Single-stranded (ss)DNA binding (SSB) proteins play essential roles in genome maintenance, binding transiently, but with high affinity, to ssDNA intermediates to protect them during DNA replication, recombination, and repair (1). SSB proteins also interact with a large array of other proteins (1–7), to bring them to their sites of action on DNA (1). Escherichia coli (Ec) SSB is a homotetramer (8, 9) that binds ssDNA in several binding modes differing in occluded site size and cooperativity depending on salt conditions and SSB concentration (10). Once bound, SSB proteins also function to remove interfering DNA secondary structure (e.g., hairpins) (2, 11). EcSSB binds ssDNA with very high (picomolar to femtomolar) affinities (10, 12, 13), and can undergo direct intra- or intersegment transfer to other DNA sites (13, 15, 16).

DNA helicases/translocases are motor proteins that can translocate directionally and processively along ssDNA at high rates in reactions tightly coupled to ATP binding and hydrolysis (17). Well-studied examples are the Superfamily 1A (SF1A) translocases Rep and UvrD (both 3′–5′ ssDNA translocases), results in the appearance of isolated, irregularly spaced saw-tooth FRET spikes only in the presence of ATP. These FRET spikes result from translocase-induced directional (5′ to 3′) pushing of the SSB toward the 3′ ssDNA end, followed by displacement of the SSB from the DNA end. Similar ATP-dependent pushing events, but in the opposite (3′ to 5′) direction, are observed with EcRep and EcUvrD (both 3′ to 5′ ssDNA translocases). Simulations indicate that these events reflect active pushing by the translocase.

The ability of translocases to chemo-mechanically push heterologous SSB proteins along ssDNA provides a potential mechanism for reorganization and clearance of tightly bound SSBs from ssDNA.

SSB proteins | SF1 translocases | DNA motors | dynamics

Can displace histones (45) and also push dsDNA bound proteins along dsDNA (46, 47).

Although SF1 translocases can displace proteins from ssDNA, there has been no demonstration that an ssDNA translocase can remodel ssDNA–protein complexes by “pushing” them directionally along ssDNA. Here we show that the SF1 ssDNA translocases (ScPif1, EcUvrD, and EcRep) can push a high affinity ssDNA binding protein (EcSSB) along ssDNA, eventually displacing it from ssDNA. Such an activity is likely functionally important in a variety of contexts in genome maintenance.

Results

SSB Diffusion and Dynamics on ssDNA. To examine the results of an encounter between a directional translocase and an EcSSB tetramer bound to ssDNA, we designed a single molecule total internal reflection fluorescence (smTIRF) microscopy assay. We used a 140-nt oligodeoxynucleotide [(dT)140] labeled with Cy3 at the 3′ end attached to an 18-bp mixed sequence duplex with a biotin on the 3′ end of the shorter strand (Fig. 1A). This DNA was immobilized on the surface of a slide coated with PEG via a biotin-neutravidin linkage (Materials and Methods). Experiments were conducted at 25 °C in 20 mM Tris-HCl, pH 8.1, 100 mM NaCl, and 5 mM MgCl2, conditions under which a single EcSSB tetramer binds in its fully wrapped (SSB)6c binding mode in which all four subunits interact with ssDNA (48). When excited with 532-nm laser light, the DNA alone displays a stable Cy3 fluorescence signal with no significant fluctuations (Fig. 1A). On addition of 1 nM Cy3-labeled SSB tetramer (labeled at A122C with an average of one Cy5 per tetramer), anticorrelated Cy3 and Cy5 fluorescence fluctuations are observed, indicating a fluctuating FRET signal (Fig. 1B) consistent with SSB diffusion along the ssDNA as previously described (2). This FRET signal persists after washing with 100 μL imaging buffer (~10-fold slide volume), indicating that can displace histones (45) and also push dsDNA bound proteins along dsDNA (46, 47).

