mass spectrometry from bands or interband regions in A. Each fragment is shown as a pink bar under its corresponding position in E. coli RecQ, SSB, RecJ, or ExoI. Interband labels in RecJ and ExoI refer to the region between bands 3 and 4 in A. Schematic diagrams of proteins display colored boxes for folded domains of known structure (RecQ (42, 63), SSB (54), RecJ inferred from Thermus thermophilus RecJ structure (71)), and lines symbolize disordered regions.

minator (200 base pairs) was subcloned downstream of the sequence encoding the TAP tag to mimic recQ transcriptional regulatory elements on the plasmid.

After transformation of pTAP-RecQ into MG1655, growth of the strain in rich medium and purification as described in under “Experimental Procedures,” the TAP eluent was analyzed by SDS-PAGE. Coomassie staining revealed the presence of eight discrete bands with regions of light staining interspersed between several of the bands (Fig. 1A, lane 2). Gel mobility of the bands compared with a molecular mass standard indicated that their masses were between ~20 and ~70 kDa, with the slowest migrating band corresponding to the predicted mass of 73 kDa for TAP-RecQ.

MALDI-TOF mass spectrometry of trypsin-derived peptides was used to identify the protein(s) in each band. This analysis showed that the seven slowest migrating bands were RecQ or RecQ fragments that were presumably generated through proteolysis of the full-length protein (Fig. 1B). The faster migrating RecQ bands were generally enriched for more C-terminal elements of the RecQ protein (consistent with the C-terminal attachment for the TAP tag), and all appeared to have the central RecQ C-terminal domain intact. Some of the RecQ bands in Fig. 1A appear to be mixtures of different breakdown products rather than a single species. For example, band 6 has an estimated mass of ~36 kDa but contains peptides that map across most of RecQ; it is likely that this band is comprised of multiple RecQ fragments with similar mobilities. Observations described below support the idea that some RecQ fragments that lack the TAP tag could co-purify with TAP-RecQ by binding a common heterologous protein.

In addition to RecQ, heterologous proteins were also found in the TAP-RecQ eluent. The fastest migrating band (Fig. 1A, Band 8) was identified as SSB. Mass spectrometric analysis revealed several peptides from SSB in the band (Fig. 1B), and its gel mobility was consistent with that of full-length SSB (47). Diffusely stained interband regions were also analyzed to determine whether additional proteins were present but in lower abundance than SSB and RecQ. Mass spectrometric analysis of the region between bands 3 and 4 revealed tryptic peptides from the RecJ exonuclease (4 peptides) and ExoI (2 peptides) (Fig. 1B). Consistent with their very faint Coomassie staining, the number of peptides identified for RecJ and ExoI were less than for RecQ or SSB. However, the molecular masses of RecJ (63 kDa) and ExoI (54 kDa) correspond well to their migration between bands 3 and 4. These results are consistent with recent findings from a large scale TAP tag experiment in E. coli that indicated that RecQ associates with SSB, RecJ, and ExoI, among other proteins (48), but differ significantly from a genome-wide His tag-based screen for protein complexes in E. coli that failed to identify these interactions (49). This could be due to the differences in the affinity purification tags used in the two approaches, which led us to test for in vitro protein-protein interaction between purified RecQ and SSB.

