The RSC chromatin remodelling ATPase translocates DNA with high force and small step size

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ATP-dependent chromatin remodelling complexes use the energy of ATP hydrolysis to reposition and reconfigure nucleosomes. Despite their diverse functions, all remodelers share highly conserved ATPase domains, many shown to translocate DNA. Understanding remodelling requires biophysical knowledge of the DNA translocation process: how the ATPase moves DNA and generates force, and how translocation and force generation are coupled on nucleosomes. Here, we characterize the real-time activity of a minimal RSC translocase ‘motor’ on bare DNA, using high-resolution optical tweezers and a ‘tethered’ translocase system. We observe on dsDNA a processivity of ~35 bp, a speed of ~25 bp/s, and a step size of 2.0 (± 0.4, s.e.m.) bp. Surprisingly, the motor is capable of moving against high force, up to 30 pN, making it one of the most force-resistant motors known. We also provide evidence for DNA ‘buckling’ at initiation. These observations reveal the ATPase as a powerful DNA translocating motor capable of disrupting DNA–histone interactions by mechanical force.

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Introduction

Remodellers antagonize DNA–histone interactions to reposition nucleosomes, alter nucleosomal DNA configurations, change histone compositions, or eject histones from DNA (Hamiche *et al*, 1999; Langst *et al*, 1999; Whitehouse *et al*, 1999; Boeger *et al*, 2004; Mizuguchi *et al*, 2004; Smith and

Peterson, 2005; Yang *et al*, 2006; Clapier and Cairns, 2009; Dechassa *et al*, 2010). Current mechanistic models for chromatin remodelling depend on the magnitude of the DNA translocation force provided by remodeller motors relative to the resistant force imposed by the histone–DNA contacts (Luger *et al*, 1997). A low driving force can remodel nucleosomes, but requires either of the two constraints in the remodelling mechanism: (1) the remodeller disrupts only a portion of the DNA–histone contacts at a time via translocation to form a DNA ‘loop/wave’, which then propagates around the histone surface (Langst *et al*, 1999; Saha *et al*, 2005), or (2) the remodeller globally weakens DNA–histone interactions upon binding nucleosomes (Chaban *et al*, 2008; Lorch *et al*, 2010; Shukla *et al*, 2010), which then translocates DNA over the entire octamer surface, thus bypassing a DNA loop intermediate. In contrast, a high DNA translocation force enables more diverse remodelling mechanisms and functions, as many or all DNA–histone interactions can be disrupted in either a stepwise or a concerted manner (Clapier and Cairns, 2009; Bowman, 2010). Therefore, the magnitude of the forces generated by remodellers informs and limits the spectrum of their possible mechanisms and functions. Furthermore, the step size of RSC translocating DNA has not been well characterized, which may set the minimum size of DNA loops/waves present on the histone surface as remodelling intermediates, or DNA slid over the surface per ATP hydrolysis (Langst and Becker, 2001; Saha *et al*, 2005; Clapier and Cairns, 2009). Thus, direct measurements of the driving and resistant forces and other DNA translocation properties will provide important insights into the molecular mechanisms and possible biological functions of chromatin remodelling. The forces required to mechanically disrupt the nucleosome structure have been well characterized, which range from 10 to 30 pN (Brower-Toland *et al*, 2002). However, the driving forces generated by remodellers have not been well measured.

We recently utilized optical tweezers to characterize DNA translocation parameters and driving forces for the SWI/SNF and RSC remodelers on nucleosomal DNA (Zhang *et al*, 2006). Related work involved a study of translocation parameters by RSC complex on bare DNA using magnetic tweezers. These two studies differed greatly in their measurements of RSC translocation velocity (13 versus >200 bp/s) and processivity (100 versus 700 bp), and the ability to translocate against force. Our prior studies revealed resistance to forces of ~12 pN, whereas other studies did not observe translocation events at forces >~1 pN (Supplementary Table S1). Here, the differences could involve the alternative systems for measurement, substrates, or preparations of the 15-subunit RSC complex. Particularly, the histone octamer in the nucleosomal context may affect the translocation properties of the remodeller motor, or help tether the RSC complex to the histone octamer, which may provide greater

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ability to create a constrained loop (see below) on nucleosomal substrate than on bare DNA. To help resolve these different measurements for the same remodeller, we developed a novel ‘tethered’ motor assay that allows us to monitor the translocation kinetics of the RSC ATPase with unprecedented resolution based on high-resolution optical tweezers. Using this new assay, we clarify the different measurements, provide a new maximum force parameter, characterize the kinetic step size of the motor, and identify a new translocation initiation process indicative of DNA buckling.

Results

Experimental setup

To help clarify the true biophysical properties of RSC and reconcile the disparate measurements, we reasoned it crucial to determine the DNA translocation properties of a ‘core’ translocation module derived from recombinant versions of RSC components. Pilot experiments involving deletion derivatives of the RSC ATPase subunit Sth1, along with previous work (Yang *et al*, 2007; Szerlong *et al*, 2008), defined a core module that comprises amino acids 301–1097 of Sth1 and two actin-related proteins (Arp7 and Arp9), which bind to Sth1 and are required for Sth1 stability and ATPase activity

(Szerlong *et al*, 2008) (Figure 1A). To monitor translocation on bare DNA and the accompanying force generation, we developed a novel ‘tethered’ translocase system. This system involves the fusion of the Sth1 ATPase fragment to the site-specific DNA-binding protein TetR (Figure 1A), which normally binds as a homodimer to its cognate binding site (tetO) with subnanomolar affinity (Orth *et al*, 2000). Co-expression of four proteins in bacteria, including tagged TetR–Sth1–FLAG fusion, a 7xHis–TetR derivative and both Arp7 and Arp9, followed by double affinity tandem purification (Ni-NTA and anti-FLAG), yielded a pure four-protein complex: Sth1–ARPs–TetR, termed StART (Figure 1A and B). This strategy generated a TetR heterodimer with a single ATPase motor capable of binding a single tetO site in the middle of a long DNA molecule. Bulk assays showed that the DNA-dependent ATPase activity ($V_{\max}$) of StART is similar to the complete RSC complex.