Significance

Cellular processes take place in dynamic, crowded environments. Single-stranded DNA binding (SSB) proteins are ubiquitous in cells, serving to protect the transient single-stranded (ss)DNA formed during DNA replication, recombination, and repair and recruit numerous other proteins to the ssDNA. SSBs must be displaced or otherwise moved in order for DNA to be replicated or repaired. The motor protein activity of ssDNA translocases could serve in this capacity to facilitate directional movement of SSBs along ssDNA. In this work, we show that high-affinity SSBs can be moved directionally along ssDNA and eventually displaced via the ATP-driven action of ssDNA translocases. This process occurs via nonspecific chemo-mechanical pushing of the SSB along the ssDNA in the direction of translocation.

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Single molecule TIRF time trajectories showing SSB pushing by Pif1.

(A) 3′-dT-Cy3-(dT)_{140} DNA immobilized on the slide surface via a biotin-Neutravidin-biotin linkage displays only Cy3 fluorescence. (B) On addition of Cy5-SSB(A122C) (1 nM) and washing out free protein, anticorrelated Cy3 and Cy5 fluorescence fluctuations are observed indicating ssDNA diffusion along the ssDNA. (C) Addition of Pif1 (100 nM) to the 3′-dT-Cy3-(dT)_{140} DNA, followed by ATP (5 mM) and washing out free protein, results in repetitive Cy3 enhancement (PIFE) spikes. (D) Addition of Pif1 (100 nM) with ATP (5 mM) to the 3′-dT-Cy3-(dT)_{140} DNA prebound with Cy5-S SB results in a replacement of SSB-diffusing FRET signals with Pif1 translocating PI FE signals and intermittent asymmetric FRET spikes reflecting Pif1 pushing of SS B in a 5′ to 3′ direction. Green, Cy3 fluorescence; red, Cy5 fluorescence; blue, FRET efficiency calculated from the Cy3 and Cy5 signals. Solution conditions: 30 mM Tris-HCl, pH 8.1, 100 mM NaCl, 5 mM MgCl_2, 1 mM DTT, 0.1 mM BSA, 0.5% (wt/vol) dextrose, 3 mM Trolox, 1 mg/mL glucose oxidase, and 0.4 mg/mL catalase, 25 °C.

Directional Pushing of SSB Along ssDNA by a Translocating Pif1 Monomer.

Surface immobilized 3′-dT-Cy3-(dT)_{140} prebound with no more than a single Cy5-SSB tetramer shows the FRET fluctuations characteristic of ssDNA diffusion (Fig. 1B). On addition of Pif1 (100 nM) and ATP (5 mM), the population of single molecule traces showing SSB diffusion rapidly diminished and was replaced by traces displaying repetitive Pif1 translocation (Fig. S3), indicating clearance of SSB from the ssDNA, Pif1 does not show clearance of SSB in the absence of ATP (Fig. S4). In 16.5% (55/332) of the DNA molecules showing Pif1 translocation activity, anticorrelated Cy3/Cy5 FRET spikes are also observed at irregularly spaced time intervals (Fig. S2D and Fig. S3d). These FRET spikes are asymmetric in shape with a gradual increase to a maximum FRET value followed by a sudden decrease to baseline. These intermittent FRET spikes are consistent with transient binding of a Cy5-SSB to the DNA followed by directional (5′ to 3′) pushing by the Pif1 motor of the Cy5-SSB toward the 3′-Cy3 end of the (dT)_{140}. The frequency distributions shown in Fig. S3 suggest that many pushing events have occurred by the time recording was started, as indicated by the decrease in the number of time trajectories showing SSB diffusion behavior and the increase in the number of Pif1 repetitive translocation events (Fig. S2A).

On reversing the order of addition by first adding Pif1 (100 nM) and then Cy5-SSB (1 nM) and ATP (50 μM or 5 mM), we observed similar asymmetric FRET spikes (Fig. S3d). Eventually, after ~5 min of incubation, the repetitive Cy3 PI FE signals reflecting Pif1 translocation are replaced by fluctuating FRET signals reflecting Cy5-SSB diffusion on the Cy3 ssDNA. This behavior reflects loss of Pif1 from the DNA and replacement by SSB either due to direct dissociation of Pif1 or possibly a Pif1-SSB collision that results in Pif1 dissociation.