E. coli RecQ and SSB Form a Complex in Vitro—We hypothesized that RecQ binds SSB directly. To test this possibility, RecQ and SSB were purified and assayed for direct interaction using two different methods. The first experiment was a qualitative co-precipitation assay that takes advantage of the insolubility of SSB in low concentrations of ammonium sulfate (39). E. coli SSB is efficiently precipitated in 150 g/liter ammonium sulfate, whereas most other proteins, including E. coli RecQ, remain soluble (Fig. 2A, compare lanes 5 and 9). To test whether RecQ and SSB directly interact with one another, the two purified proteins were mixed, incubated in 150 g/liter ammonium sulfate, and centrifuged. If RecQ physically associates with SSB, it would be predicted to be in the pellet fraction of the sample when mixed with SSB, but in the supernatant in the absence of SSB. Indeed, a significant amount of RecQ was found in the pellet of the ammonium sulfate precipitated SSB/RecQ mixture (Fig. 2A, lane 13). Some RecQ remains in the soluble fraction, which could be indicative of the interaction occurring with a dissociation constant (K_d) near the concentrations of RecQ and SSB in the reaction (see below). To determine whether RecQ interaction with SSB was facilitated by DNA, ethidium bromide or DNase was added to binding assays in control reactions (Fig. 2B). Interaction between the two proteins appeared unaltered by either treatment, demonstrating that contaminating DNA was not the cause of their co-precip-
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To quantify and plot the interaction between RecQ and SSB, we used ITC to measure the stoichiometry and stability of their association. In this experiment, SSB was titrated into a solution of RecQ, and the heat evolved by their interaction was measured (Fig. 2C and Table 1). The data fit well to a simple model in which one monomer of SSB binds one monomer of RecQ with a $K_D$ of $\sim$6 $\mu$M. Because SSB is a stable tetramer (50–52), these results indicate that four RecQ proteins can associate with each SSB tetramer. These data offer a quantitative confirmation of the physical interaction between RecQ and SSB.

The Conserved C-terminal Tail of SSB Forms Its RecQ-binding Site—Previous proteolytic (53) and crystallographic (54–57) studies have divided E. coli SSB into two regions: an N-terminal oligonucleotide/oligosaccharide-binding (OB) domain and a C-terminal tail of $\sim$60 residues that ends in a highly conserved sequence (Met-Asp-Phe-Asp-Asp-Ile-Pro-Phe) (Fig. 3A). The OB domain is a well ordered module and serves as the ssDNA-binding site on SSB. In contrast, the E. coli SSB C-terminal domain is a highly dynamic element that is required for interactions with heterologous proteins, including ExoI (39), uracil DNA glycosylase (58), the $\chi$ subunit of DNA polymerase III (59), and PriA DNA helicase (60). Interestingly, the modest RecQ-SSB $K_D$ of $\sim$6 $\mu$M was similar to the 2 $\mu$M $K_D$ measured for PriA helicase binding the SSB C-terminal tail (60). Given this similarity and the proven importance of the C-terminal regions of SSB as a protein interaction element, we hypothesized that the SSB C terminus plays a role in the physical interaction between RecQ and SSB.

To test the role of the SSB C-terminal tail in the RecQ-SSB interaction, we assayed RecQ binding to a variant of E. coli SSB that lacked its C-terminal eight residues, SSB-C8. As was the case with full-length SSB, SSB-C8 precipitated readily in 150 $\mu$M ammonium sulfate (Fig. 3B, lane 9). However, in contrast to the result with full-length SSB, co-precipitation experiments with RecQ and SSB-C8 resulted in greatly diminished levels of RecQ in the insoluble pellet fraction (Fig. 3B, lane 13). Therefore, the C terminus of SSB forms a critical binding determinant for RecQ.

To more directly test the importance of the SSB C-terminal tail for RecQ binding, ITC experiments were carried out using a peptide, “wt,” which comprises the C terminus of E. coli SSB in RecQ binding experiments (Fig. 3C). Remarkably, the stability and stoichiometry of the wt peptide-RecQ interaction are indistinguishable from those of the SSB-RecQ interaction (Table 1).
Two control peptides, “ssb113” and “mixed,” were used to
determine whether the wt peptide-RecQ interaction was spe-
cific. The ssb113 peptide substitutes a Ser residue for a Pro at
the penultimate residue of the SSB tail sequence, which
mimics a previously characterized ssb mutation that leads to
temperature-dependent growth deficiencies (61, 62) and
alters physical interaction of SSB with DNA polymerase III
subunit (59), ExoI (39), and PriA (60). Unlike the wt peptide, the
ssb113 peptide binds RecQ much more weakly, indicating a
greatly diminished affinity for this sequence (Fig. 3C).

