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Torque-limited RecA polymerization on dsDNA

Thijn van der Heijden, John van Noort, Hendrikje van Leest, Roland Kanaar[1,2], Claire Wyman[1,2], Nynke Dekker and Cees Dekker*

Kavli Institute of Nanoscience, Delft University of Technology, Lorentzweg 1, 2628 CJ Delft, The Netherlands, [1]Department of Cell Biology and Genetics and [2]Department of Radiation Oncology, Erasmus Medical Center, PO Box 1738, 3000 DR Rotterdam, The Netherlands

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ABSTRACT

The assembly of RecA onto a torsionally constrained double-stranded DNA molecule was followed in real time using magnetic tweezers. Formation of a RecA–DNA filament on the DNA tether was stalled owing to different physical processes depending on the applied stretching force. For forces up to 3.6 pN, the reaction stalled owing to the formation of positive plectonemes in the remaining DNA molecule. Release of these plectonemes by rotation of the magnets led to full coverage of the DNA molecule by RecA. At stretching forces larger than 3.6 pN, the twist induced during filament formation caused the reaction to stall before positive supercoils were generated. We deduce a maximum built-up torsion of 10.1 ± 0.7 k_bT. *In vivo* this built-up torsion may be used to favor regression of a stalled replication fork or to free the chromosomal DNA in *E.coli* from its condensing proteins.

INTRODUCTION

In *Escherichia coli*, the RecA protein plays a central role in repair of damaged DNA through homologous recombination by mediating homologous DNA pairing and DNA strand-exchange reactions, in which a single-stranded DNA (ssDNA) replaces the homologous strand on a double-stranded DNA (dsDNA) (1). The structural modification of DNA by RecA during this genetic process is a key step in sequence recognition and specificity (2). Biochemical and electron microscopy studies have shown that RecA binds cooperatively to ss- and dsDNA with a stoichiometry of 1 RecA molecule per 3 nt or base pairs, respectively (3). The resulting complex is stretched by a factor of 1.5 with respect to B-form DNA and has a twist angle of 20° per base pair (4) instead of 35° per base pair in bare dsDNA. In B-form DNA, a single helical pitch measures 3.5 nm occupying 10.4 bases (5), whereas in a RecA–DNA filament this single helical pitch changes to 9.5 nm occupying 18.6 bases (4). Owing to these different helical pitches the binding of RecA causes unwinding of dsDNA. Electron microscopic images of RecA–DNA complexes suggest that RecA is unable to fully cover covalently closed circular DNA (4). Instead, it forms a partial filament, leaving the remaining uncovered DNA in a highly supercoiled state. The production of additional negative twist in the RecA–DNA filament is thus countered by the formation of positive writhe in the remaining DNA molecule. In contrast, open circular DNA can be fully covered by RecA. *In vivo*, however, the topology of dsDNA molecules is usually constrained. A torsional study allows us to explore the energetics involved in the assembly of RecA on topologically constrained DNA.

In this paper, we used magnetic tweezers to study the dynamics of assembly of a RecA–DNA filament on a torsionally constrained DNA molecule in real time. As RecA changes the helical pitch upon binding, it generates torsion in the remainder of the DNA molecule. Depending on the applied stretching force, this generated torsion is stored either as writhe, in plectonemes, or as twist. In both the cases, RecA was found to form only partial filaments on the DNA molecule. Controlled release of the superhelical torsion resulted in a fully covered DNA molecule. From the torsion exerted by RecA on dsDNA (up to 10.1 k_bT), an estimate of 7 k_bT is deduced for the binding energy of a RecA monomer to dsDNA. Fulconis *et al*. (6) have recently reported a magnetic-tweezers study, in which they study the disassembly of a RecA–DNA filament in the presence of an external torque. They demonstrated that a DNA molecule can be fully covered by RecA when negatively unwinding a torsionally constrained DNA molecule prior to the introduction of RecA.

*To whom correspondence should be addressed. Tel: +31 15 2786094; Fax: +31 15 2781202; Email: dekker@mb.tn.tudelft.nl
Present address:
John van Noort, Department of Biophysics, Huygens Laboratory, Leiden University, 2300 RA Leiden, The Netherlands

MATERIALS AND METHODS

Magnetic tweezers

In the magnetic-tweezers setup used in these experiments (see Figure 1a), a DNA molecule is attached at one end to a glass surface and at the other end to a superparamagnetic bead (7). The tethered molecule can consequently be stretched and supercoiled by translating or rotating a pair of external magnets, respectively. The applied force is calculated by quantifying the thermal motion of the DNA-tethered bead (8). By using image processing, 5 nm position accuracy of the bead was obtained in three dimensions. To minimize the effect of thermal drift, all positions were measured relative to polystyrene beads that were fixed to the bottom of the flow cell.