We used four criteria for scoring a Cy5 FRET spike as resulting from a Pif1 translocase-SSB collision: (i) The Cy5 fluorescence increase must be accompanied by an anticorrelated Cy3 fluorescence decrease; (ii) the FRET spike must be symmetric with a gradual rise to a maximum followed by a sudden drop to zero FRET; (iii) the FRET spike must be preceded by and followed by the repetitive Cy3 PI FE spikes reflecting Pif1 translocation along ssDNA; and (iv) the FRET spikes must be isolated from other FRET events. Fig. 2 shows an example of the standard shape of a FRET spike resulting from a Pif1 collision with SSB. The gradual increase in FRET with time indicates a decrease in the distance between the Cy5-labeled SSB and the Cy3 at the 3′ end of the (dT)_{140} consistent with directional (5′ to 3′) pushing of SSB. The subsequent sharp loss of FRET reflects displacement of SSB from the DNA. Other examples of FRET spikes resulting from translocase-induced pushing of SSB are shown in Fig. 5.
Pf1 can push SSB off the end of ssDNA. (Fig. 1B). Addition of ScPf1 (100 μM) with ATP (50 μM) results in loss of the bound Cy5-SSB FRET signal and replacement by the repetitive Cy3 enhancement spikes characteristic of Pf1 translocation, reflecting clearance of the SSB from the DNA (Fig. S4). Displacement of SSB by Pf1 is observed as a sudden drop in Cy5 emission and the coincident onset of a saw-tooth-shaped repetitive Cy3 fluorescence enhancement (Fig. 3 B and C). With this shorter ssDNA substrate, no saw-tooth-shaped FRET spikes are observed as each Pf1-SSB encounter results in SSB displacement because there is no free ssDNA along which to push the SSB. When a displaced Cy5-SSB rebinds ssDNA, this is accompanied by a sudden increase from no FRET to high FRET (Fig. 3C). The SSB remains bound until another Pf1 can establish a foothold and displace the SSB once again.

We also examined whether the 3’ to 5’ SF1A ssDNA translocases, E. coli Rep and E. coli UvrD can push SSB along ssDNA. As a monomer, Rep is a 3’ to 5’ processive ssDNA translocase with an average translocation rate of 280 ± 50 nt/s on a poly(dT) track at saturating [ATP] (25). A UvrD monomer is a slower 3’ to 5’ ssDNA translocase with a rate of 190 ± 5 nt/s on a poly(dT) track at saturating [ATP] (21, 22, 24). When added to a 5’-Cy3-labeled (dT)140 bound with Cy5-SSB, both of these enzymes show the characteristic FRET spikes, indicating that they can also push SSB, but in the opposite 3’ to 5’ direction (Figs. S7 and S8). Hence, ssDNA

Note that these isolated, asymmetric FRET spikes differ significantly from the clustered, symmetric FRET fluctuations reflecting diffusion of Cy5-SSB on ssDNA (Fig. S6E). The use of these stringent criteria to identify pushing events likely underestimates the actual number of translocase–SSB collisions. For example, events where an SSB binds initially in the 3’-half of the ssDNA and is then pushed by the translocase would not meet these criteria because such events would start at a FRET value higher than baseline.

The shapes of the FRET spikes suggest that Pf1 pushes the SSB uni-directionally along the ssDNA, eventually displacing SSB from the 3’ end of the DNA. Once displaced, it is difficult for SSB to rebind due to the constant repetitive ssDNA translocation of Pf1, explaining the long time intervals between FRET spikes. Once an SSB rebinds to the ssDNA, it is again pushed off the end of the DNA by Pf1. Most of the FRET spikes display a sharp drop in FRET after reaching its maximum value; however, some display a longer dwell time (pause) in the maximum FRET (typically less than 100 ms but sometimes as long as a second). These pauses could result from saturation of the FRET signal as the SSB approaches the Cy3, as we see in some of our simulations discussed below. However, the pauses may also result from the increased energy required to displace SSB from an ssDNA end compared with pushing SSB along ssDNA, which is a much slower process. Multiple ATP hydrolysis cycles may be needed to displace SSB from the ssDNA end.