The WH Subdomain of E. coli RecQ Is Necessary and Suf-
cient for Interaction with SSB—E. coli RecQ can be divided into
two structural domains: the N-terminal catalytic core domain
and the HRDC domain (Fig. 1B) (41). The RecQ catalytic core
domain is responsible for ATP-dependent DNA unwinding in
the enzyme, whereas the HRDC domain forms an auxiliary
DNA-binding element (42, 63). To map regions of RecQ that
are important for binding SSB, we first tested whether its cata-
lytic core or HRDC domain could interact with SSB as isolated
recombinant fragments. When incubated with SSB, the RecQ
catalytic core was efficiently precipitated by the addition of 150
g/liter ammonium sulfate (Fig. 4A, lane 13), whereas the HRDC domain was not (Fig. 4B, lane 3). Neither the RecQ catalytic core nor the HRDC was precipitated in control experiments in which SSB was omitted (Fig. 4A, lane 5, and data not shown), indicating that ammonium sulfate precipitation of the catalytic core domain required SSB. Thus, the RecQ catalytic core domain is necessary and sufficient for interaction with SSB.

RecQ catalytic core domain truncation variants were tested for interaction with SSB to better define the SSB interaction site on the RecQ catalytic core domain. The RecQ catalytic core domain comprises both helicase and RecQ C-terminal subdomains, the latter of which contains both Zn\(^{2+}\)-binding and WH elements (Fig. 1B). The first truncation variant tested, RecQΔC202, encodes the Helicase and Zn\(^{2+}\)-binding subdomains of the catalytic core but removes the WH subdomain. When RecQΔC202 was used in the SSB co-precipitation assay, undetectable levels of the RecQ variant were found in the ammonium sulfate pellet with SSB (Fig. 4C, lane 3). This result indicates that the WH subdomain is necessary for RecQ interaction with SSB. To determine whether the RecQ WH subdomain is sufficient for interaction with SSB, a recombinant fragment containing only the WH element (RecQ-WH, residues 408–523) was tested. When incubated with SSB, RecQ-WH was co-precipitated by ammonium sulfate (Fig. 4D) consistent with the RecQ-WH subdomain being sufficient for association with SSB (Fig. 4E). However, the co-precipitation was not as robust as was observed for full-length RecQ, with significant amounts of the RecQ-WH subdomain in the soluble fraction and a loss of protein through washes that resulted in a less intense band in the ammonium sulfate precipitated lane. These differences could reflect a somewhat weakened association between the isolated RecQ-WH subdomain and SSB.

ITC binding experiments were used to more quantitatively assess the RecQ-WH subdomain interaction with SSB. Initial ITC experiments where full-length SSB was titrated into a concentrated solution of the RecQ-WH subdomain led to protein precipitation (data not shown), which made interpretation of the data impossible. We therefore examined the binding properties of the SSB C-terminal peptide to the RecQ-WH subdomain. Unlike the case with full-length SSB, titration of the SSB peptides did not lead to precipitation. As was observed with full-length RecQ, the wt SSB peptide binds robustly to the RecQ-WH subdomain, whereas the ssb113 and mixed peptides do not (Fig. 4F). The stoichiometry of the wt SSB-peptide/RecQ-WH subdomain interaction was ~1:1, whereas the stability of the interaction (K\(_d\) = ~17 μM) was diminished ~3-fold relative to full-length RecQ binding either SSB or the wt SSB peptide (Table 1). Taken together, these results indicate that the WH subdomain of RecQ is necessary and sufficient for interaction with SSB. Additional elements of the RecQ structure could augment the interaction by providing additional interaction surfaces for the SSB C terminus or, indirectly, by stabilizing or optimally positioning the RecQ WH domain for SSB binding.