DNA substrates

For assays involving rotationally constrained DNA, pSFV1 (Invitrogen) was cleaved with SpeI and BamHI, resulting in a 10 kb linear fragment, which was ligated to two 700 bp PCR fragments, one containing several biotin-modified dUTP bases and the other PCR fragment containing several digoxigenin-modified dUTP bases. Using the magnetic tweezers, we selected molecules that were rotationally constrained. As shown in Figure 1b, for forces below 0.5 pN, a torsionally constrained DNA molecule shows a symmetrical behavior upon rotation of the magnets. However, at higher forces the DNA tether shows an asymmetric behavior for positive and negative rotations by the magnets (e.g. the curve at $F = 3.8$ pN in Figure 1b), as described by Strick *et al*. (8). For experiments involving freely rotatable DNA, pSFV1 was cleaved with SpeI and XhoI, resulting in an 8 kb linear fragment. The biotin-modified PCR fragment was pre-treated with SAP to dephosphorylate the 5′ end. After ligation, the resulting construct therefore has a nick near the magnetic bead.

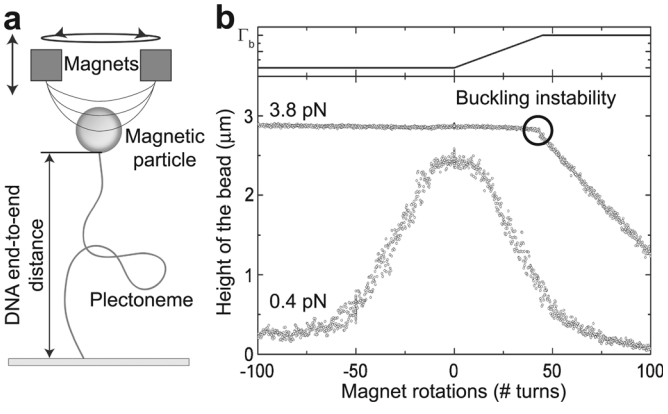

Figure 1. (**a**) Schematic drawing of the magnetic tweezers. A DNA molecule is attached at one end to the bottom of the flow cell and at the other end to a magnetic bead. This molecule can be pulled and twisted using small magnets placed above the flow cell. The position of the magnetic bead is measured using an inverted microscope placed beneath the flow cell. The bead position and thus the end-to-end distance of the DNA molecule is determined using video microscopy and image analysis. (**b**) Extension of a DNA molecule versus the number of turns applied by the magnets for various stretching forces. At low force, contraction of the molecule is symmetrical under positive and negative applied turns. At higher force, the molecule's extension initially remains constant for positive applied turns. The induced torque increases linearly with the number of applied turns, as depicted in the top graph until a buckling transition allows the system to saturate its torsional constraint through the formation of plectonemes.

Flow cell

Polystyrene beads, as well as DNA constructs tethered to a magnetic bead at one end, were anchored to the bottom of a flow cell as described previously (7). The force-extension curve of a single DNA molecule was measured. After confirmation of the correct contour and persistence lengths, experiments were started with RecA. All measurements were carried out at 25°C.

RecA/DNA reactions

The flow cell final volume is ~100 µl. All reactions were carried out in a buffer of 20 mM MOPS, pH 6.2, 10 mM $MgCl_2$, 0.5 mM EDTA and 1 mM DTT. For the start of a measurement, the cell contents were replaced by 1 µM RecA (Roche) and 1 mM ATP in the same buffer, while the DNA molecule was relaxed. RecA polymerization in saturating conditions was monitored through measurement of the height of the magnetic bead, which measures the end-to-end distance of the DNA molecule.

RESULTS

Torsionally unconstrained dsDNA

For nicked dsDNA, we observe the expected RecA-induced length extension of 50% (9–12). After the introduction of RecA and ATP in the flow cell, the height of the bead attached to a nicked DNA molecule was initially unaffected. However, after a short time delay (~100 s), RecA assembly commenced, as observed from the extension of the DNA molecule (see Figure 2a). This was followed by a steady linear increase in the height of the bead. Finally, this increase ceased and the height of the bead remained constant. Stretching the DNA tether at different forces ($0.5 < F < 9.0$ pN) did not change the behavior of RecA polymerization on dsDNA (data not shown). The growth velocity appeared constant at ~10 nm/s, or 20 monomers/s. The linear increase in height indicates a cooperative growth process. The observed growth profile is consistent with optical tweezer experiments by Shivashankar *et al*. (10), who stated that RecA assembly starts from a single nucleation site and proceeds linearly in one direction. The construct used for our experiment contained a nick, which is a preferable binding site to start nucleation, close to the magnetic bead. The change in contour length induced by RecA polymerization was 49 ± 3%, which is in agreement with results reported in literature (9–12).

Torsionally constrained dsDNA

In the case of torsionally constrained dsDNA, we observed a more complex behavior showing both elongation and shortening of the DNA tether, dependent on the applied force. At forces exceeding 3.6 pN, introduction of RecA into the flow cell initially showed the same behavior as torsionally unconstrained DNA (see Figure 2b). After a time lag, a linear increase in the height of the bead was observed with the same rate as above. The observed time lag was 2.9 ± 0.6 min ($N = 14$), allowing us to determine a nucleation rate of RecA onto torsionally constrained dsDNA of $3.4 \pm 0.7 \times 10^{-5}$ min^{-1} bp^{-1}, which is in agreement with the results reported in literature (13). The DNA tether, however, was not extended by 50%,