To more directly determine whether Pf1 can push an SSB from the end of ssDNA, we used a shorter 3’-dT-Cy3-(dT)70 DNA (Fig. 3). On this shorter ssDNA, the Cy5-SSB does not have room to undergo significant diffusion, so a bound Cy5-SSB results in a high stable FRET signal that does not exhibit the rapid large-scale fluctuations observed for Cy5-SSB on 5’-dT Cy3-(dT)140 DNA (Fig. 1B). Addition of ScPf1 (100 μM) with ATP (50 μM) results in loss of the bound Cy5-SSB FRET signal and replacement by the repetitive Cy3 enhancement spikes characteristic of Pf1 translocation, reflecting clearance of the SSB from the DNA (Fig. S4).

**Fig. 2.** Analysis of the translocase-induced FRET spikes reflecting SSB pushing. (A) Asymmetric FRET spikes identified as a gradual increase in FRET followed by a sharp decrease in FRET. The spike is preceded and followed by spikes in Cy3 fluorescence due to Pf1 translocation. The time-to-peak is determined from the time, $t_1$, at which the signal increases above the baseline average to the time, $t_2$, where the FRET value reaches its maximum. (Left) Raw Cy3 and Cy5 fluorescence emission time trajectories. (Right) Corresponding FRET signal. (B) Histogram of time-to-peak values for Pf1–SSB collisions at 5 μM ATP. The median value for the distribution is 0.272 s; mean value = 0.4 s; SD = 0.3 s ($n = 112$ events). (C) Histogram of time-to-peak values for Pf1–SSB collisions at 50 μM ATP. Median value for the distribution is 0.512 s; mean value = 0.7 s; SD = 0.6 s ($n = 136$ events). (D) Histogram of time-to-peak values for Rep–SSB collisions at 5 μM ATP. Median value for the distribution is 0.192 s; mean value = 0.2 s; SD = 0.1 s ($n = 42$ events). (E) Histogram of time-to-peak values for UvrD–SSB collisions at 5 mM ATP. The median value for the distribution is 0.256 s; mean value = 0.3 s; SD = 0.2 s ($n = 45$ events).

**Fig. 3.** Pf1 can push SSB off the end of 3’-dT-Cy3-(dT)$_{70}$ DNA. (A) Cartoons representing the three states of the DNA after Pf1 and ATP are added to 3’-dT-Cy3-(dT)$_{70}$ DNA prebound with Cy5-SSB: (i) Cy5-SSB bound to 3’-dT-Cy3-(dT)$_{70}$. (ii) Pf1 binds at the ssdsDNA junction and pushes SSB off the 3’-DNA end using its ATP driven 5’ to 3’ translocation. (iii) Cy3 fluorescence. (B) Representative time trajectory showing the Cy3, Cy5 and FRET signals resulting from each of the three states depicted in A. The region marked (ii) shows the ATP-dependent displacement of Cy5-SSB from the ssDNA end by Pf1 translocation indicated by a stable, high FRET signal that is replaced by repetitive Cy3 enhancement with no Cy5 emission. (C) Representative time trajectory showing (iii) repetitive Pf1 translocation along 3’-dT-Cy3-(dT)$_{70}$ DNA, followed by (i) binding of Cy5-SSB to the DNA and (ii) subsequent displacement of Cy5-SSB from the DNA by another Pf1. Green, Cy3 fluorescence; red, Cy5 fluorescence; blue, FRET efficiency. Solution conditions as in Fig. 1.

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translocases from two different organisms with opposite directionalities and different rates display chemo-mechanical pushing of SSB along ssDNA.

