

# Transition dynamics and selection of the distinct S-DNA and strand unpeeling modes of double helix overstretching

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## ABSTRACT

Recent studies have revealed two distinct pathways for the DNA overstretching transition near 65 pN: ‘unpeeling’ of one strand from the other, and a transition from B-DNA to an elongated double-stranded ‘S-DNA’ form. However, basic questions concerning the dynamics of these transitions, relative stability of the two competing over-stretched states, and effects of nicks and free DNA ends on overstretching, remain open. In this study we report that: (i) stepwise extension changes caused by sequence-defined barriers occur during the strand-unpeeling transition, whereas rapid, sequence-independent extension fluctuations occur during the B to S transition; (ii) the secondary transition that often occurs following the overstretching transition is strand-unpeeling, during which the extension increases by 0.01–0.02 nm per base pair of S-DNA converted to single-stranded DNA at forces between 75 and 110 pN; (iii) even in the presence of nicks or free ends, S-DNA can be stable under physiological solution conditions; (iv) distribution of small GC-rich islands in a large DNA plays a key role in determining the transition pathways; and (v) in the absence of nicks or free ends, torsion-unconstrained DNA undergoes the overstretching transition via creation of S-DNA. Our study provides a new, high-resolution understanding of the competition between unpeeling and formation of S-DNA.

## INTRODUCTION

The DNA overstretching transition occurs at forces around 65 pN, after which the double helix becomes about 1.7 times the B-DNA contour length (1). The transition is sharp (a few pN wide) and cooperative near room temperature (1,2). A series of experiments have been used to support an interpretation that this transition is exclusively force-induced strand separation, resulting in single-stranded DNAs (ssDNA) (3–8). In these experiments, the dependence of the transition force on factors that affect base pair stabilities was reported to be in agreement with the strand-separation hypothesis. In addition, ssDNA binding proteins and glyoxal were found able to directly interact with overstretched DNA (7,8), suggesting that the overstretched state involves exposed base pairs. Notably, this ‘force-induced DNA melting’ interpretation of overstretching was initially proposed to involve two, parallel ssDNAs (3–5). However, subsequent theoretical analysis (9) and experiment (7,10) have suggested that force-driven strand separation occurs *via* ‘unpeeling’ of one strand from the other starting from a DNA nick or end, leaving one ssDNA to support the pulling force.

A separate series of experiments suggested that instead of always leading to force-induced melting, overstretching can result in formation of a novel double-stranded (ds) form of DNA called ‘S-DNA’. These experiments showed that: (i) the DNA tether was not broken after the transition in experiments where force was applied to the two opposing strands, as would be expected if strand separation were to occur (1); (ii) a second transition at a higher force was often observed that did lead to final strand separation after the 65-pN overstretching transition

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(11–14); (iii) S-DNA is only partially unwound, with a well-defined helix repeat near 35 bp (15); and (iv) the force-extension curve of S-DNA was inconsistent with that of either one or two ssDNAs (9). Despite these inconsistencies between experiments observing S-DNA and an interpretation of those experiments in terms of force-induced melting, advocates of force-induced melting have asserted that the overstretching transition is always strand separation (16).

The conflicts between the two views were largely resolved by a recent study that revealed the existence of two distinct overstretching transitions: a slow hysteretic ‘unpeeling’ of one strand off the other, and a fast, non-hysteretic transition to an elongated ds form, i.e. formation of S-DNA (17). Either can be selected or can co-exist via small changes in salt concentration, temperature, and DNA sequence composition (17). Overall, higher salt concentration, lower temperature, and higher GC base pair percentage can bias the transition toward forming S-DNA, in general agreement with prior theoretical predictions (9,18,19). This result suggests that special attention must be paid to temperature control when optical tweezers are used in such experiments (20,21), since a temperature increase by only a few degrees Celsius can completely switch the transition from forming S-DNA to causing unpeeling (17). Similarly, caution should be applied when ssDNA binding ligands or proteins are used, since they likely can bias the transition toward the strand-unpeeling transition.

Despite the elucidation of the existence of two distinct types of overstretching transition, many other important questions remain unanswered: (i) The strand-unpeeling/re-annealing process requires the unpeeling fork between the ssDNA and B-DNA regions to move forward/backward (see sketch in Supplementary Figure S1), while the B to S transition does not. This suggests a distinction between the unpeeling and B to S transition dynamics which has not been