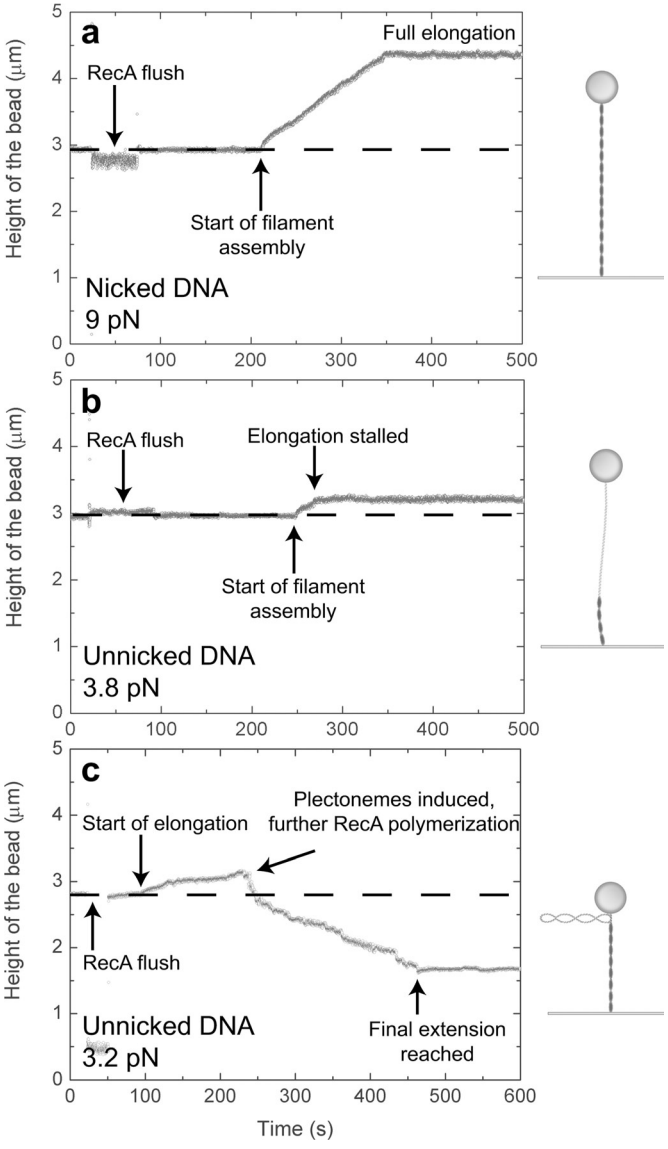

Figure 2. RecA polymerization on dsDNA. (**a**) RecA extends a torsionally unconstrained dsDNA molecule by 50% in the presence of 1 mM ATP at a stretching force of 9 pN. For torsionally constrained DNA, a very different behavior is observed. (**b**) At $F = 3.8$ pN, a torsionally constrained construct is only extended by 6%, after which elongation is stalled. (**c**) At low forces ($F = 3.2$ pN), the height of the bead first increases, but then is followed by a reduction in length. This reduction in length is caused by the formation of positive plectonemes in the remaining part of the uncovered DNA molecule. The dashed lines denote the end-to-end distance of the bare dsDNA molecule at the given stretching force.

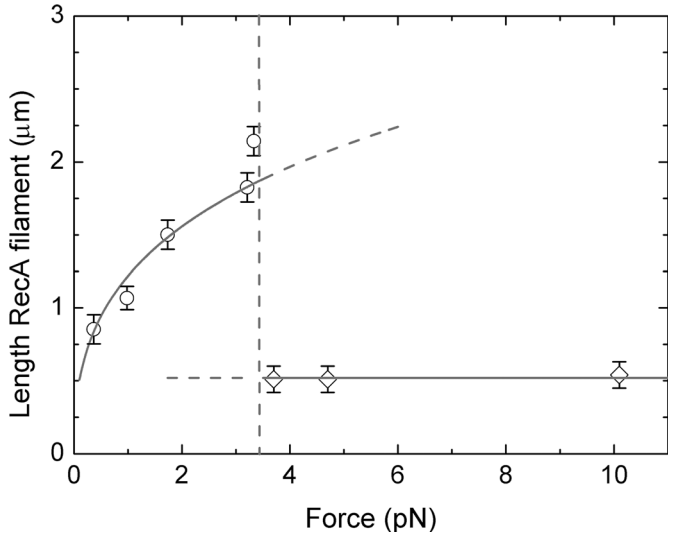

Figure 3. Dependence of the length of the RecA–DNA filament on the applied stretching force for torsionally constrained DNA. Data are taken at the end of the filament formation when the assembly reaction has stalled. Up to 3.6 pN, the length of the RecA–DNA filament increases (circles). Above 3.6 pN, the size of the RecA–DNA filament remains constant (diamonds). Solid line is the outcome of the model described in 'Discussion'. Note that the model curve is plotted without any adjustable parameters. Above 3.6 pN, the model does not apply because the assembly reaction is not stalled by the formation of plectonemes but by a built-up torsion in the remaining DNA molecule.

but by a much smaller value of $6.5 \pm 1.1\%$ ($N = 8$), independent of force between 3.6 and 10 pN. Apparently, at these forces RecA was not able to form a complete filament on the torsionally constrained dsDNA molecule. The height increase of 0.18 ± 0.03 μm ($N = 8$) implied that a RecA–DNA filament with a length of only $0.36 + 0.18 = 0.54$ μm formed on the DNA molecule, i.e. a 0.36 μm fragment of DNA, was extended by 50%. The key difference with the previous experiment was that a torsionally constrained DNA molecule accumulates twist, which it could not release. During filament assembly, the binding of each RecA monomer induced a small twist in the remaining dsDNA. Therefore, as the filament grew in length, the twist stored in the remaining dsDNA molecule increased. After some point, the accumulated twist prevented the next RecA monomer from assembling, and extension of the dsDNA molecule was therefore stalled.