**SSB Physical Interaction with RecQ Stimulates Its DNA Helicase Activity**—With the co-purification and identification of a physical interaction between SSB and RecQ, we next determined the consequences of this interaction on RecQ DNA unwinding activity. Previous studies have shown that SSB stimulates RecQ DNA unwinding (28, 29). However, whether this stimulation requires physical association between RecQ and SSB or relies exclusively on the ssDNA binding properties of SSB has not been defined. To test whether direct physical association is important for SSB stimulation of RecQ DNA unwinding, a RecQ DNA substrate to which SSB can also bind was created. This substrate contains a 30-base pair duplex with a 70-base 3’ ss extension. E. coli RecQ preferentially binds to DNA structures with 3’ ssDNA extensions (23, 25), and the substrate ssDNA is sufficiently long to allow SSB binding, making it useful for determining the contributions of SSB to RecQ DNA unwinding.

In the assay, SSB (or buffer in control experiments) was incubated with the DNA substrate prior to the addition of RecQ. In the absence of SSB, RecQ was able to unwind the DNA substrate in an enzyme concentration-dependent manner (Fig. 5A, lanes 1–5). Approximately 0.5 nM RecQ was required to catalyze unwinding of 50% of the ~1 nM substrate (Fig. 5, A and C). When SSB was included in the helicase reactions, however, the amount of RecQ required for 50% DNA unwinding was reduced 5-fold to ~0.1 nM (Fig. 5, A, lanes 6–10, and C), consistent with earlier observations of SSB stimulation of RecQ helicase activity (28, 29). To test whether direct physical interaction was required for SSB stimulation of RecQ unwinding, SSBΔC8 was substituted for SSB in the reaction (Fig. 5, A, lanes 11–13, and C). SSBΔC8 not only failed to stimulate RecQ activity but also significantly inhibited unwinding, indicating that the SSB C-terminal tail is critical for stimulation of RecQ DNA unwinding. Experiments that substituted bacteriophage T4 gp32 or S. cerevisiae RPA proteins for SSB inhibited the RecQ activity as well (Fig. 5, B and C), further indicating that E. coli SSB specifically stimulates RecQ helicase activity.

To test whether the effects of SSB and SSBΔC8 on RecQ helicase activity were linked to differential recruitment of RecQ to the SSB/DNA and SSBΔC8/DNA substrates, electrophoretic mobility shift assays were used. In these assays, RecQ was incubated with DNA, SSB/DNA, or SSBΔC8/DNA substrates, and the resulting complexes were separated on a nondenaturing polyacrylamide gel. RecQ was able to bind the SSB/DNA substrate in a concentration-dependent manner, with a mobility shift of ~50% of the substrate apparent with 8 nM RecQ (Fig. 5D, lanes 7–12). In contrast, RecQ binding to the SSBΔC8/DNA substrate in this assay was greatly impaired, with only smearing apparent in lanes with the highest RecQ concentrations (Fig. 5D, lanes 13–18). Thus, SSBΔC8 appears to impede RecQ DNA binding. Interestingly, binding by RecQ to SSB-free DNA was distinct from the SSB-bound DNA substrate, with several different shifted substrate observed at all RecQ concentrations (Fig. 5D, compare lanes 1–6 with lanes 7–12). These bands are consistent with multiple RecQ proteins binding simultaneously to individual substrate molecules, an effect that is only observed in the highest RecQ concentrations tested for SSB/DNA substrates. These data indicate that SSB helps define RecQ DNA binding in a C-terminal tail-dependent manner.
Interaction between E. coli RecQ and SSB

(A) RecQ catalytic core

(B) RecQ HRDC domain

(C) RecQΔC202

(D) RecQ-WH

(E) WH subdomain

(F) wt SSB peptide  ssb113 peptide  mixed peptide
Interaction between *E. coli* RecQ and SSB