Next, we used high-resolution dual-trap optical tweezers (Supplementary data; Supplementary Figure S1) to monitor the translocation kinetics of single StART complexes. In a typical experiment, a single, tetO-containing DNA molecule was tethered between two polystyrene beads in a torsionally unconstrained manner and stretched to a tension of 1–3 pN. Then, StART was added at a low concentration (≤ 10 nM)

Figure 1 The tethered minimal RSC complex translocates along bare DNA. (A) Experimental setup (not drawn to scale). Shown in the centre is a scheme of the subunit composition for the tethered minimal RSC complex, Sth1–Arp7–Arp9–TetR, or StART complex. The histidine and Flag tags used for protein purification are also indicated. A single StART complex was specifically tethered to DNA through TetR. The DNA molecule was attached between two beads each held in an optical trap. As the remodeller translocase moves along the DNA template, a loop is formed, resulting in a decrease in the end-to-end distance and an increase in the tension of the DNA molecule, which was detected in real time using high-resolution optical tweezers. (B) In all, 12% SDS–PAGE gel of purified StART complex, stained with Coomassie blue, with components are listed at right. (C) The remodeller motor generated a series of prominent spikes (marked with asterisks) in a representative time-dependent DNA tension or contour length (insets) trace. Throughout this work, the contour length refers to the DNA segment directly stretched by optical traps, excluding the possible contribution from the looped DNA portion. The translocation speeds and distances are calculated from linear fits (red solid lines) of the translocation phases and sizes of the resultant loops (Figure 2), respectively. Note that the translocation speed generally remains the same in the different rounds of translocation within the same burst, but varies significantly among different bursts (insets).

such that a single StART complex bound tetO at a proper frequency. As the remodeller ATPase 'motor' Sth1 translocates along DNA, it shortens the end-to-end distance of the DNA to form a loop, pulls the attached beads away from their corresponding trap centres, and compels the motor to move against an increasing force proportional to the bead displacement. Thus, the kinetics of motor translocation is detected by the accompanying DNA end-to-end distance and tension changes in real time.

Translocation kinetics of the tethered RSC motor

Figure 1C shows representative DNA tension or contour length (Figure 1C, insets) changes induced by StART in the presence of 2 mM ATP and nominally (Zhang *et al*, 2006) 10 nM StART, while both traps were kept in fixed positions. After StART addition (within 100 s), the quiet force baseline at ~ 2.7 pN was interrupted by individual or bursts of upward spikes (marked by red stars) with variable amplitudes. Each spike contains a phase of approximately continuous force increase, or length decrease (Figure 1C, insets), followed by a sudden drop in force, or jump in length, to the respective baseline. The overall occurrence frequency of these spikes was 0.66 per minute measured over an accumulated detection time of 431 min under the same experimental conditions. This frequency decreased with a decrease in the StART concentration, whereas the average size of the spikes remained the same within experimental error, indicating that each spike was induced by a single StART complex. Further control experiments showed that both the frequency and average size of the spikes became negligible when TetR was removed or ATP was omitted (≤ 0.03 per minute based on accumulated measurement time of > 138 min). Taken together, we conclude that the spikes were generated by ATP-dependent remodeller translocation along DNA (see also Figure 2) while tethered to the tetO site, consistent with the expectation from our experimental design.

The sudden drop to the baseline observed in a spike suggests complete and instantaneous loop dissipation, due to dissociation of the motor and/or the TetR from the DNA molecule. If only the motor disengaged, it could quickly rebind to DNA and start translocation again, generating a burst of spikes (Figure 1C, insets). Indeed, about 60% of the spikes appeared in such a burst mode. In contrast, if the StART complex completely dissociates from DNA, a delay is expected until a new complex occupies the tetO site and generates new spikes, because of the low StART concentration under our experimental conditions. This likely underlies the relatively long time interval between individual or burst spikes (Figure 1C). Finally, besides the common sudden DNA loop dissipation, $\sim 9\%$ of the loops were partially or completely released through an alternative mode, a continuous process with approximately the same speed and ATP-dependence as the translocation that produced the loops (Figure 3). Therefore, the continuous loop dissipation mode has properties consistent with active translocation of the remodeller, but with a reversal in its translocation direction (or reverse translocation). This dynamic switch in translocation direction has been found for the full RSC complex and other motors (Lia *et al*, 2006; Zhang *et al*, 2006; Abbondanzieri *et al*, 2008).

We also obtained histogram distributions of the speed and distance of remodeller translocation at different ATP concentrations (Figure 2) by scoring all the spikes described above

Figure 2 Speed and processivity of the remodeller motor translocation along DNA. (A) Speed distributions of the remodeller translocation at ATP concentrations of 2 mM (black square), 0.1 mM (red circle), and 0.075 mM (blue triangle), respectively. Each distribution can be fitted by a Gaussian function (solid line), with its centre designated as the average speed. Shown in the insert is the ATP-dependent average speed (black symbols with error bars as standard errors), which can be fitted with a Michaelis-Menten equation (red line). (B) Size distributions of the DNA loops generated by the motor translocation at three ATP concentrations. Each distribution can be fitted with a single-exponential function (with the first point corresponding to the smallest loop size excluded). In the last bin were grouped all loops with sizes > 250 bp.

in the DNA length-time traces. We found that the remodeller motor does not lose its speed when moving against high forces (Figure 1C), as was observed previously for the full RSC complex (Zhang *et al*, 2006) and several other motors such as RNA polymerases (Galbur *et al*, 2007). Interestingly, different single StART complexes tend to have significantly different speeds (Figure 1C, insets), indicating heterogeneities among enzymes, a property often seen in single-molecule experiments (Lu *et al*, 1998; English *et al*, 2006). Overall, the speed approximately follows a Gaussian distribution with a centre (or an average) of $25.0 (\pm 0.7, \text{s.e.m.})$ bp/s at 2 mM ATP (Figure 2A). As ATP concentration is reduced, the average speed decreases in a manner predicted by the Michaelis-Menten equation (Figure 2A, inset). A fit of the equation to the experimental data yields a maximum average translocation speed of 25.4 bp/s, which is consistent with the