A strikingly different behavior was observed for forces below 3.6 pN. In these cases, an initial increase in the height of the bead was followed by a large decrease (Figure 2c). At very low forces (below 0.5 pN), the molecule's end-to-end distance did not even show the initial increase but directly started to reduce. The process reached an equilibrium where no further shortening was observed (e.g. near 470 s in Figure 2c). The end-to-end distance of the molecule at which the reaction finally stalled varied strongly with different stretching forces. In Figure 3, these stalling heights are plotted versus force. These stalling heights correspond to the length of a partially RecA-coated dsDNA molecule as will be shown below. As can be seen, the stalling height increased monotonically with the applied stretching force up to 3.6 pN.

However, further RecA assembly can be induced at the stalling position by negative rotation of the magnets (see Figure 4) that released (some of) the positive supercoils in the remaining uncovered DNA molecule. At constant stretching force, the end-to-end distance of the molecule increased with increasing negative rotation of the magnets to a final elongation of ~50% (compared with the original DNA contour length). Beyond this point, the molecule's extension started to decrease owing to twist-induced RecA depolymerization as also shown by Fulconis *et al.* (6). In contrast, the application of positive instead of negative rotation of the magnets at the stalling position caused a decrease in the end-to-end distance of the molecule (data not shown).

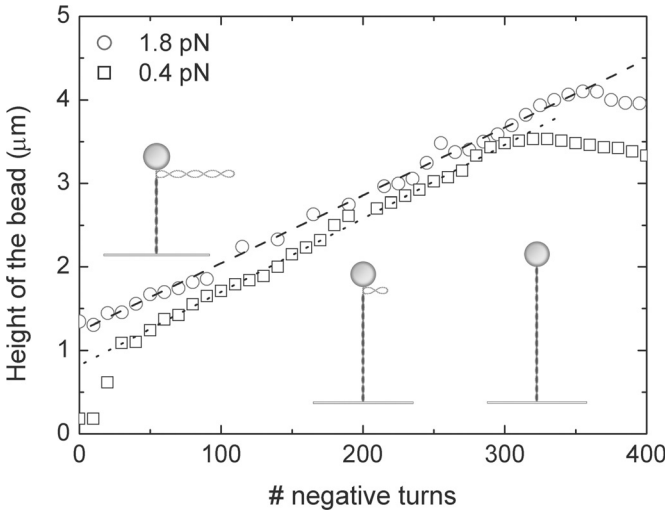

Figure 4. On torsionally constrained dsDNA, RecA–DNA filament formation stalls at a certain length that is dependent on the applied stretching force. This is due to the formation of positive plectonemes in the remaining uncovered DNA. Removal of these positive plectonemes in the remaining DNA molecule by rotation of the external magnets allows the assembly reaction of a RecA–DNA filament to continue. The molecule reached its maximal extension after 355 ± 10 and 320 ± 10 negative turns by the magnets at a stretching force of 1.8 and 0.4 pN, respectively. From force-extension measurements in both the cases, an effective length increase of 51 ± 2% is obtained compared with the DNA contour length.

DATA ANALYSIS AND DISCUSSION

When a RecA monomer binds to dsDNA, the base-to-base distance increases by 50% and the helix is unwound by 15° per base pair, because the twist angle per base pair changes from 35° to 20°. In the case of a torsionally unconstrained DNA molecule, this results in elongation because the torsion is released by free rotation of one DNA strand. However, when the DNA molecule is not allowed to freely rotate around its helical axis, the torsional energy is stored as either twist or writhe depending on the applied stretching force. At high forces, the DNA extension initially remains unchanged upon rotation (see the data for 3.8 pN in Figure 1b) and the torsional energy is stored as twist. At low stretching forces (see the data for 0.4 pN in Figure 1b), this torsional energy is stored as writhe (plectonemes) and the extension of the DNA molecule decreases upon rotation, because the plectonemes consume a significant length of DNA.

This behavior explains why, in the low-force regime ($0.5 < F < 3.5$ pN), both elongation and shortening are observed during RecA assembly on torsionally constrained DNA. First, elongation is observed because RecA polymerization proceeds and the induced torsional energy is initially consumed as twist. When the DNA molecule reaches its buckling instability, twist cannot be increased further and additional torsional energy is consumed as writhe causing a reduction in the bead height owing to the formation of plectonemes in the DNA molecule. This process continues until the DNA molecule is fully consumed in one part that is covered by RecA and another part that is plectonemically supercoiled. At very low forces ($F < 0.5$ pN), the net effect is that the height of the bead decreases directly, because the length reduction caused by plectoneme formation dominates.