**FIGURE 5.** *E. coli* SSB stimulates and SSB△C8 inhibits *E. coli* RecQ DNA unwinding and binding. A, buffer (lanes 1–8), SSB (lanes 9–14), or SSB△C8 (lanes 15–20) was incubated with 3’ overhang DNA substrate in the presence of 1 mM ATP. Unwinding reactions were initiated by the addition of *E. coli* RecQ as indicated above each lane and terminated as described under “Experimental Procedures.” Control lanes with boiled substrate (lane 2) or where no protein was added (lane 1) are indicated. Unwound DNA product was separated from substrate on a 12% nondenaturing gel as indicated. B, T4 gp32 or *S. cerevisiae* RPA were substituted for SSB proteins in RecQ DNA unwinding reactions similar to those described in A. C, unwinding data from SSB-free DNA (RecQ alone) or with prebound SSB, SSB△C8, gp32, or RPA are plotted as the averages of three replicate reactions; error bars reflect one standard deviation. Lines are presented to guide the eye. D, buffer (lanes 1–6), SSB (lanes 7–12), or SSB△C8 (lanes 13–18) was incubated with 3’ overhang DNA substrate and *E. coli* RecQ as indicated above each lane in the absence of ATP. Protein/DNA species were separated on a 10% nondenaturing gel as indicated.

**DISCUSSION**

In this study, we tested whether the prototypical *E. coli* RecQ DNA helicase binds to heterologous proteins in vivo and in vitro as has been observed for its eukaryotic RecQ counterparts. Two of the best studied human RecQ proteins, WRN and BLM, each associate with numerous genome maintenance proteins that are important for targeting and modulating their functions (1, 3). To extend such findings into structurally simpler bacterial RecQ systems, we identified the *E. coli* RecQ binding partners through a rapid affinity purification scheme. SSB was abundant in RecQ-containing complexes, whereas RecJ and Exol were present but at substoichiometric levels. We reasoned that, because SSB was plentiful in the preparations, and Exol and RecJ had previously been shown to bind SSB and SSB/DNA complexes (38–40), respectively, the primary RecQ interaction could be to SSB and that RecQ interactions with RecJ and Exol could be mediated by SSB.

As predicted by this model, purified *E. coli* RecQ and SSB were found to associate in two different assays. The first assay, which was originally developed to analyze ExoI interaction with SSB (39), used co-precipitation to qualitatively assess the RecQ-SSB association. The second assay (ITC) measured the enthalpy of RecQ-SSB binding to determine a quantitative thermodynamic description of the interaction. Both methods showed that RecQ binds SSB directly in vitro, complementing detection of their in vivo interaction by TAP. The ITC results indicated that RecQ association with SSB is stoichiometric and modest in affinity (with a $K_d$ of ~6 µM).

Observation of a direct RecQ-SSB interaction led to a systematic dissection of the structural elements from each protein that support their association. First, the C-terminal tail of SSB facilitates its interaction with RecQ. This places RecQ among a growing list of genome maintenance enzymes (including ExoI (39), uracil DNA glycosylase (58), DNA polymerase III $\Gamma$ subunit (59), and PriA DNA helicase (60)) that associate with SSB by binding the conserved C-terminal tail of SSB. Other known SSB-binding proteins might also interact via the SSB C terminus (e.g. RecJ), but mapping experiments have not yet identified the SSB structural elements required for interaction in all such cases. Additional mapping experiments showed that the WH subdomain of RecQ is necessary and sufficient for association with SSB.