Figure 3 Time-dependent DNA contour length trace, showing three modes of DNA loop formation (A–C) and abrupt (indicated with upward arrow) or continuous loop dissipation. The loop formation modes are categorized into continuous loop formation (A), abrupt loop formation (B, indicated with red arrows), and abrupt loop formation (green arrow) followed by continuous loop formation (C). Although the last loop formation event has a relatively noisy initial phase, close inspection shows that it is more appropriate to be categorized into mode A.

speed measured at the saturated 2 mM ATP concentration, and a Michaelis–Menten constant $K_m = 0.03$ mM. The size of the loop generated by StART (Figure 2B) is stochastic, ranging from ~ 5 bp, the signal detection limit of the tweezers under the experimental conditions, up to 1500 bp (Figure 4A). Its distribution can be approximately fitted with a single-exponential function (Ali and Lohman, 1997), yielding a translocation processivity of ~ 35 bp for the remodeler motor (Fischer *et al*, 2007). Note that the processivity is less than the measured average loop size (~ 65 bp), which may overestimate the real translocation distance due to the threshold (10 bp) used to identify the translocation signal from the measurement noise (Supplementary data). The processivity is not affected by the presence of force (< 6 pN in general) or force change (with an average of 2.2 pN) in our measurement, probably because the force range falls well below the maximum force that the motor is able to produce (~ 30 pN, see the following section). Furthermore, the measured processivity is only minimally affected by TetR dissociation, due to predominant translocation in the burst phase and the high-mechanical strength of TetR–tetO association (> 30 pN). Finally, the processivity decreases with a decrease in ATP concentration (Figure 2B). In conclusion, the RSC translocase has low translocation speed and processivity compared with other dsDNA translocases in the SF2 helicase and translocase family (Pyle, 2008), such as Rad54 (301 bp/s speed and 11 500 bp processivity) (Amitani *et al*, 2006) and EcoR124I (560 bp/s and 1320 bp) (Seidel *et al*, 2004), which likely reflects its use in nucleosome remodelling in a smaller, defined chromatin region.

Measuring maximum force generation by the RSC translocase

As the tethered translocase extends a small DNA loop during translocation, the motor will experience a decreasing internal dragging force exerted by the constrained loop (see the following section) (Shroff *et al*, 2008) and an increasing

Figure 4 The RSC translocase is one of the strongest molecular motors. (A) Time-dependent DNA force induced by remodeler motor translocation in the presence of 2 mM ATP. (B) Force-time trace in a force-jump experiment, showing the maximum force (30 pN) generated by the remodeler motor. The motor initiated DNA loop formation at a tension of ~ 6 pN and then was quickly stretched to a high tension of ~ 25 pN. After the force jump, the motor continued moving against high forces up to 30 pN until the DNA loop dissipated. Subsequently, the DNA template was relaxed to the initial tension to start a new round of force-jump experiment.

external force applied by the optical traps. When the size of the DNA loop exceeds about 200 bp, the optical force dominates the total opposing force for remodeler translocation, thus providing a way to measure the maximum force generated by the remodeler motor. The maximum force measured in our standard assay as described above is 26 pN (Figure 4A). To reach this force, the remodeler motor had translocated an unusually long distance of 1500 bp. Most of the translocating StART complexes fell off before they reached high forces, due to their low processivity and the low force-loading rate used in our assay (~ 0.1 pN/nm). Thus, this assay only provides a lower bound of the maximum force generated by the remodeler motor. To overcome its limitation, we performed a new force-jump experiment (Figure 4B) to better mimic the expected high force-loading rate during nucleosome remodelling by remodellers. In this experiment, we first allowed the remodeler motor to initiate loop formation at a low DNA tension, and then quickly (within ~ 5 ms) pulled the DNA molecule to high tension by increasing the trap separation. Most of the remodellers (30 out of 44, or 68%) survived the force jump and continued to translocate

above 15 pN, with one prominent example shown in Figure 4B. Here after the force jump the remodeller motor continued translocating over 100 bp to reach the highest force, or 30 pN. Compared with other molecular motors with single ATPases, the RSC motor is the second strongest motor tested (Supplementary Table S2). Once the DNA loop was released (in three steps in this case), no additional loop formation activity was observed at high DNA tension, suggesting the existence of a force-sensitive loop initiation step. Finally, the DNA template was relaxed to the initial low tension before the force jump to facilitate a new round of DNA loop initiation. Taken together, we reveal Sth1 as an exceptionally force-resistant DNA translocating motor.

A small step size for the RSC translocase

The step sizes of double-strand DNA translocases have not been well characterized in general (Chemla, 2010), but these values greatly impact the functional models of the translocases (Clapier and Cairns, 2009). We optimized our assay to measure the step size of the remodeller translocation by conducting the translocation assays under conditions with improved spatial resolution (Abbondanzieri *et al*, 2005; Moffitt *et al*, 2006), especially at high force range (>7 pN) and at lower ATP concentration (0.1 mM). Because of the remodeller's low processivity, we further adopted two experimental approaches. First, we repeated our standard translocation assay extensively, and selected rare events with both long translocation distances ($\geq \sim 120$ bp) and large force generation (≥ 7 pN). Second, we performed the force-jump experiment again (see Figure 4B for an example). Both approaches yielded time-dependent DNA length traces with