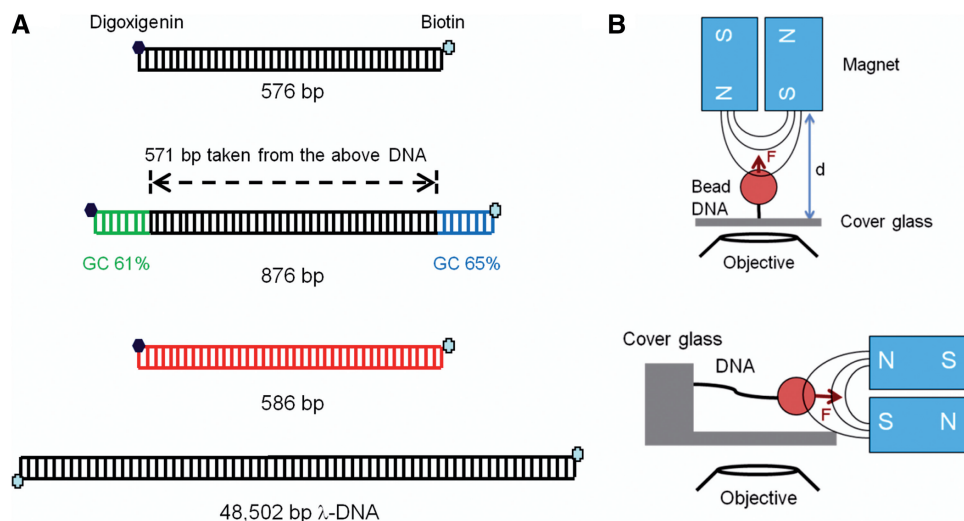
systematically investigated. (ii) A secondary transition often occurs following the overstretching transition (11,13). Whether it is a strand-unpeeling transition directly from B-DNA or from S-DNA is unclear (7,16). (iii) The properties of S-DNA have not been carefully studied. In particular, it has been questioned whether S-DNA is merely a transient intermediate state before the DNA is finally unpeeled. (iv) It remains unknown whether nicks or free ends are necessary for DNA overstretching at  $\sim 65$  pN.

Since extended double helices and strands displaced from a nick are both transition states in DNA recombination, replication and repair processes, understanding the tendency of different sequence and different conditions to control the relative stability of these overstretching modes is of basic biological importance. The above outstanding questions have become gaps persisting in our understanding of the overstretching transition and the resulting over-stretched DNA structures. In this article, these questions are answered by overstretching various DNA constructs under different conditions using magnetic tweezers.

## MATERIALS AND METHODS

### DNA constructs

The four DNA constructs used in our experiments are shown in Figure 1A: (i) 48 502 bp  $\lambda$ -DNA (New England Biolabs), (ii) a 576 bp DNA of 53% GC content, (iii) a 586 bp DNA of 60% GC content and (iv) an 876 bp DNA that contains a central 571 bp sequence identical to that in the 576 bp DNA. Two GC-rich DNA ‘handles’, biotin labeled 153 bp DNA (GC = 61%) and digoxigenin labeled 152 bp DNA (GC = 65%), were ligated to each end of this 571 bp DNA. For all the short DNA constructs, biotin and digoxigenin were labeled to the two ends of the same DNA strand. Therefore, the force is applied by 3’–5’ stretching. For the longer  $\lambda$ -DNA, the force is applied to the two opposite strands (3’–3’). More



**Figure 1.** (A) DNA constructs. (B) Sketches of the magnetic tweezers used in the experiments. The vertical magnetic tweezers (top) were used to stretch short DNA, while the transverse magnetic tweezers (bottom) were used for stretching long  $\lambda$ -DNA.

details of construct fabrication are in 'DNA constructs' in the Supplementary Data.

### Magnetic tweezers measurements

A vertical magnetic tweezers setup was used to stretch the short DNA constructs (Figure 1B). The digoxigenin end of the DNA was fixed to an anti-digoxigenin (Roche) coated cover glass surface, and the biotin end of the DNA was fixed to a streptavidin coated 2.8- $\mu\text{m}$  paramagnetic bead (Dynal M-280, Invitrogen). The bead position in the focal plane was determined by the self-correlation method at a resolution of  $\sim 2$  nm (22). The bead position perpendicular to the focal plane was determined at resolution  $\sim 2$  nm by analyzing the diffraction pattern of the defocused bead image at different defocusing planes (22). More details are in 'Vertical magnetic tweezers' in the Supplementary Data.

Forces were applied to the opposite strands (3'-3') of the  $\lambda$ -DNA by a transverse magnetic tweezers setup (23). One biotin end of the DNA was fixed to a streptavidin-coated edge of a #0 cover glass, and the other was attached to an 8.2-micron-diameter streptavidin-coated paramagnetic bead (Bangs Laboratories Inc.) to achieve high force. The DNA was inside a narrow flow channel so that the buffer could be conveniently replaced. A permanent magnet outside the channel applies controlled forces from 0.04 up to 180 pN to the paramagnetic bead in the focal plane, and the extension of DNA was determined to be the distance from the bead to the edge of the cover glass in the force direction (Figure 1B). The persistence length of DNA was measured in the force range 1–10 pN by fitting force-extension data to the expected response for a worm-like chain (24). The DNA was determined to be a single DNA if the measured persistence length was 44–53 nm. Temperatures below room temperature were achieved using an ice-water container on the top of the flow channel and were measured using the TM-902C Prober Digital Thermometer (Lutron). More details of the setup can be found in our previous article (17).