**FIGURE 4.** Dissection of the RecQ structural elements required for association with SSB. A and B, co-precipitation of *E. coli* RecQ structural domains and SSB. The symbols are the same as in Fig. 2A except that RecQ catalytic core (A) or HRDC domains (B) are used in the assay. C, co-precipitation of RecQ△C202 with SSB. The symbols are the same as in Fig. 2A except that RecQ△C202 is used in the assay. D, co-precipitation of the RecQ-WH subdomain with SSB. The symbols are the same as in Fig. 2A except that the RecQ-WH subdomain is used in the assay. E, ribbon diagram of the crystal structure of *E. coli* RecQ catalytic core domain (63). The color scheme is the same as in Fig. 18 with the minimal SSB-interacting subdomain (WH subdomain) indicated. F, calorimetric analysis of RecQ-WH SSB C-terminal peptide interaction. Top panels, heat of interaction evolved by wt SSB (left panels), ssb113 (center panels), or mixed (right panels) peptides into a solution of RecQ-WH as described under “Experimental Procedures.” Bottom panels, binding isotherms corresponding to the data from the top panels. The line in the wt SSB peptide experiment is the calculated curve from the nonlinear least squares fit. The sequence of each peptide used is shown in the inset.
with the C terminus of SSB, which is remarkably similar to the observed functions of the human WRN WH domain (64). Implications of the conservation of RecQ-SSB complexes and of the biological roles of SSB that extend from this work are discussed further below.

Finally, the functional consequence of the RecQ-SSB interaction was tested using a model helicase substrate to which SSB can bind. RecQ unwinds this DNA in the absence of SSB, whereas its helicase activity is stimulated —5-fold by the addition of SSB. In contrast to full-length SSB, an SSB variant that is unable to bind to RecQ (SSBΔC8) as well as heterologous SSBs from bacteriophage T4 and *S. cerevisiae* strongly inhibit RecQ helicase activity, indicating that direct physical association is important for SSB stimulation of RecQ. The concentrations of SSB and RecQ used in these assays were several orders of magnitude below their $K_M$, implying that this constant underestimates the stability of the RecQ-SSB complex in the presence of DNA. This apparent difference may be accounted for by high effective concentrations of RecQ and SSB molecules bound to a common DNA molecule and/or to DNA binding inducing conformation changes in SSB and RecQ that support higher affinity interaction than in their apo forms.

Our DNA binding studies showed that RecQ readily associates with SSB-bound DNA but that RecQ binding to SSBΔC8-bound DNA is greatly diminished. This suggests that the difference in the affinity of RecQ for SSB- and SSBΔC8-bound DNA leads to respective stimulatory and inhibitory effects on RecQ helicase activity. Previous studies have shown that SSB assists RecQ unwinding of plasmid-length double-strand DNA (25) and partial duplex DNA with ssDNA regions of several thousand bases (24). In these systems, SSB ssDNA binding appears to promote RecQ unwinding in two ways: 1) by preventing reannealing of the ssDNA products of the helicase reaction and 2) by precluding formation of RecQ/ssDNA complexes that strongly inhibit RecQ helicase activity. The results presented in this report using a DNA substrate with only 70 bases of ssDNA indicate that physical interaction between RecQ and SSB is an additional key feature that mediates SSB stimulation of RecQ in the absence of extensive stretches of ssDNA.

A simple model that accounts for the stimulatory effect of SSB on RecQ is that *E. coli* RecQ not only recognizes and acts on particular DNA structures as has been noted in earlier studies (23–25, 65), but that SSB also forms an important determinant for defining RecQ nucleoprotein substrates (Fig. 6). If RecQ stimulation by SSB was based entirely on SSB binding of ssDNA, then SSBΔC8 would assist RecQ DNA unwinding similarly to SSB, because C-terminal truncation variants of *E. coli* SSB retain ssDNA binding activity (Ref. 53 and Fig. 5D). However, SSBΔC8 strongly inhibits the helicase activity of RecQ. Moreover, T4 gp32 and *S. cerevisiae* RPA also impeded RecQ DNA unwinding, further demonstrating that ssDNA binding alone is not sufficient to stimulate RecQ DNA unwinding in this assay. Instead, direct contact between SSB and RecQ appears to be required for SSB stimulation of RecQ activity under our conditions. Our binding experiments indicate that SSBΔC8 inhibition could be caused by SSBΔC8 tetramers blocking RecQ DNA binding. If so, full-length SSB would also have this property, but the stimulatory effects of the C-terminal tail more than compensate for such blockage (Fig. 6).