improved signal-to-noise ratio, with a typical trace shown in Figure 5 (blue trace). However, the expected individual steps corresponding to motor stepping were only rarely discernable from these traces. To determine the step size, we analysed individual translocation events using a hidden Markov model (HMM) (Milescu *et al*, 2006; Park *et al*, 2010; Syed *et al*, 2010a, b). In this method, the likelihood of each experimental trace is calculated based on a Poisson model for the motor stepping process (Ali and Lohman, 1997) and a Gaussian distribution for the measurement noise. The model is characterized by a step size and an average dwell time between successive steps. These parameters can then be optimized by maximizing the likelihood, yielding the best-fit step size (1.9 bp, inset in Figure 5) and the idealized and noiseless motor stepping trace. To verify that the HMM analysis can reveal step sizes from data with relatively low signal-to-noise ratio, we simulated the motor stepping process (Supplementary Figure S1) at input step sizes of 1 bp (cyan), 2 bp (green), and 3 bp (black), under otherwise identical conditions as motor translocation. These step sizes were correctly identified to be $1.1 (\pm 0.1, \text{s.d.})$ bp, $1.9 (\pm 0.1)$ bp, and $3.0 (\pm 0.1)$ bp, respectively (see also Supplementary Figure S2). Accordingly, more extensive measurements revealed a best estimated step size of $2.0 (\pm 0.4, \text{s.e.m.})$ bp for the remodeller motor. Nucleosome remodelling reactions have been interpreted as involving large (> 40 bp), moderate (~ 10 bp), or small (1–3 bp) step sizes (Saha *et al*, 2005; Lia *et al*, 2006; Blosser *et al*, 2009; Clapier and Cairns, 2009). This interpretation is important as it sets the lower limit for the size of postulated DNA loops/waves present on the histone surface as remodelling intermediates and the distance of

Figure 5 Step size analyses of the remodeller motor translocation. The time-dependent loop size (partially shown here in the blue trace) was analysed with the hidden Markov model, yielding a best-fit step size of 1.9 bp and the idealized motor stepping trace (red). Here, the best-fit step size maximizes the likelihood (relative to its maximum) of the trace as a function of step size (inset). To validate the HMM analysis, we simulated the motor stepping by a stepwise increase in the separation between the two traps based on a Poisson process (see also Supplementary Figure S1). The dwell time between successive steps is stochastic, but follows a single-exponential distribution. The mean dwell time (τ) is chosen to give a speed of trap separation same as the average translocation speed of the motor (V), that is, $\tau = d/V$, where d is the step size. For simulations with input step sizes of 1 bp (cyan), 2 bp (green), and 3 bp (black), HMM revealed the correct step size inputs (see text) and the corresponding idealized stepping traces (red). The time-length traces are shown here at 50 Hz.

remodeller-catalysed nucleosome sliding (Langst and Becker, 2001; Schwanbeck *et al*, 2004; Saha *et al*, 2005; Zhang *et al*, 2006). Thus, our measurement defines a fundamental kinetic step size to ~ 2 bp and excludes large steps, except by accumulation of these smaller steps. Interestingly, the Sth1 motor has a step size close to that of the ATPase ISWI, the motor subunit of an alternative remodeller family (involving ACF and NURF, both ~ 3 bp) (Schwanbeck *et al*, 2004; Blosser *et al*, 2009), suggesting a similar DNA translocation mechanism for remodellers. Moreover, the step size is also similar to that of the DNA packaging motor in bacteriophage $\phi 29$ (2.5 bp) (Moffitt *et al*, 2009).

Abrupt DNA loop formation

The remodeller translocation kinetics described above is most often characterized by continuous DNA loop formation, which accounts for 62% of all observed signals (loop formation mode A). We discovered two additional modes of DNA loop formation, in which DNA loops are completely (mode B) or partially (mode C) formed in a discontinuous manner (Figures 3 and 6A). Mode B is represented by a sudden drop in DNA length followed by a sudden jump back to the baseline after a short delay. This mode of abrupt DNA loop formation and dissipation accounts for about 32% of all signals, with an overall frequency of 0.33 per minute. The remaining 8% signals

combine the above two modes of loop formation and starts with a small abruptly formed loop followed by continuous loop growth (mode C). The size of the abruptly formed loop in both modes B and C has a similar unimodal distribution, indicating a common mechanism of loop formation, with the former shown in Figure 6B for 2 mM ATP. The average size of all such loops is $25.6 (\pm 0.4, \text{s.e.m.})$ bp. Once formed by mode B, the loop could remain approximately the same size for various times ranging from several milliseconds to 3 s until it was suddenly dissipated. The duration has a single-exponential distribution with a time constant of 0.4 s (Figure 6C), indicating that the translocase entered a unique state without further translocation. Interestingly, neither the average size nor the duration of the loop changes with ATP concentration (Figure 6D). However, the occurrence frequency of loop formation in modes B and C increases with ATP concentration, suggesting that such loop formation is also generated by the remodeller motor in an ATP-dependent process.

The abrupt DNA loop formation observed here resembles the DNA bulging process mediated by the DNA translocase EcoR124I at translocation initiation (Seidel *et al*, 2004). To test if the abrupt DNA contour length shortening observed by us is also caused by a similar DNA conformation change induced by coupled motor binding to both DNA and ATP, we repeated our translocation assay by replacing ATP with ATP γ S. However, we did not detect any appreciable signals in a total measurement time of 283 min. Thus, we conclude that the abrupt DNA loop induced by StART must be formed by an alternative mechanism, probably DNA buckling (Supplementary Figure S3; Supplementary Table S3) (Shroff *et al*, 2008).

Regardless of its mode, the DNA loop formation has a force-sensitive and rate-limiting initiation step. This conclusion is supported by the reduced signal occurrence frequency at high DNA tension (> 7 pN; Figure 4B), the ATP-dependence of the abrupt loop formation (Figure 6D), and the observation of bursts of loop formation in mode B (Figure 6A, top black trace). To initiate a DNA loop, the remodeller motor needs to generate significant force to bend a short segment of DNA, in addition to the pulling force exerted by optical traps. The force required to form a DNA loop is sensitive to its size, from > 10 pN for 57 bp DNA to < 4 pN for 101 bp DNA (Shroff *et al*, 2008). Thus, the remodeller motor faces a large energy barrier to initiate a DNA loop in our experimental format, which causes its sensitivity to any external force.