Depending on the applied stretching force, the induced torsional energy owing to RecA polymerization is stored in twist or writhe (plectonemes), because the linking number (Lk) of a torsionally constrained construct remains constant (14). The linking number can either be increased or decreased by applying positive or negative magnet turns, respectively. Because a single turn of the magnets implies one turn on the molecule, we have $\Delta Lk = r$, where r is the number of turns the magnet rotates. As shown in Figure 4, the molecule reached its maximum length after 355 ± 10 negative rotations of the magnets at an applied stretching force of 1.8 pN, whereas 320 ± 10 rotations were needed to reach the maximum length at $F = 0.4$ pN. From force-extension measurements in both the cases, we obtained a contour length of 4.10 ± 0.05 μm. Prior to the introduction of RecA, the contour length of the relaxed DNA molecule was 2.72 ± 0.02 μm. Therefore, the effective length increase is 51 ± 2%. The linking number of a helical molecule is obtained by dividing its contour length by its helical pitch. In the case of the bare DNA molecule, the linking number equals 2720/3.5 = 777. A RecA–DNA filament on the same (length of DNA) has a linking number of 4100/9.5 = 432. The difference in linking number of $432 - 777 = -345$ corresponds very well to the experimental values of the negative turns of the magnets, which is 340 ± 10 ($N = 5$).

Our data show that the RecA binding reaction reaches an equilibrium that is force dependent, as shown in Figure 3. The end-to-end distance of the molecule at which the reaction finally stalled varied strongly with different stretching forces. The measured extension corresponded to the length of a partially RecA-coated dsDNA molecule with the remaining uncovered dsDNA in a positively supercoiled state. Because highly supercoiled dsDNA has an end-to-end distance of approximately zero (see Figure 1b), we take the height of the bead as a measure for the length of the partially formed RecA–DNA filament. It should be noted that all torsion is assumed to be absorbed by the remaining free dsDNA; in other words, we assume that the rotational stiffness of a RecA–DNA filament is much larger than that of bare dsDNA. This is a reasonable assumption because Stasiak and Di Capua (4) showed using electron microscopy that the torsion generated during RecA assembly is indeed almost entirely consumed by the remaining DNA in the covalently closed plasmid and that the partially formed RecA–DNA filament hardly consumed any torsion.

We now introduce a model that describes the influence of plectoneme formation on RecA assembly on a torsionally constrained DNA molecule. Given an equilibrium situation in which DNA is partitioned into RecA-coated and highly supercoiled portions, the base pairs of the DNA molecule are either consumed in positive plectonemes or covered with RecA. The overall number N_{DNA} of base pairs in the DNA molecule is a constant: $N_{DNA} = N_{RecA} + N_{pl}$, where N_{RecA} is the number of base pairs covered with RecA and N_{pl} the number of base pairs that is not covered by RecA and which is consumed in plectonemes. As mentioned in the introduction, a single RecA monomer covers 3 bp and therefore $N_{RecA} = 3n$, where n is the number of bound RecA monomers. The size of a single plectoneme l_p is given by $l_p = 2\pi R$, where R is the radius of a plectoneme (15). The amount of base pairs stored in a single plectoneme is $N_{l_p} = (2\pi R)/(d_{DNA})$, in which d_{DNA} is the base pair rise of dsDNA. The heavily supercoiled state

exists of multiple positive plectonemes, therefore $N_{pl} = mN_{l_p}$ with m the number of plectonemes. Binding of a single RecA monomer (covering 3 bp) induces a change φ in the helical pitch of approximately $\varphi = 3\,(20^\circ - 35^\circ) = -45^\circ$. As soon as a torsionally constrained DNA molecule has passed its buckling instability, the torsion induced by RecA is converted into the formation of plectonemes. The number of plectonemes m can therefore be expressed as $m = (n\varphi - \Omega_b)/2\pi$, where Ω_b is the twist angle at which the DNA molecule starts to buckle, and both φ and Ω_b are expressed in radians. Before a DNA molecule reaches its buckling instability upon twisting at a given stretching force, its extension remains unchanged and pure twist accumulates (see the data for 3.8 pN in Figure 1b). In this regime, Hooke's law relates the accumulated twist angle Ω and the torsion Γ as follows

$$\Gamma = C\frac{\Omega}{L}, \qquad \mathbf{1}$$

in which C is the torsional modulus and L the contour length of the molecule. Therefore, the number of base pairs of DNA stored in plectonemes is

$$N_{pl} = mN_{l_p} = \left(n\varphi - \frac{\Gamma_b L}{C}\right) \times \frac{R}{d_{DNA}}. \qquad \mathbf{2}$$

Both the radius of a plectoneme and the buckling instability Γ_b are force-dependent, given by (16)

$$R = \left(\frac{Pk_bT}{2F}\right)^{\alpha}, \qquad \mathbf{3}$$

and

$$\Gamma_b = \sqrt{2Pk_bTF}, \qquad \mathbf{4}$$

where P is the persistence length, k_b Boltzmann's constant, T the absolute temperature and F the applied stretching force. For the value α, Strick *et al*. (16) found experimentally a value of 0.4, whereas simple mechanics yields a value of 0.5 (17). In our calculations, we adopt the experimental value of 0.4. Using Equations 2–4, we can determine how many RecA monomers n cover the torsionally constrained DNA molecule with a contour length L ($= N_{DNA}\, d_{DNA}$) for a given stretching force F as only the amount of base pairs stored in plectonemes is force-dependent. The overall number of base pairs in the DNA molecule becomes