### Kinetics simulation of unpeeling dynamics

The kinetics simulation was performed assuming strand-unpeeling from B-DNA using Gillespie's stochastic kinetics simulation algorithm (25). In our model, tensile force was applied to one strand of a dsDNA. Meanwhile, the DNA has two free ends, so two ways of unpeeling that start from two ends are considered according to fork dynamics (26). The conformational free energy of B-DNA was determined by the extensible worm-like-chain model (2,9,24), and the conformational free energy of ssDNA was determined by the model in (1,9,26). The B-DNA base pairing and stacking energy was determined by Santa Lucia's model that includes sequence, salt and temperature dependence (27). The details of the simulation can be found in Supplementary Information S1.

## RESULTS

### Two distinctive modes of overstretching: irreversible unpeeling versus reversible formation of S-DNA

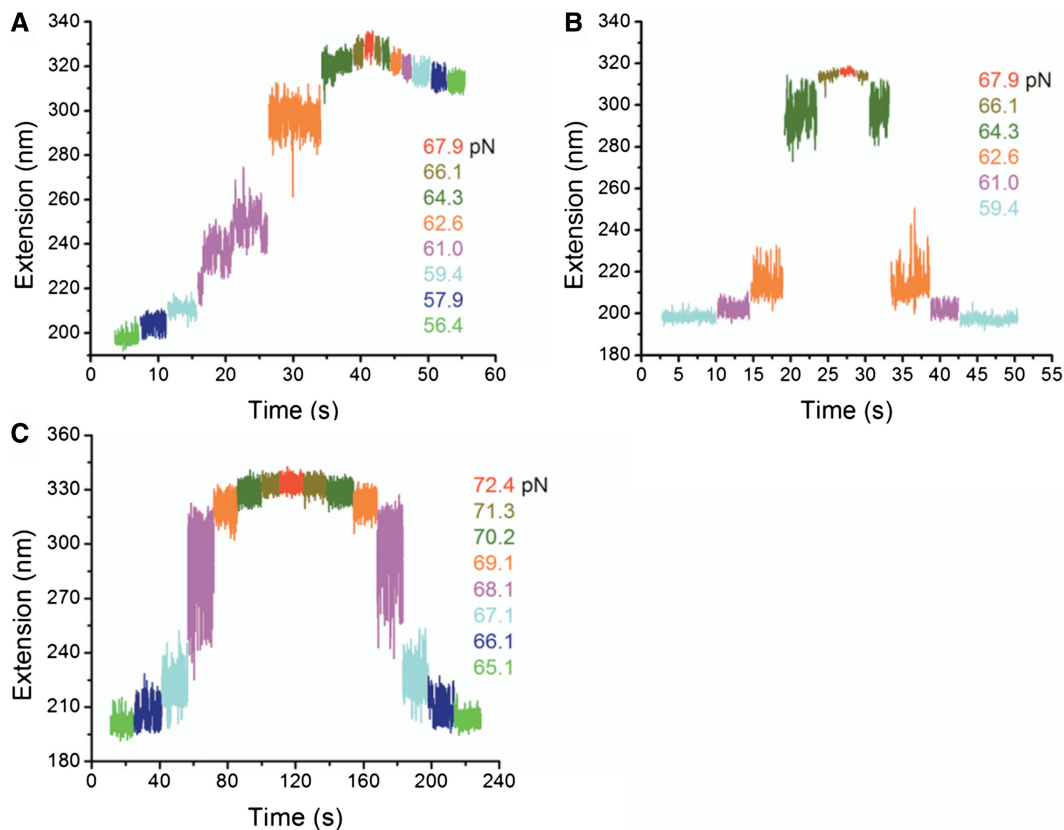
We first investigated how short DNAs respond during the unpeeling transition and the B to S transition. Beginning with a moderate-salt buffer favoring unpeeling, 10 mM Tris pH 7.5, 150 mM NaCl and 24°C, we slowly increased the force through the overstretching transition for a 576 bp DNA that contained 53% GC base pairs. We paused for a few seconds or more at each force level; we then decreased force through the same set of force levels. Figure 2A shows the whole time course of one such experiment. After the transition the tether became an ssDNA, indicated by the non-reversibility as force was reduced through the same set of forces. Given that 3'- and 5'-ends of the same strand are tethered, this experiment shows that in the moderate-salt buffer, the non-tethered strand unpeeled from the tethered strand at a force near to 65 pN.