Quantitative Analysis of Pushing Events. We quantified the translocase-induced SSB pushing events by measuring the time for the asymmetric FRET signal to increase from baseline to its peak value (Fig. 2A). These time-to-peak were measured for Pif1 at several ATP concentrations, as well as for Rep and UvrD to evaluate the role of translocation rate on the kinetics of SSB pushing. Ensemble studies have shown that Pif1 translocates on poly(dT) with an average rate of ~81 ± 8 nt/s at 5 mM ATP, but decreases to ~15 nt/s at 50 μM ATP under similar buffer conditions used in our assay (50 mM Tris-HCl, pH 8.3, 100 mM NaCl, 5 mM MgCl2) (28). At 5 mM ATP, the mean translocation rate was 0.4 ± 0.3 s with a median value of 0.272 s (n = 112 events; Fig. 2B), increasing slightly at 500 μM ATP to a mean time-to-peak of 0.4 ± 0.2 s with a median of 0.352 s (n = 85), and increasing significantly at 50 μM ATP where the mean time-to-peak was 0.7 ± 0.6 s, with a median value of 0.512 s (N = 136 events; Fig. 2C). This increase in median time-to-peak at lower ATP concentration indicates that the rate of SSB pushing is dependent on the rate of Pif1 translocation along ssDNA. The time-to-peak values did not depend on [NaCl] (Fig. S5B). SSB pushing by Rep (Fig. 2D) and UvrD (Fig. 2E) at 5 mM ATP both show shorter time-to-peak than Pif1 at 5 mM ATP, consistent with their faster translocation rates [280 ± 50 for Rep (25) and 190 ± 5 for UvrD (23, 52)], a further indication that the rate of pushing is dependent on the rate of translocation of the motor.

Mechanism for Translocase-Induced Pushing of SSB. Because ssDNA translocation by these motors is tightly coupled to ATP hydrolysis (1 ATP/nt translocated even at low [ATP] (23, 52), these motors operate as “power stroke” motors rather than as Brownian ratchets (53). However, this does not necessarily mean that these motors push SSB using a power stroke mechanism. We considered two limiting mechanisms for how a translocating motor could push an SSB protein directionally along ssDNA (Fig. 4). The first case, the active pushing (power stroke) model, considers that, on collision, the translocating motor directly exerts a chemomechanical force and pushes the SSB along ssDNA as one unit at the same rate as on ssDNA alone (Fig. 4A). The second case, the moving barrier to diffusion (Brownian ratchet) model, assumes that the translocase is unable to push the SSB but serves as a barrier to SSB diffusion (Fig. 4B). In this model, collision of the translocase with SSB prevents motor translocation. The motor can only resume translocation if the SSB diffuses away from the motor (in the 5′ to 3′ direction in the case of Pif1). As SSB diffuses, Pif1 then follows, gradually decreasing the length of ssDNA accessible for SSB diffusion. Eventually the Cy5-SSB will end up at the 3′-Cy3 end of the DNA. To compare these two models, we performed Monte Carlo simulations of pushing trajectories as a function of the translocation rate of the motor as described in *SI Text* and Fig. S9, and the results are shown in Fig. 4A and B. The simulated times-to-peak for the moving barrier to diffusion model are independent of ATP concentration (Fig. 4B) due to the fact that the average stepping rate of SSB diffusion [0.0592 s/step on average with Dss = 76 nm^2/s at 25 °C and a 3-nt step size (2)] is always slower than the motor translocation rate in the range studied, and thus diffusion will limit the apparent rate of SSB pushing. However, for the active pushing model, the simulated times-to-peak show a clear increase with decreasing motor translocation rate (decreasing ATP concentration) in agreement with our experimental observations (Fig. 4A). Thus, our experiments and simulations support a model in which the translocase can use its chemo-mechanical motor to actively push SSB along ssDNA.

Discussion

SF1 helicases/translocases can displace proteins from ssDNA (20, 32–36, 38, 40, 54) and this ability is thought to be important in overcoming barriers to replication (40). We show here that translocases are also able to push a heterologous ssDNA binding protein along ssDNA. Three different SF1 translocases, ScPif1, EcRep, and EcUvrD, can push a tightly bound Es.SSB tetramer along ssDNA (poly[dT]) with the directionalality and rate of pushing determined by the translocase. Moreover, each pushing event, monitored by a FRET increase as the Cy5-SSB is pushed toward the Cy3-labeled end of ssDNA, is always followed by a sudden loss of FRET signal, suggesting that the translocase can force the SSB off the ssDNA end. This interpretation is supported by simulations that predict the same asymmetric FRET spikes (Fig. S9B). The random nature and large time period between FRET spikes suggest that the displaced SSB proteins have difficulty rebinding to the ssDNA in the presence of repetitive translocase movement. When an SSB protein is occasionally able to rebind the ssDNA, it is rapidly pushed off the ssDNA end.