Figure 6 Abrupt DNA loop formation induced by remodeller motor translocation. (A) DNA length-time traces, indicating abrupt loop formation (marked by red arrows) in modes B (left black traces) and C (right cyan trace), respectively. (B) Histogram distribution of the DNA loop size in the loop formation mode B at 2 mM ATP, with an average of $26 (\pm 7, \text{s.d.})$ bp. (C) The lifetime distribution of the loop corresponding to (B) has a single-exponential distribution (red line). (D) Comparison of the average loop size, lifetime, and occurrence frequency (or percentage) of the abruptly formed DNA loops under different ATP concentration and loop formation mode.

Discussion

Insights on DNA translocation through a 'tethered' translocase system

The translocation properties of the minimal RSC complex (StART) on bare DNA show both similarities and differences when compared with the full RSC complex on nucleosomal DNA (Zhang *et al*, 2006). They share the properties of a relatively low processivity, the ability to translocate against high forces, and to occasionally reverse their directions. Moreover, both have a force-sensitive loop or translocation initiation step, but a force-insensitive translocation speed. These similarities suggest that the translocation properties of the full RSC complex are mainly determined by this core translocase complex. However, the minimal and the full remodeller complexes exhibit modest differences in translocation kinetics. First, the minimal StART complex translocates about

two-fold faster on bare DNA than the full complex on nucleosomal DNA. Note that the difference is not caused by the subtle differences in experimental conditions, such as different optical tweezer instruments (Supplementary Figure S4). This difference in the translocation rate suggests that other remodeller subunits and/or different substrates (bare DNA and nucleosomes) may tune the translocation properties of the motor, which provide an important way to regulate the chromatin remodelling activity (Qin *et al*, 2000; He *et al*, 2008; Goldman *et al*, 2010). Second, the measured maximum force is higher for the minimal complex on bare DNA than the full complex on nucleosomal DNA. The difference does not necessarily mean that the translocase motor within RSC constitutively produces lower force, because part of the force output from the motor may be transmitted to disrupt the nucleosome structure and not measured in the nucleosome-dependent assay. Finally, the abrupt DNA loop formation is only generally observed here on bare DNA, but not on nucleosomal DNA. Whereas the tethered remodeller motor has to bend a segment of straight DNA to initiate a loop, the Sth1 ATPase within the full RSC complex engages bent nucleosomal DNA for translocation, bypassing the abrupt DNA loop formation process. Consistent with this notion, the ATPase binds the DNA internally, about two helical turns from the nucleosomal dyad (Schwanbeck *et al*, 2004; Saha *et al*, 2005; Zofall *et al*, 2006). Taken together, we suggest that, despite its robustness, the translocation properties of the remodeller motor are likely regulated by various attendant subunits in the complex and by its different translocation substrates. This regulation of the translocase motor activity may be the key to understanding the diverse functions of remodellers (Clapier and Cairns, 2009). By adding back additional subunits from RSC complex, our minimal RSC complex should provide an important platform to dissect the functions of the 15-subunit RSC complex, subunit-by-subunit.

Abrupt DNA loop formation in translocation initiation

Our observation of the abrupt loop formation by the RSC motor corroborates and extends the notion that translocation initiation constitutes a distinct phase of DNA translocation, which has been observed for several other DNA translocases, including EcoR124I and ACF. In ACF-catalysed nucleosome sliding, both ACF binding to nucleosome and subsequent translocation initiation depend on ATP (Blosser *et al*, 2009). Furthermore, ACF takes a greater initial step (7 bp) than any successive steps (3–4 bp), indicating a distinct translocation initiation process. Because of the high energy barrier to form a small DNA loop involved in these processes, translocation initiation tends to be a rate-limiting step for productive DNA translocation and often has to couple to ATP binding or hydrolysis. Many DNA-based motors engages DNA through large conformation changes in both protein and DNA (Fitzgerald *et al*, 2004; Durr *et al*, 2005; Lee and Yang, 2006; Lewis *et al*, 2008), which may activate the otherwise idle ATPases and lead to coupling between ATP binding or hydrolysis and DNA bending required for translocation initiation (van Noort *et al*, 2004). However, our model for the translocation initiation of the remodeller motor on linear DNA suggests an alternative coupling mechanism (Supplementary Figure S3), in which repetitive and short DNA translocation proceeds productive DNA translocation and loop formation. Such DNA translocation is used first to deform part of the protein complex (illustrated as peptide linker stretching in Supplementary Figure S3). Then the corresponding deforma-

tion energy is transferred to bend the DNA and complete the translocation initiation process. Similar spring-loaded mechanism has been proposed for coupling of DNA translocation to DNA unwinding by NS3 helicase (Myong *et al*, 2007).