Figure 2B shows that overstretching of the same DNA in 1 M NaCl (other conditions were unchanged) became completely reversible. This transition could not be explained by strand unpeeling or formation of two, parallel ssDNAs, since then an ssDNA tether should be expected after the transition. The overstretched molecule was not a mixture of ssDNA and B-DNA either, as this would have resulted in hysteresis during the reverse transition. Further, it could not be a mixture of two, parallel ssDNAs and B-DNA, because the resulted extension would be much shorter than that observed in experiments. The reversible transition could also be obtained by increasing the GC percentage. As shown in Figure 2C, a 586 bp DNA containing 60% GC base pairs in 150 mM NaCl and 24°C displayed the same behavior as in Figure 2B. These experiments (Figure 2A–C) unambiguously indicated two distinct overstretched DNA structures: ssDNA via the strand-unpeeling transition when DNA base pairs are less stable and S-DNA via the B to S transition when DNA base pairs are more stable. These results were in agreement with our recently reported findings obtained from stretching a 48 502 bp  $\lambda$ -DNA (17).

### Distinctive transition dynamics of unpeeling and S-DNA formation

Earlier experiments for long DNA have shown that the strand-unpeeling transition is slow and hysteretic (17). The cause of the slow kinetics in the unpeeling transition was hypothesized to be the energy barriers present in the transition due to sequence heterogeneity. This hypothesis was examined in detail by recording extension at constant forces in the range where overstretching occurred (Figure 3A). This revealed interesting stepwise dynamics under various constant forces during the transition plateau, similar to that seen in DNA/RNA unzipping experiments (28,29).

In order to understand the stepwise dynamics, we carried out kinetic simulations for strand-unpeeling starting from the free ends of the DNA (Supplementary Figures S1–S3 and Supplementary Information S1).



**Figure 2.** Overstretching transition of short DNA in 10 mM Tris buffer (pH 7.5, 24°C) at different salt concentrations. (A) DNA (576 bp) (GC 53%) in 150 mM NaCl; in these moderate-salt conditions, force-induced melting occurs leading to strong hysteresis. (B) The same 576 bp DNA as (A) in 1 M NaCl; these conditions suppress melting, and a reversible overstretching transition is observed. (C) DNA (586 bp) (GC 60%) in 150 mM NaCl; the sequence composition suppresses force-induced melting at physiological salt concentration, and a reversible transition is observed. Each force is denoted by a unique color in each panel.

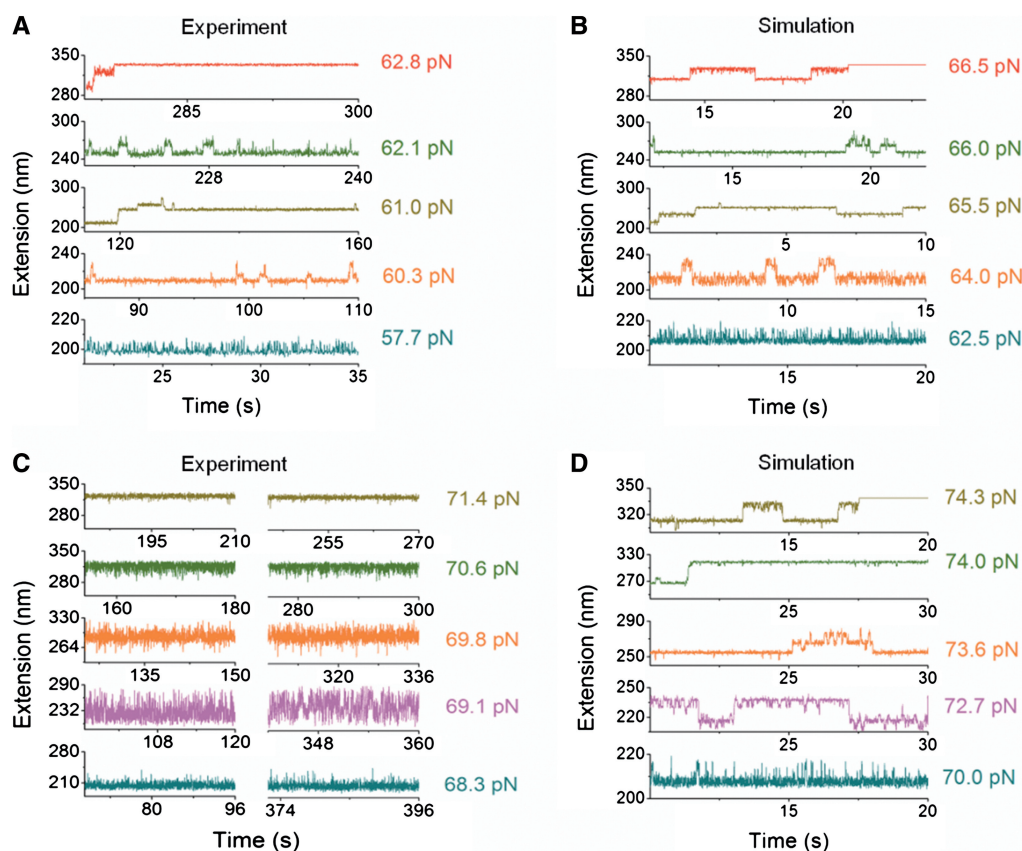
Figure 3B shows that, for parameters corresponding to the experimental conditions of Figure 3A, similar stepwise dynamics were observed in the simulation. The agreement between experiment and simulation suggested that the stepwise dynamics was resulted from overcoming sequence-dependent energy barriers in the unpeeling process from DNA ends. In contrast, the time courses recorded in 1 M NaCl completely lacked such stepwise dynamics (Figure 3C). However, the corresponding strand-unpeeling kinetic simulation (Figure 3D) carried out with parameters corresponding to experimental conditions of Figure 3C still predicted stepwise dynamics, but at higher forces due to more stable DNA base pairs at higher salt concentration. We emphasize that formation of S-DNA was not included in our simulation. The disagreement between Figure 3C and Figure 3D indicates that the transition in Figure 3C was not strand-unpeeling of B-DNA.