$$N_{DNA} = \left(n\varphi - \frac{\Gamma_b N_{DNA} d_{DNA}}{C}\right) \times \frac{R}{d_{DNA}} + 3n, \qquad \mathbf{5}$$

which can be rewritten as

$$n = \left(N_{DNA} + \frac{\Gamma_b N_{DNA} R}{C}\right)\left(3 + \frac{\varphi R}{d_{DNA}}\right)^{-1}, \qquad \mathbf{6}$$

where the radius of a plectoneme and the buckling instability are given by Equations 3 and 4, respectively. The length of the RecA–DNA filament is $L_{RecA} = 3nd_{RecA}$, where d_{RecA} is the base pair rise in a RecA–DNA filament, and the amount of DNA consumed in plectonemes is $L_{pl} = N_{pl}\, d_{DNA}$. Up to forces of 3.6 pN in Figure 3, the RecA–DNA filament length increases monotonically with applied stretching force. The solid line in Figure 3 denotes the length of the RecA–DNA filament as derived from the above model using a base pair rise in a RecA–DNA filament of 0.51 nm (3) and a torsional modulus of DNA of 86 k_bT·nm (18). The persistence length of the naked DNA molecule (53 ± 2 nm) was obtained from force-extension measurements. Note that the model curve is plotted without any adjustable fitting parameters. The torsional modulus of DNA, however, varies between 75 and 120 k_bT·nm depending on the applied analytical method (19). Such a variation shifts the theoretical curve of Figure 3 up or down by only 10%. In this force regime, the proposed model corresponds very well to measured stalling positions.

In the model, RecA assembly is stalled by the formation of (positive) plectonemes in the remaining uncovered dsDNA molecule. However, for forces beyond 3.6 pN, a different behavior is observed. The length of the partially formed RecA–DNA filament becomes approximately constant at a low value of about $L_{RecA} = 0.54$ μm, much lower than the predicted values from the proposed model. We can understand this as follows. In order to create the first plectoneme, the buckling instability of the DNA molecule has to be overcome. Whether this is possible or not depends on the torsion produced by the partially formed RecA–DNA filament. To determine this torsion using Equation 1, the twist angle and the length in which the twist accumulates have to be deduced. As stated in the model, every RecA monomer covers 3 bp and induces a change in the helical pitch of approximately $\varphi = -45^\circ$. The DNA molecule that is partially covered by n RecA monomers is, therefore, unwound by $\Omega = n\varphi = L_{RecA}/3d_{RecA}\varphi = 44$ rotations, which is consumed by the remaining uncovered DNA molecule with a length $L_{free\ DNA}$ given by $L_{free\ DNA} = L - \frac{1}{1.5} \times L_{RecA} = 2.72 - \frac{1}{1.5} \times 0.54 = 2.36$ μm. The torsion produced by the partially formed RecA–DNA filament on the remaining uncovered DNA is therefore calculated from Equation 1 using a torsional modulus of DNA of 86 k_bT·nm (18) to equal 41 ± 3 pN·nm or about 10.1 ± 0.7 k_bT. This is indeed equal to the value of 9.6 ± 0.2 k_bT needed to overcome the buckling instability at stretching forces up to 3.6 pN (Equation 4). Our calculations thus confirm that RecA assembly on a torsionally constrained DNA molecule generates an amount of torsion that is insufficient to overcome the buckling instability at forces above 3.6 pN. Using a similar setup, Fulconis *et al*. (6) demonstrated that applying an external torque on a RecA-covered DNA molecule forced the filament to depolymerize. In the presence of ATP, they estimated a RecA depolymerization torque between 3 and 5 k_bT. This value, however, appears to be a lower bound, as the buckling force in their experiments is between 1 and 2 pN, which, using Equation 4, yields a RecA depolymerization torque between 5 and 7 k_bT. Also, the method by Fulconis *et al*. (6) to determine the depolymerization torque is rather sensitive to fluctuations in the measured value of the slope of their rotation curves; a change in this measured value of 10% causes the obtained RecA depolymerization torque to change by 100%.

The energy that a RecA monomer gains for binding to dsDNA is used to unwind and stretch the dsDNA molecule. The energy E required to locally unwind dsDNA by a single RecA monomer is $E \cong \Gamma\varphi$, as the induced torsion in the remaining uncovered DNA remains approximately constant. The maximum built-up torsion in dsDNA during RecA

assembly is $\Gamma = 10.1\ k_bT$ (as calculated above) and the change in helical pitch is $\varphi = 45^\circ \times 2\pi/360^\circ = \pi/4$, which together yield a torsional energy of $E = 7.9 \pm 0.5\ k_bT$. The elongation of DNA by a single RecA monomer reduces the torsional energy by 3 ($d_{RecA} - d_{DNA}$) F, which is 0.1–1.5 k_bT for F = 1–10 pN due to the work carried out by the applied stretching force. The energy to unwind and stretch DNA by a single RecA monomer is therefore about 7 k_bT. This provides a lower bound for the binding energy of RecA. Using biochemical assays, the binding energy of a RecA monomer to ssDNA has been determined to be 10 k_bT (20), whereas in the experiments of Fulconis *et al*. (6) a binding energy of RecA to dsDNA of ~6 k_bT was obtained.