Summary

In summary, we have developed a novel tethered motor assay that utilizes high-resolution optical tweezers to measure the real-time kinetics of a remodeller translocase moving along bare DNA. We characterized its translocation speed (25 bp/s), processivity (35 bp), step size (2.0 bp), and the corresponding three loop formation modes. We note that the processivity of the RSC motor determined here on DNA is close to the average distance of nucleosome sliding (28 bp) in a single remodelling event catalysed by SWI/SNF (Shundrovsky *et al*, 2006), but is lower than our previous measurement of RSC processivity on a nucleosome (~100 bp) (Zhang *et al*, 2006). Thus, a minimal remodelling event on a nucleosome may consist of the remodeller ATPase translocating DNA to its intrinsic processivity limit (~35 bp), and then disengaging from DNA. However, as the ATPase (within the full RSC complex) remains naturally tethered to a nucleosome, it may simply conduct additional rounds of translocation, allowing for a longer measurement of processivity on a nucleosome. Furthermore, we discovered that the RSC motor is an intrinsically powerful DNA translocase capable of generating high forces (up to 30 pN). Considering the previous force measurements for SWI/SNF complex (~12 pN) on nucleosomal DNA and the highly conserved structures of all remodeller motors, we suggest that other remodellers may also contain strong DNA translocases, though this prediction remains to be tested. The maximum force generated by these translocases can be higher than the typical forces required to mechanically disrupt nucleosomes (~23 pN) (Brower-Toland *et al*, 2002). This comparison suggests a fundamental mechanism for nucleosome remodeling, by which remodeller motors generate mechanical forces to alter DNA–histone interactions by translocating DNA. To apply such high forces to DNA, remodellers have to anchor their translocase motors on histones, in analogy with our experimental design and as suggested previously (Langst *et al*, 1999; Schwanbeck *et al*, 2004; Saha *et al*, 2005; Zofall *et al*, 2006), where the motors stay on a fixed position on the histone octamer and pump DNA around the histone surface. This anchoring can be implemented with the assistance of many subunits in the remodeller complexes and through the recognition of various histone modifications. Like the repetitive cycles of loop formation and dissipation observed in our assay, the tethered remodellers can iteratively slide DNA around histones to achieve nucleosome sliding (Blosser *et al*, 2009; Dechassa *et al*, 2010). Moreover, the high force generation of the RSC motor also qualifies its role in assisting an RNA polymerase II (with a maximum force output of ~12 pN) in transcribing through nucleosomes (Soutourina *et al*, 2006; Galburt *et al*, 2007; Hodges *et al*, 2009). Furthermore, we show that the robust translocase motor is versatile with respect to its translocation properties, revealing modes for regulating the remodelling process. Finally, we believe that our tethered motor assay can be applied to a wide variety of other remodellers or DNA translocases (Singleton *et al*, 2007; Pyle, 2008; Clapier and Cairns, 2009), whose high force generation has crucial roles in regulating protein–DNA

interactions (Byrd and Raney, 2004; Sprouse *et al*, 2006; Finkelstein *et al*, 2010).

Materials and methods

Protein and DNA preparation

The StART complex was expressed in *Escherichia coli* BL21 (DE3) RIL from two duet vectors: one contains *Arp7* and *Arp9* genes and the other bears *TetR*-(His)₇ and *TetR*-*Sth1*_(301–1097)-Flag genes (Figure 1A). The properly assembled complex contains a heterodimer of TetR with one copy fused to Sth1 core and another TetR copy (not fused) expressed separately but under the same promoter. Between TetR and Sth1 is inserted an eight-amino-acid sequence Gly-Gly-Ala-Gly-Gly-Ala-Gly-Gly. However, the exact linker length between Sth1 and TetR, illustrated in Figure 1A, is unknown due to possible further contribution of the disordered region from Sth1 or the TetR protein. The StART complex was purified by two successive affinity purifications. First, the cleared cell extracts were mixed with Ni-NTA agarose resin (Qiagen), which captures the StART complex (and unwanted TetR homodimer complexes) through the histidine tag fused to one of the TetR copy. The sample eluted from the resin was further purified using the Flag tag and anti-Flag M2 affinity gel and eluted with 3 × FLAG peptide (Sigma) to obtain the desired StART complex. Finally, the purity of StART complexes was refined by a gel filtration step on a S200GL 10/300 (Amersham, GE) (Figure 1B). Two DNA templates containing the tetO site (tctatcattagatag) in the middle were used, one with 5063 bp (Figure 1A) and the other with 4031 bp. The latter was specifically used for the step size measurement in combination with ~1 µm-diameter polystyrene beads (Figure 5), whereas the former was used for all other experiments with ~2 µm-diameter beads. The remodeller translocation experiments were conducted in a buffer containing 20 mM Tris-acetate, 10 mM magnesium acetate, 50 mM potassium acetate, 1 mM DTT, 6% glycerol, 0.1 mg/ml BSA, supplemented with ATP.

High-resolution optical tweezers and data analysis

The dual-trap high-resolution optical tweezers were built, calibrated, and tuned to the same basepair resolution mainly as previously described (Moffitt *et al*, 2006) (Supplementary Figure S1). One notable improvement was the use of new water-immersion objectives (N.A. = 1.2) with over 80% transmission for the 1064-nm trapping light (Olympus). Data were acquired at 20 kHz, filtered online, and saved at 5 kHz. If not specified, the time-dependent traces shown were further filtered to 20 Hz using moving-box averaging. The DNA loop formation signals were identified as previously described (Zhang *et al*, 2006), except that a 10-bp threshold was used to distinguish translocation signals from the background noise, mainly from Brownian motion of the beads. The contour length of the DNA tether was calculated from the measured force and extension using the worm-like-chain model (Supplementary Figure S1).

Step size analysis based on the HMM

The step size analysis was mainly adapted from the work by Sigworth and coworkers (Syed *et al*, 2010a,b). Briefly, the HMM

quantitatively describes both the motor stepping process and the measurement noise. We model the motor stepping as a Poisson process, a special Markov process. The model contains only two parameters: the motor step size and the stepping probability (*P*) during each sampling interval (Δt). The stepping probability is related to the average dwell time (τ) between two successive steps by $P = \Delta t / \tau$. To apply HMM analysis, we typically filtered the time-dependent displacement trace (Figure 5) to 50 Hz, yielding an effective sampling time of 20 ms, or $\Delta t = 20$ ms. The measurement noise from optical tweezers is dominated by bead's Brownian motion, and has a normal distribution, i.e.,

$$P(x) = \frac{1}{(2\pi\sigma^2)^{1/2}} \exp\left[-\frac{(x-\mu)^2}{2\sigma^2}\right],$$

where *x* is the displacement at any time point and σ is the standard deviation of the displacement around the corresponding motor position at μ . In general, the noise amplitude for the dual-trap optical tweezers decreases monotonically as force increases at a low force range and then becomes flat at a high force range (Moffitt *et al*, 2006). Under our experimental conditions for the step size measurement, the noise amplitude is approximately constant within the force range between 7 and 22 pN. In this case, the displacement noise level is close to the minimum and barely changes with force or average displacement. Therefore, the noise amplitude σ in our calculations is treated as a constant independent of motor position μ and DNA tension. Furthermore, the noise is uncorrelated between the data sampled with the 20-ms sampling time, a crucial requirement for HMM. All HMM parameters were optimized by the Baum–Welch algorithm and the gradient-based algorithms that yielded consistent results.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (<http://www.embojournal.org>).