#### The secondary transition is unpeeling from S-DNA

A secondary transition often occurs following the 65 pN overstretching transition which results in unpeeling from nicks or free ends of DNA (11,13). Depending on the pulling rate and sequence, the transition plateau can be much larger than 65 pN (slower pulling biases toward

smaller secondary transition force). Such a secondary transition has often been observed for DNA containing significant GC percentage, while AT-rich DNA goes through a single unpeeling-like transition (11,13). Although it is clear that this secondary transition is a strand-unpeeling transition, it has been debated whether it is unpeeling from S-DNA or directly from small remaining B-DNA islands that prevent the two DNA strands from being dissociated. In other words, there has been a debate over whether the observation of the secondary transition can be used as evidence to support the existence of S-DNA (7,10,16).

In order to answer this question, we investigated the extension increase per base pair of S-DNA converted to unpeeled DNA at forces in the range of 75–110 pN at 24°C. To do this, we increased the force from 61 to >75 pN in <0.3 s, during which the DNA was over-stretched (as an example, Figure 4A shows part of a time course obtained from cycling between 61 and 110 pN). Following this, the DNA was held at the higher force for a few seconds or more, during which the secondary transition occurred as indicated by the gradual elongation at the higher force. This secondary transition was an unpeeling transition, and the amount of base pairs unpeeled determined the number of melted base pairs to be re-annealed when the force was dropped



**Figure 3.** Detailed dynamics of 576 bp DNA during the overstretching transition in 10 mM Tris buffer (pH 7.5, 24°C) at different salt concentrations. (A) Stepwise dynamics observed in an experiment in 150 mM NaCl (see the full-time course in Supplementary Figure S4). (B) Similar stepwise dynamics observed for the same DNA sequence in a kinetic simulation of unpeeling of B-DNA in 150 mM NaCl (see details in Supplementary Data). (C) Rapid fluctuation dynamics observed for experiment in 1 M NaCl (see the full-time course in Supplementary Figure S5). (D) Stepwise dynamics observed during kinetics simulation of unpeeling of the same DNA sequence in 1 M NaCl. The transition occurred at higher forces due to more stable base pairs in 1 M salt concentration.

back down to 61 pN. Therefore, cycling between the two forces allowed us to obtain the correlation between the extension at the higher force right before dropping the force, and the extension right after dropping the force which marked the re-annealing starting position. Figure 4B shows that a linear relationship exists between the two extensions for the data in Figure 4A.