The translocase-induced pushing of SSB can be quantified by measuring the time it takes for the rising FRET spike to reach its maximum. At saturating [ATP], the three translocases in our studies
display different ensemble translocation rates: EcRep (280 ± 50 nt/s) (25), EcUvrD (190 ± 5 nt/s) (21, 23, 52), and Pif1 (81 ± 8 nt/s) (28). The time-to-peak values of the single molecule FRET spikes are inversely correlated with these translocation rates indicating that the rate of SSB pushing is controlled by the rate of motor translocation. This conclusion is further supported by comparing at two [ATP] the relative rates of Pif1 pushing of SSB to the relative rates of Pif1 translocation in the absence of SSB. The ratio of the median time-to-peak for Pif1 pushing of SSB at 50 μM and 5 mM ATP (0.512 s/0.272 s) is 1.88, whereas the ratio of the median PIFIE peak intervals reflecting repetitive Pif1 translocation in the absence of SSB at the same ATP concentrations is 1.61 (1.440 s/0.892 s). This comparison also indicates that SSB pushing is dominated by motor protein translocation rate rather than SSB protein diffusion. We considered two models for how a translocase might move an SSB protein directionally along ssDNA, active pushing by the translocase or a moving barrier to SSB diffusion. Our simulations show that only the active pushing model predicts the dependence of SSB pushing rate on [ATP] that we observe experimentally. Although we cannot unambiguously determine the number of SF1 translocases required to push an SSB, our results suggest that a single monomeric translocase is sufficient to push an SSB tetramer. We cannot assess from our data whether the motor translocation rate is slowed by SSB pushing. However, the general agreement between the experiments and the simulations, that assume no change in motor translocation rate, suggests no major reduction in rate (less than a factor of 2) when the translocase is pushing the SSB load.

Our finding that SF1 translocases can actively push EcSSB along ssDNA is somewhat surprising. Under the solution conditions of our experiments, a single EcSSB tetramer binds to (dT)$_{10}$ in (SSB)$_{10}$, and in which the ssDNA interacts with all four SSB subunits (55, 56) with a wrapping topology resembling that of the seams on a baseball (8, 57). The affinity of EcSSB for poly(dT) in this mode is less than picomolar under our solution conditions, with a half-life greater than 20 min. However, all three SF1 ssDNA translocases are able to push EcSSB at rates that are not distinguishably different from their translocation rates on isolated ssDNA. One possibility for how this might be accomplished is outlined in Fig. 5 for a 5' to 3’ translocase such as Pif1. When the translocase encounters the SSB tetramer, it may partially peeled the 3’-sided ssDNA away from a region of the translocase as it continues to translocate. The now unoccupied DNA binding site of the tetramer could then be rapidly occupied by the 3’-sided ssDNA. By this mechanism, only a subset of the ssDNA-SSB contacts are broken at any time and the SSB is “rolled” along the ssDNA. An isolated SSB tetramer will not bind stably in its (SSB)$_{10}$ binding mode on ssDNA of lengths ≥70 nucleotides or in the presence of 5 mM Mg$^{2+}$, as we use in this study (48, 58); hence, we are unable to examine whether SSB can be pushed when bound in this mode.

Our experiments and simulations indicate that the chemomechanical pushing of SSB is only dependent on the rate and directionality of the translocase motor with no need for a specific interaction between the translocase and SSB protein. Hence, such pushing events could occur whenever any ssDNA translocase encounters an SSB protein. Translocase-induced directed motion of SSB proteins offers an additional mechanism by which SSB proteins could be reorganized along ssDNA during replication, repair, and recombination. Our results also indicate that SF1 translocases can continue to translocate even after colliding with a tightly bound SSB protein. It has previously been shown that RecBCD helicase can push DNA binding proteins along dsDNA (47). One difference is that as RecBCD translocates, it also unwinds the dsDNA, thus eliminating the dsDNA binding site for the protein. Another difference is that the model proposed for RecBCD-induced pushing suggested transient dissociation of the protein during pushing (47), whereas in the SSB-ssDNA case the tightly bound SSB maintains contact with the ssDNA throughout the pushing process.