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Author contributions: YZ and BC designed the experiments; GS, YZ, and YG built the tweezers and performed the tweezer experiments; CC and RV purified StART proteins and developed the tethered translocase system; GS and YZ analysed the data; and YZ, GS, CC, and BC wrote the paper.

Conflict of interest

The authors declare that they have no conflict of interest

References

- Abbondanzieri EA, Bokinsky G, Rausch JW, Zhang JX, Le Grice SFJ, Zhuang XW (2008) Dynamic binding orientations direct activity of HIV reverse transcriptase. *Nature* **453**: 184–189
- Abbondanzieri EA, Greenleaf WJ, Shaevitz JW, Landick R, Block SM (2005) Direct observation of base-pair stepping by RNA polymerase. *Nature* **438**: 460–465
- Ali JA, Lohman TM (1997) Kinetic measurement of the step size of DNA unwinding by *Escherichia coli* UvrD helicase. *Science* **275**: 377–380
- Amitani I, Baskin RJ, Kowalczykowski SC (2006) Visualization of Rad54, a chromatin remodeling protein, translocating on single DNA molecules. *Mol Cell* **23**: 143–148
- Blosser TR, Yang JG, Stone MD, Narlikar GJ, Zhuang XW (2009) Dynamics of nucleosome remodelling by individual ACF complexes. *Nature* **462**: 1022–1028
- Boeger H, Griesenbeck J, Strattan JS, Kornberg RD (2004) Removal of promoter nucleosomes by disassembly rather than sliding *in vivo*. *Mol Cell* **14**: 667–673
- Bowman GD (2010) Mechanisms of ATP-dependent nucleosome sliding. *Curr Opin Struct Biol* **20**: 73–81
- Brower-Toland BD, Smith CL, Yeh RC, Lis JT, Peterson CL, Wang MD (2002) Mechanical disruption of individual nucleosomes reveals a reversible multistage release of DNA. *Proc Natl Acad Sci USA* **99**: 1960–1965
- Byrd AK, Raney KD (2004) Protein displacement by an assembly of helicase molecules aligned along single-stranded DNA. *Nat Struct Mol Biol* **11**: 531–538
- Chaban Y, Ezeokonkwo C, Chung WH, Zhang F, Kornberg RD, Maier-Davis B, Lorch Y, Asturias FJ (2008) Structure of a RSC-nucleosome complex and insights into chromatin remodeling. *Nat Struct Mol Biol* **15**: 1272–1277