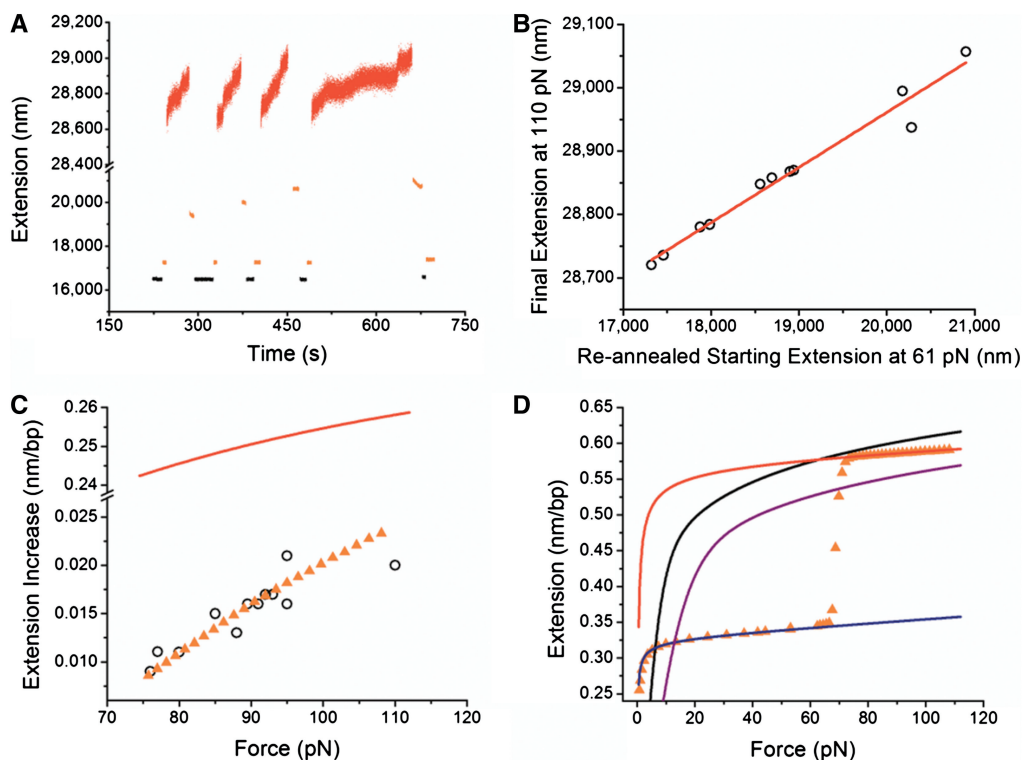
A linear relationship was also observed for cycling between 61 pN and other forces >75 pN (Supplementary Figure S6). From the fitting slopes, the extension increase by converting one base pair of the overstretched DNA at the higher force to an ssDNA nucleotide was determined to be 0.01–0.02 nm/bp in the force range of 75–110 pN (empty circles in Figure 4C. Also see Supplementary Information S2 for the method of conversion). This value was one order of magnitude smaller than the predictions assuming that it is unpeeling from a B-DNA (red curve in Figure 4C. Also see Supplementary Information S2), suggesting that this secondary transition must be unpeeling from S-DNA rather than from B-DNA.

The extension increase by converting one base pair of S-DNA to an ssDNA nucleotide could also be obtained by comparison of the force-extension curve of the S-DNA and the ssDNA. Figure 4D shows the force extension

curve of a DNA recorded from 1 to 110 pN recorded at 17°C. In the force range of 75–110 pN, the DNA was nearly pure S-DNA, indicated by the negligible re-annealing signal (Figure 5B). The difference of the force-extension between the ssDNA and the measured S-DNA was plotted in Figure 4C (orange triangles) (see Supplementary Information S2 for the ssDNA force-extension model), which was comparable to the values obtained by direct unpeeling from S-DNA (empty circles). In Figure 4D, the extensible WLC model was used to fit the data measured before (blue) and after (red) the transition. In addition, the theoretical force-extension of ssDNA was plotted to compare with that of S-DNA (see Supplementary Information S2 for the ssDNA force-extension model).

### Stability of S-DNA

Another point of debate concerns the question of whether S-DNA can be a stable state or just a short-lived transient intermediate structure before the DNA is finally unpeeled (10,16). To address this question, we overstretched a  $\lambda$ -DNA in 150 mM NaCl, 10 mM Tris pH 7.5 at 17°C and held it at a constant force of 83 pN. As shown in Figure 5A, we did not observe an apparent secondary



**Figure 4.** The secondary transition and the mechanical response of S-DNA. (A) Cycling between 61 pN (orange) and 110 pN (red) for a  $\lambda$ -DNA molecule in 150 mM NaCl, 24°C. A lower force of 35 pN (black) was used to facilitate the re-annealing. (B) A linear relationship exists between the extensions just before and just after reducing force from 110 to 61 pN. The red line shows the linear fitting of the data (circles) with a slope of 0.085. For the same DNA, cycling between 61 pN and various other forces larger than 75 pN yielded linear relations with different slopes (Supplementary Figure S6). (C) The extension increase associated with unpeeling one base pair of the overstretched DNA was calculated from the fitting slopes (circles). The solid triangles show the extension difference between ssDNA and S-DNA plotted in Figure 4D. The red line shows the theoretical extension difference between ssDNA and B-DNA. (D) Force-extension curve of  $\lambda$ -DNA measured in 150 mM NaCl at 17°C (solid triangle). The blue curve shows the fitting by the extensible WLC model with persistence length of 46 nm, stretching modulus of 1230 pN, and contour length per base pair 0.332 nm/bp. The red curve shows the fitting by the extensible WLC model with a persistence length of 11.6 nm, stretching modulus of 3030 pN, and contour length per base pair of 0.58 nm/bp. The black curve shows the fitting by the ssDNA force-extension curve using formula in ref. (9). The purple curve shows the force-extension curve of two, non-interacting parallel ssDNAs.