In the presence of ATP, RecA is able to bind and dissociate from dsDNA owing to ATP hydrolysis. RecA disassociation is blocked in the presence of ATPγS, a non-hydrolyzable ATP analog (21). Applying an external torque depolymerizes filaments assembled in the presence of ATPγS (6). In this case, the external torque at which the RecA–DNA filament starts to depolymerize is, therefore, a measure for the unbinding energy of ATPγS bound RecA from dsDNA. The question arises whether the binding energy is equivalent to the unbinding energy. In the presence of ATPγS, an external torque of more than 13 k_bT had to be applied to dissociate RecA from dsDNA giving a lower bound for the unbinding energy of 10 k_bT (22). This value is comparable with the energy that is available upon ATP hydrolysis of 12.3 k_bT.

When exposed to high torque ($\Gamma \gtrapprox 8\ k_bT$) DNA can undergo a structural transition from regular B-DNA into supercoiled P-DNA (Pauling-like structure with exposed bases) (23). As RecA assembly creates up to 10 k_bT of torsion in the remaining DNA molecule, the formation of supercoiled P-DNA could hinder further RecA binding. However, this structural transition is highly dependent on the ionic conditions in the flow cell. Addition of 5 mM $MgCl_2$ shifts the critical torque at which the structural transition occurs to 13 k_bT (22). Owing to the presence of 10 mM $MgCl_2$ in our experiments, it is therefore unlikely that supercoiled P-DNA has formed.

We consider two possible biological roles for the DNA torsion induced by RecA filament formation on dsDNA. RecA filaments on dsDNA occur as a result of strand invasion and joint molecule formation in homologous recombination. Torsion introduced here in the target DNA molecule, usually the chromosome, could function to displace the *E.coli* proteins responsible for chromosome condensation in order to complete the recombination process. Chromosomal DNA in *E.coli* is condensed by architectural proteins, including HU, H-NS, IHF and Fis (24). Though several studies report force spectroscopy analysis of these proteins bound to DNA (7,25), none has addressed a critical torque, as described here for RecA. Such critical torque values would be interesting for comparison with RecA to determine whether the architectural proteins could be displaced. RecA filaments also form on dsDNA nucleated at sites of damage, such as ultraviolet (UV) lesions. Under normal growth conditions, bacterial replication forks are stalled by DNA damage (26,27), such as UV-irradiation-induced lesions (28). The repair of such stalled replication forks is a significant function of recombination systems (29). One mechanism for rescuing stalled replication involves fork reversal resulting in a Holliday junction structure called a 'chicken foot'. Postow *et al*. (30) have shown that positive torsional strain is absorbed by a regression of the replication fork and calculated that this would require very little energy, only 5 kcal/mol (8.4 k_bT). Formation of RecA filaments at sites of DNA damage ahead of the replication fork could generate up to 4.1 kcal/mol (7 k_bT) of positive torsional energy, which would be sufficient to drive the fork reversal and is in accordance with the value estimated by Postow *et al*. (30).

CONCLUSION

In this paper, we quantitatively described the binding of RecA to torsionally constrained dsDNA. Upon binding, a RecA monomer both stretches and twists the backbone of the DNA molecule. The built-up of torque is stored either in writhe (plectonemes) or in pure twist depending on the applied force. A torsional constraint of the DNA molecule leads to stalling of RecA assembly because the remaining uncovered DNA cannot absorb more torsion. Rotation of the magnets results in the expected elongation of 50%. The amount of magnet turns is a measure for the change in linking number accompanied with the binding of RecA. The assembly reaction stalls whenever the extra torsion stored in the remaining free dsDNA reaches a value of 10.1 k_bT. At this amount of torsion, it is not energetically favorable for RecA to bind to its substrate. We have discussed the possible biological role of RecA-induced torsion in regression of replication forks and displacement of condensing proteins.

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REFERENCES

1. Lusetti,S.L. and Cox,M.M. (2002) The bacterial recA protein and the recombinational DNA repair of stalled replication forks. *Annu. Rev. Biochem.*, **71**, 71–100.
2. Yu,X., VanLoock,M.S., Yang,S., Reese,J.T. and Egelman,E.H. (2004) What is the structure of the recA–DNA filament? *Curr. Protein Pept. Sci.*, **5**, 73–79.
3. Di Capua,E., Engel,A., Stasiak,A. and Koller,T. (1982) Characterization of complexes between recA protein and duplex DNA by electron microscopy. *J. Mol. Biol.*, **157**, 87–103.
4. Stasiak,A. and Di Capua,E. (1982) The helicity of DNA in complexes with recA protein. *Nature*, **299**, 185–186.
5. Watson,J.D. and Crick,F.H.C. (1953) Molecular structure of nucleic acids: a structure for deoxyribose nucleic acid. *Nature*, **171**, 737–738.
6. Fulconis,R., Bancaud,A., Allemand,J.F., Croquette,V., Dutreix,M. and Viovy,J.L. (2004) Twisting and untwisting a single DNA molecule covered by recA protein. *Biophys. J.*, **87**, 2552–2563.
7. van Noort,J., Verbrugge,S., Goosen,N., Dekker,C. and Dame,R.T. (2004) Dual architectural roles of HU: formation of flexible hinges and rigid filaments. *Proc. Natl Acad. Sci. USA*, **101**, 6969–6974.