What may be the functional consequences of translocase-induced SSB reorganization? SSB pushing may play a role in replication–transcription conflicts (59) as the two machineries collide either head-on on the lagging strand or directionally on the leading strand. The consequences of a joint translocase–SSB entity moving along ssDNA could also be functional. It is well established that monomeric SF1 helicases, such as Rep, UvrD, and Pif1, are rapid translocases, but very poor helicases on their own that must be activated either by self-assembly or through interactions with an accessory protein (60–62). However, SSB proteins can use their ability to diffuse along ssDNA to transiently destabilize short duplex hairpin loops (2, 11). Our results suggest that a translocase pushing an SSB could apply a directed force on an SSB at a ss/dsDNA junction to promote helicase activity beyond the ~8-bp limit previously observed for a diffusing SSB (2, 11). The chemo-mechanical pushing of SSBSs by SF1 translocases adds to the growing list of functions for this class of motor proteins, including the recent demonstration that UvrD is able to use its motor activity to cause backtracking of an RNA polymerase elongation complex stalled at a DNA lesion (63).

Materials and Methods
Oligodeoxynucleotide Synthesis. All oligodeoxynucleotides were synthesized on a MerMade 4 synthesizer (Bioautomation) using phosphoramidite reagents from Glen Research and purified as described (11). Sequences used in this study are shown in Table S1. DNA concentrations were determined as described (11). DNA duplexes were annealed in a buffer containing 10 mM Tris-HCl, pH 8.1, 50 mM sodium chloride, and 0.1 mM disodium EDTA.

Protein Purification. E. coli SSB and SF1 translocases were purified as described (2, 14). Labeling with Cy5 maleimide was performed using underlabeling conditions (6). EcSSB concentrations were determined spectrophotometrically (2, 14). The ratio of Cy5 dye to SSB tetramer was 1.1. SSB was purified and concentrated as described (64). EcRep and EcUvrD were purified as described (25, 62, 65).

smTIRF Microscopy. An objective-type TIRF microscope (IX71 inverted microscope; Olympus) with an oil immersion objective (60×/1.45 NA PlanApo; N; Olympus) was used for the smTIRF experiments as described (11, 66). The sample slide was illuminated with a 532-nm laser (CrystalLaser) fiber-optically coupled to the TIR microscope. The temperature of the slide was maintained at 25 °C using both a temperature-controlled stage (BC-110 Bioinnomic controller; 20/20 Technology) and an objective heater (Biotechs). The TIRF signal was observed using an Andor iXon EMCCD camera (Model DU890E). Data were collected using SINGLE, a custom program provided by the laboratory of Taekjip Ha (Johns Hopkins University). Raw data files were processed with IDL (Exelis Visual Information Solutions) and individual intensity time-trajectory plots were analyzed with MATLAb (Mathworks) as described (11). Details on the simulations of translocase/SSB collisions resulting in SSB pushing can be found in SI Text.

All smTIRF experiments with EcPif1 were conducted in 30 mM Tris buffer (pH 8.1), 100 mM sodium chloride (total concentration of NaCl from all components, including protein dilution buffers and oxygen scavenger solutions, ranged from 110 to 120 mM depending of disodium ATP concentration), 5 mM magnesium chloride, 0.1 mM disodium EDTA, 1 mM DTT, 0.5% (vol/vol) dextrase, 0.1 mg/mL BSA, and 3 mM 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox). The smTIRF experiments with EcRep were conducted in the buffer above but with the presence of 10% (vol/vol) glycerol and the absence of added sodium chloride (total concentration of NaCl from all components ranged from 10 to 20 mM depending on disodium ATP concentration). EcUvrD smTIRF experiments were done in the same buffer as the SsbPf1 experiments but with the addition of 10% (vol/vol) glycerol. Immediately before all TIRF measurements, glucose oxidase (1 mg/mL final concentration) and catalase (0.4 mg/mL final concentration) were added to the samples to serve as an oxygen scavenging system.

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