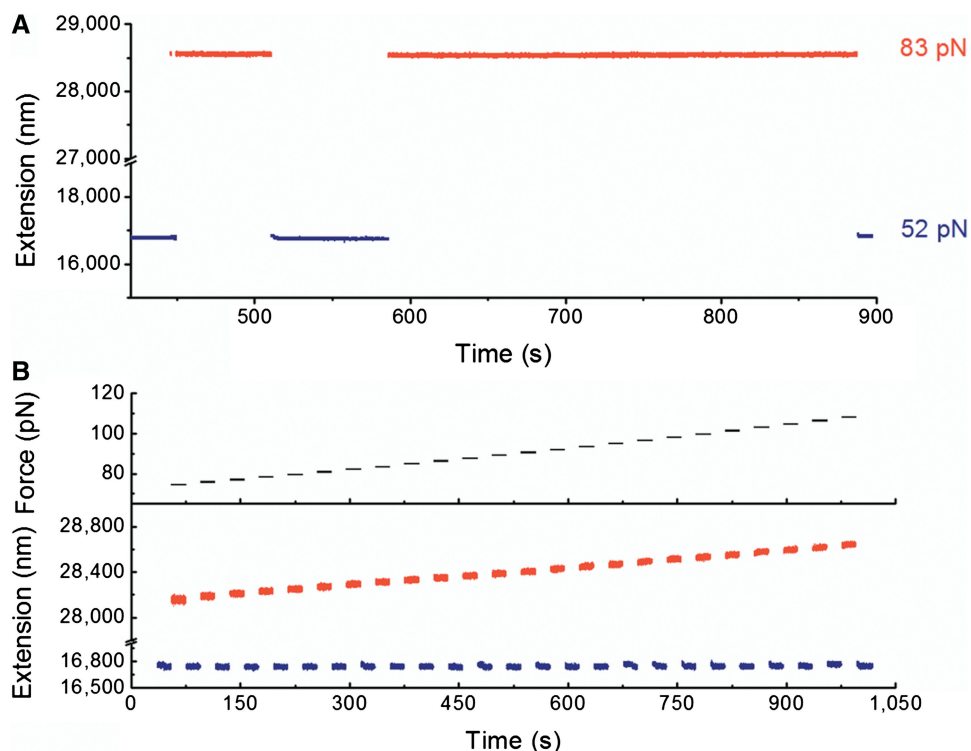
unpeeling transition over a time scale up to five minutes (it was difficult to record for a longer time scale due to biotin-streptavidin linkage breakage at such high force). Accordingly, when force was dropped to <60 pN, the DNA extension immediately returned to the B-DNA extension without significant re-annealing. This result suggested that the S-DNA is a stable structure that can exist over at least a time scale of minutes. Figure 5B shows that, in 150 mM NaCl and 17°C, S-DNA was stable over a wide force range seen by cycling between 53 pN and various forces in the range of 75–110 pN. At each force >75 pN, we held the DNA for 20 s to check whether there was the secondary transition, and then the force was dropped to 53 pN to check whether there was re-annealing transition. We found the unpeeling from S-DNA was negligible until force was increased to above 110 pN.

#### Stabilization of S-DNA in an unnicked molecule with GC-rich ends

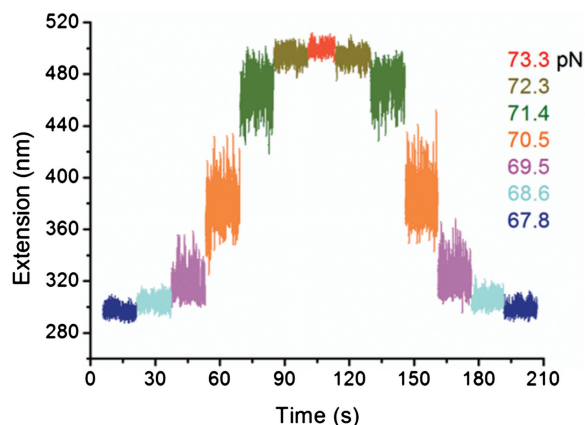
It was reported that torsion-constrained DNA that did not have free ends and nicks could not undergo the overstretching transition at ~65 pN (7,15). This raised a question as to whether free ends or nicks in DNA are

necessary for the 65-pN overstretching transition (7,16). In Figure 2A, we showed that the 576 bp DNA (53% GC) underwent the strand-unpeeling transition in 150 mM NaCl and 24°C; while under the same condition, we showed that the 586 bp GC-rich DNA (60%) converted to S-DNA (Figure 2C). This made it possible to construct a new 876 bp DNA which was essentially the 576 bp DNA sandwiched between two short GC-rich DNA handles (>60% GC) at its ends. These two DNA handles were used to make sure that strand-unpeeling could not occur from the two ends of DNA in 10 mM Tris pH 7.5, 150 mM NaCl and 24°C. We then ask whether the 576 bp DNA in the middle, which does not have free ends but torsion unconstrained, still can undergo the 65-pN transition.