8. Strick,T.R., Allemand,J.F., Bensimon,D. and Croquette,V. (1998) Behavior of supercoiled DNA. *Biophys. J.*, **74**, 2016–2028.
9. Stasiak,A., Di Capua,E. and Koller,T. (1981) Elongation of duplex DNA by recA protein. *J. Mol. Biol.*, **151**, 557–563.
10. Shivashankar,G.V., Feingold,M., Krichevsky,O. and Libchaber,A. (1999) RecA polymerization on double-stranded DNA by using single-molecule manipulation: the role of ATP hydrolysis. *Proc. Natl Acad. Sci. USA*, **96**, 7916–7921.
11. Hegner,M., Smith,S.B. and Bustamante,C. (1999) Polymerization and mechanical properties of single recA–DNA filaments. *Proc. Natl Acad. Sci. USA*, **96**, 10109–10114.
12. Bennink,M.L., Scharer,O.D., Kanaar,R., Sakata-Sogawa,K., Schins,J.M., Kanger,J.S., de Grooth,B.G. and Greve,J. (1999) Single-molecule manipulation of double-stranded DNA using optical tweezers: interaction studies of DNA with recA and YOYO-1. *Cytometry*, **36**, 200–208.
13. Sattin,B.D. and Goh,M.C. (2004) Direct observation of the assembly of recA/DNA complexes by atomic force microscopy. *Biophys. J.*, **87**, 3430–3436.
14. White,J.H. (1969) Self-linking and Gauss-integral in higher dimensions. *Am. J. Math.*, **91**, 693–728.
15. Marko,J.F. and Siggia,E.D. (1995) Statistical-mechanics of supercoiled DNA. *Phys. Rev. E: Stat., Nonlinear, Soft Matter Phys.*, **52**, 2912–2938.
16. Strick,T.R., Charvin,G., Dekker,N.H., Allemand,J.F., Bensimon,D. and Croquette,V. (2002) Tracking enzymatic steps of DNA topoisomerases using single-molecule micromanipulation. *C. R. Phys.*, **3**, 595–618.
17. Strick,T., Allemand,J.F., Croquette,V. and Bensimon,D. (2000) Twisting and stretching single DNA molecules. *Prog. Biophys. Mol. Biol.*, **74**, 115–140.
18. Bouchiat,C. and Mezard,M. (1998) Elasticity model of a supercoiled DNA molecule. *Phys. Rev. Lett.*, **80**, 1556–1559.
19. Neukirch,S. (2004) Extracting DNA twist rigidity from experimental supercoiling data. *Phys. Rev. Lett.*, **93**, 198107.
20. Menetski,J.P. and Kowalczykowski,S.C. (1985) Interaction of recA protein with single-stranded DNA. Quantitative aspects of binding affinity modulation by nucleotide cofactors. *J. Mol. Biol.*, **181**, 281–295.
21. Mcentee,K., Weinstock,G.M. and Lehman,I.R. (1981) Binding of the recA protein of *Escherichia coli* to single-stranded and double-stranded DNA. *J. Biol. Chem.*, **256**, 8835–8844.
22. Strick,T.R. (1999) Mechanical supercoiling of DNA and its relaxation by topoisomerases. PhD Thesis, Universite Paris VI, Paris, France.
23. Allemand,J.F., Bensimon,D., Lavery,R. and Croquette,V. (1998) Stretched and overwound DNA forms a Pauling-like structure with exposed bases. *Proc. Natl Acad. Sci. USA*, **95**, 14152–14157.
24. Dame,R.T. and Goosen,N. (2002) HU: promoting or counteracting DNA compaction? *FEBS Lett.*, **529**, 151–156.
25. Ali,B.M.J., Amit,R., Braslavsky,I., Oppenheim,A.B., Gileadi,O. and Stavans,J. (2001) Compaction of single DNA molecules induced by binding of integration host factor (IHF). *Proc. Natl Acad. Sci. USA*, **98**, 10658–10663.
26. Lu,C., Scheuermann,R.H. and Echols,H. (1986) Capacity of recA protein to bind preferentially to UV lesions and inhibit the editing subunit (epsilon) of DNA polymerase III: a possible mechanism for SOS-induced targeted mutagenesis. *Proc. Natl Acad. Sci. USA*, **83**, 619–623.
27. Rosenberg,M. and Echols,H. (1990) Differential recognition of ultraviolet lesions by recA protein. Possible mechanism for preferential targeting of SOS mutagenesis to (6-4) dipyrimidine sites. *J. Biol. Chem.*, **265**, 20641–20645.
28. Cox,M.M., Goodman,M.F., Kreuzer,K.N., Sherratt,D.J., Sandler,S.J. and Marians,K.J. (2000) The importance of repairing stalled replication forks. *Nature*, **404**, 37–41.
29. Robu,M.E., Inman,R.B. and Cox,M.M. (2001) RecA protein promotes the regression of stalled replication forks *in vitro*. *Proc. Natl Acad. Sci. USA*, **98**, 8211–8218.
30. Postow,L., Ullsperger,C., Keller,R.W., Bustamante,C., Vologodskii,A.V. and Cozzarelli,N.R. (2001) Positive torsional strain causes the formation of a four-way junction at replication forks. *J. Biol. Chem.*, **276**, 2790–2796.