**Conservation of RecQ-SSB Interactions**—Demonstration of the *E. coli* RecQ-SSB interaction illustrates the broad conservation of such assemblies among RecQ proteins. As described earlier, human BLM, WRN, and RecQ1 proteins associate with RPA, the eukaryotic equivalent of SSB (30–34). Indeed, several additional eukaryotic RecQ proteins are stimulated by RPA and/or co-localize with RPA in cells (1), consistent with the RecQ-RPA association being an important feature of eukaryotic RecQ function. The observation of *E. coli* RecQ-SSB interactions extends this conservation to the bacterial world.

Comparison of the structural elements required for RecQ-SSB and WRN-RPA interactions demonstrates a striking parallel in their mechanisms of association. WRN binds RPA through two sites (31). The first is an acidic, direct repeat sequence located in the N-terminal extension of WRN, and the second is the WRN WH subdomain. Our results show that *E. coli* RecQ also utilizes its WH domain to physically associate with SSB and relies upon an acidic stretch of residues to form the interface. However, unlike the WRN-RPA interaction, *E. coli* RecQ does not encode the acidic residues, but instead this element of the interaction is presented by the C terminus of SSB.

Interestingly, in addition to acting as sites for protein interaction, the WH domains of *E. coli* RecQ and WRN also form DNA-binding surfaces on both enzymes (64–66). Thus, it could be that RecQ WH domains coordinate binding to both SSB and DNA (Fig. 6) and that this coordination is important for targeting RecQ proteins to particular DNA substrates in cells and for regulation of their biochemical functions. Such a model is reminiscent of the role of SSB in the hand-off of RNA primers from primase to the DNA polymerase III holoenzyme during bacterial DNA replication. In this mechanism, primase
binds to an RNA primer only when it is simultaneously bound to SSB; the primer/template is handed off to the β processivity clamp through association between SSB and χ, a subunit of the γ clamp loader complex, which competes with primase for binding SSB (67). Similar mechanisms could be commonly used by other SSB-binding proteins, including RecQ, to facilitate replication, recombination, and repair dynamics (68).

Integration of SSB into RecQ-mediated Processes—As a member of the RecF recombination pathway in E. coli, the activity of RecQ is coordinated with several other proteins, including two (RecO and RecJ) that are known to bind SSB and SSB/DNA complexes (40, 69). Given the stimulatory interaction between RecQ and SSB described in this work, it is likely that the RecQ-SSB interaction is important for integrating RecQ activity with other proteins as well. Consistent with this idea, all of the E. coli RecQ known in vitro activities either require or are strongly stimulated by SSB, including DNA unwinding (28, 29), recombination initiation (25), and DNA catenation/supercoiling (26, 27).

Finally, the observation of an interaction between E. coli RecQ and SSB extends the developing view of the multifaceted role of SSB in cellular genome maintenance reactions. The ssDNA binding activity of SSB is critical for protecting exposed ssDNA, but it is becoming clear that SSB is important for assembling complexes of heterologous proteins as well. Because SSB persists at damaged structures such as stalled replication forks (70), it is perhaps not surprising that SSB has evolved to act as a scaffold onto which genome maintenance complexes can assemble; this allows for localization of important repair enzymes to DNA structures where their activities are needed and can be coordinated. In the case of RecQ, this localization is consistent with its noted role in unwinding nascent lagging strand DNA at stalled replication forks where both SSB and RecQ DNA substrates are found (15, 18, 23). Given the number of other genome maintenance enzymes that bind SSB, it will be important to consider how such SSB-mediated enzyme centers are regulated in cells.

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