Figure 6 shows that the 876 bp DNA did undergo the 65 pN transition, via the non-hysteretic reversible B to S transition. The extension increased ~200 nm after the transition, to about 1.7 times the B-form contour length of the entire molecule. Therefore, the 576 bp DNA in the middle must be overstretched to explain this extension change. In addition, due to the end blocking by the two GC-rich handles, unpeeling of the 576 bp DNA was not possible. This experiment shows that



**Figure 5.** S-DNA stability. (A) Force cycling between 52 pN (blue) and 83 pN (red) for a  $\lambda$ -DNA in 150 mM NaCl at 17°C. The DNA was held at 83 pN for up to 5 min to study its stability. (B) Force cycling between 53 pN (Blue) and various forces from 75 to 110 pN (red) for another  $\lambda$ -DNA in 150 mM NaCl at 17°C. At each force >75 pN, the DNA was held for 20 s. The top panel in (B) shows the values of forces >75 pN.



**Figure 6.** The overstretching transition of 876bp DNA in 150 mM NaCl at 24°C. Note that this DNA consists of 571 bp taken from the 576bp DNA flanked by GC-rich DNA segments (Figure 1A). Each force is denoted by a unique color in each panel. The GC-rich segments eliminate the possibility of unpeeling, and a reversible transition to S-DNA is observed in the whole molecule under physiological conditions.

torsion-unconstrained DNA without free ends or nicks still could undergo the overstretching transition at 65 pN but only via the B to S transition pathway. Equally importantly, this result showed that not only the overall percentage of GC base pairs of a DNA but also the distribution of the GC-rich islands in the DNA play critical roles in determining the transition pathway, which is in

agreement with the previous theoretical prediction that the sequence design can be used to tune the transition pathway during overstretching (30). Finally, we noted that the 876 bp DNA and the 586 bp GC-rich DNA could undergo the stand-unpeeling transition when the base pair stability was reduced by decreasing salt concentration to 10 mM NaCl (Supplementary Figure S7).

## DISCUSSION

By stretching short DNAs of only a few hundred base pairs using vertical magnetic tweezers, a dramatic distinction in dynamics between the unpeeling transition and the B to S transition was revealed with a spatial resolution of a few nanometers and a temporal resolution of  $\sim 10$  ms. In the unpeeling transition, a stepwise extension change was observed, consistent with sequence-defined barriers in the strand unpeeling process. At forces in the B to S transition, sequence-independent and rapid extension fluctuations were observed, suggesting that the transition does not require crossing of significant energy barriers and occurs locally, i.e. as a structural transformation of the double helix. Such sequence-independent and rapid extension fluctuations are in agreement with an earlier report that showed  $\sim 65$  pN overstretching transition both in lambda DNA and CG-rich DNA (13).

Importantly, we show that a nick-free DNA that undergoes the strand unpeeling transition can be switched to overstretching to form S-DNA under the

same solution conditions when its two ends are blocked and the DNA remains torsionally unconstrained. Equally importantly, our results suggest that a pair of small GC-rich islands can impose a constraint on the mode of overstretching that occurs in a nick-free DNA of arbitrary sequence between them, resulting in preferential selection of the B to S transition over the strand-unpeeling transition under conditions where the GC-rich islands do not unpeel. We conclude that force-induced melting without torsional constraint requires a DNA nick or equivalently a free end, and that the sequence composition of the molecule at the nick or end must favor strand separation.

Our results reveal that the secondary transition seen at forces well  $>65$  pN is an unpeeling transition starting from S-DNA (Figure 4). This resolves another debate regarding the nature of the secondary transition as to whether it is unpeeling from remaining B-DNA islands or from S-DNA (7,10,16). The secondary transition under constant forces in the range of 75–110 pN allow us to directly determine the extension increase by unpeeling one S-DNA base pair into one ssDNA nucleotide to be 0.01–0.02 nm/bp. We also show that this value was in agreement with the comparison between the force-extension curve of pure S-DNA and that of ssDNA.

Our results also addressed an important question regarding whether S-DNA is merely a transient structure or a stable structure within a reasonably long experimental time scale. We showed that at 17°C, the  $\lambda$ -DNA in S-form was stable in the force range of 75–110 pN for a few minutes which was the longest time scale we could achieve in the experiments before the DNA anchors became unstable. These results suggest that even in the presence of nicks or free ends, S-DNA can still be a stable structure in physiological conditions.

Overall, we have addressed and answered several important questions related to the nature of the 65-pN DNA overstretching transition, the secondary transition, and the mechanical properties of the overstretched DNA. Under physiological condition, our results suggest that the S-DNA is a stable structure in a wide force range up to 110 pN. We have not addressed the structure of S-DNA, but insight into possible structures of S-DNA have been obtained by full-atom molecular simulations and coarse-grained models (2,31,32). These calculations suggest that an overstretched dsDNA can exist with highly inclined base pairs and a narrower helical backbone, or as an under-wound flat ribbon structure. These results were in agreement with an experiment that showed that DNA overstretching required under-winding of the DNA double helix (15).

## SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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*Conflict of interest statement.* None declared.

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