- Chemla YR (2010) Revealing the base pair stepping dynamics of nucleic acid motor proteins with optical traps. *Phys Chem Chem Phys* **12**: 3080–3095
- Clapier CR, Cairns BR (2009) The biology of chromatin remodeling complexes. *Ann Rev Biochem* **78**: 273–304
- Dechassa ML, Sabri A, Pondugula S, Kassabov SR, Chatterjee N, Kladde MP, Bartholomew B (2010) SWI/SNF has intrinsic nucleosome disassembly activity that is dependent on adjacent nucleosomes. *Mol Cell* **38**: 590–602
- Durr H, Korner C, Muller M, Hickmann V, Hopfner KP (2005) X-ray structures of the *Sulfolobus solfataricus* SWI2/SNF2 ATPase core and its complex with DNA. *Cell* **121**: 363–373
- English BP, Min W, van Oijen AM, Lee KT, Luo GB, Sun HY, Cherayil BJ, Kou SC, Xie XS (2006) Ever-fluctuating single enzyme molecules: Michaelis-Menten equation revisited. *Nat Chem Biol* **2**: 87–94
- Finkelstein IJ, Visnapuu ML, Greene EC (2010) Single-molecule imaging reveals mechanisms of protein disruption by a DNA translocase. *Nature* **468**: 983–987
- Fischer CJ, Saha A, Cairns BR (2007) Kinetic model for the ATP-dependent translocation of *Saccharomyces cerevisiae* RSC along double-stranded DNA. *Biochemistry* **46**: 12416–12426
- Fitzgerald DJ, DeLuca C, Berger I, Gaillard H, Sigrist R, Schimmele K, Richmond TJ (2004) Reaction cycle of the yeast Isw2 chromatin remodeling complex. *EMBO J* **23**: 3836–3843
- Galbur EA, Grill SW, Wiedmann A, Lubkowska L, Choy J, Nogales E, Kashlev M, Bustamante C (2007) Backtracking determines the force sensitivity of RNAP II in a factor-dependent manner. *Nature* **446**: 820–823
- Goldman JA, Garlick JD, Kingston RE (2010) Chromatin remodeling by imitation switch (ISWI) class ATP-dependent remodelers is stimulated by histone variant H2A.Z. *J Biol Chem* **285**: 4645–4651
- Hamiche A, Sandaltzopoulos R, Gdula DA, Wu C (1999) ATP-dependent histone octamer sliding mediated by the chromatin remodeling complex NURF. *Cell* **97**: 833–842
- He X, Fan HY, Garlick JD, Kingston RE (2008) Diverse regulation of SNF2h chromatin remodeling by noncatalytic subunits. *Biochemistry* **47**: 7025–7033
- Hodges C, Bintu L, Lubkowska L, Kashlev M, Bustamante C (2009) Nucleosomal fluctuations govern the transcription dynamics of RNA polymerase II. *Science* **325**: 626–628
- Langst G, Becker PB (2001) ISWI induces nucleosome sliding on nicked DNA. *Mol Cell* **8**: 1085–1092
- Langst G, Bonte EJ, Corona DFV, Becker PB (1999) Nucleosome movement by CHRAC and ISWI without disruption or trans-displacement of the histone octamer. *Cell* **97**: 843–852
- Lee JY, Yang W (2006) UvrD helicase unwinds DNA one base pair at a time by a two-part power stroke. *Cell* **127**: 1349–1360
- Lewis R, Durr H, Hopfner KP, Michaelis J (2008) Conformational changes of a Swi2/Snf2 ATPase during its mechano-chemical cycle. *Nucleic Acids Res* **36**: 1881–1890
- Lia G, Praly E, Ferreira H, Stockdale C, Tse-Dinh YC, Dunlap D, Croquette V, Bensimon D, Owen-Hughes T (2006) Direct observation of DNA distortion by the RSC complex. *Mol Cell* **21**: 417–425
- Lorch Y, Maier-Davis B, Kornberg RD (2010) Mechanism of chromatin remodeling. *Proc Natl Acad Sci USA* **107**: 3458–3462
- Lu HP, Xun LY, Xie XS (1998) Single-molecule enzymatic dynamics. *Science* **282**: 1877–1882
- Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ (1997) Crystal structure of the nucleosome core particle at 2.8 angstrom resolution. *Nature* **389**: 251–260
- Milescu LS, Yildiz A, Selvin PR, Sachs F (2006) Maximum likelihood estimation of molecular motor kinetics from staircase dwell-time sequences. *Biophys J* **91**: 1156–1168
- Mizuguchi G, Shen XT, Landry J, Wu WH, Sen S, Wu C (2004) ATP-Driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. *Science* **303**: 343–348
- Moffitt JR, Chemla YR, Aathavan K, Grimes S, Jardine PJ, Anderson DL, Bustamante C (2009) Intersubunit coordination in a homomeric ring ATPase. *Nature* **457**: 446–451
- Moffitt JR, Chemla YR, Izhaky D, Bustamante C (2006) Differential detection of dual traps improves the spatial resolution of optical tweezers. *Proc Natl Acad Sci USA* **103**: 9006–9011
- Myong S, Bruno MM, Pyle AM, Ha T (2007) Spring-loaded mechanism of DNA unwinding by hepatitis C virus NS3 helicase. *Science* **317**: 513–516
- Orth P, Schnappinger D, Hillen W, Saenger W, Hinrichs W (2000) Structural basis of gene regulation by the tetracycline inducible Tet repressor-operator system. *Nat Struct Mol Biol* **7**: 215–219
- Park J, Myong S, Niedziela-Majka A, Lee KS, Yu J, Lohman TM, Ha T (2010) PcrA helicase dismantles RecA filaments by reeling in DNA in uniform steps. *Cell* **142**: 544–555
- Pyle AM (2008) Translocation and unwinding mechanisms of RNA and DNA helicases. *Ann Rev Biophys* **37**: 317–336
- Qin F, Auerbach A, Sachs F (2000) A direct optimization approach to hidden Markov modeling for single channel kinetics. *Biophys J* **79**: 1915–1927
- Saha A, Wittmeyer J, Cairns BR (2005) Chromatin remodeling through directional DNA translocation from an internal nucleosomal site. *Nat Struct Mol Biol* **12**: 747–755
- Schwanbeck R, Xiao H, Wu C (2004) Spatial contacts and nucleosome step movements induced by the NURF chromatin remodeling complex. *J Biol Chem* **279**: 39933–39941
- Seidel R, van Noort J, van der Scheer C, Bloom JGP, Dekker NH, Dutta CF, Blundell A, Robinson T, Firman K, Dekker C (2004) Real-time observation of DNA translocation by the type I restriction modification enzyme EcoR124I. *Nat Struct Mol Biol* **11**: 838–843
- Shroff H, Sivak D, Siegel JJ, McEvoy AL, Siu M, Spakowitz A, Geissler PL, Liphardt J (2008) Optical measurement of mechanical forces inside short DNA loops. *Biophys J* **94**: 2179–2186
- Shukla MS, Syed SH, Montel F, Faivre-Moskalenko C, Bednar J, Travers A, Angelov D, Dimitrov S (2010) Remosomes: RSC generated non-mobilized particles with approximately 180 bp DNA loosely associated with the histone octamer. *Proc Natl Acad Sci USA* **107**: 1936–1941
- Shundrovsky A, Smith CL, Lis JT, Peterson CL, Wang MD (2006) Probing SWI/SNF remodeling of the nucleosome by unzipping single DNA molecules. *Nat Struct Mol Biol* **13**: 549–554
- Singleton MR, Dillingham MS, Wigley DB (2007) Structure and mechanism of helicases and nucleic acid translocases. *Ann Rev Biochem* **76**: 23–50
- Smith CL, Peterson CL (2005) ATP-dependent chromatin remodeling. *Curr Top Dev Biol* **65**: 115–148
- Soutourina J, Floch VB-L, Gendrel G, Flores A, Ducrot C, Dumay-Odelot H, Soularue P, Navarro F, Cairns BR, Lefebvre O, Werner M (2006) Rsc4 connects the chromatin remodeler RSC to RNA polymerases. *Mol Cell Biol* **26**: 4920–4933
- Sprouse RO, Brenowitz M, Auble DT (2006) Snf2/Swi2-related ATPase Mot1 drives displacement of TATA-binding protein by gripping DNA. *EMBO J* **25**: 1492–1504
- Syed S, Mullner FE, Selvin PR, Sigworth FJ (2010a) Improved hidden Markov models for molecular motors. Part 1: basic theory. *Biophys J* **99**: 3684–3695
- Syed S, Mullner FE, Selvin PR, Sigworth FJ (2010b) Improved hidden Markov models for molecular motors. Part 2: extensions and application to experimental data. *Biophys J* **2010**: 3696–3703
- Szerlong H, Hinata K, Viswanathan R, Erdjument-Bromage H, Tempst P, Cairns BR (2008) The HSA domain binds nuclear actin-related proteins to regulate chromatin-remodeling ATPases. *Nat Struct Mol Biol* **15**: 469–476
- van Noort J, van der Heijden T, Dutta CF, Firman K, Dekker C (2004) Initiation of translocation by Type I restriction-modification enzymes is associated with a short DNA extrusion. *Nucleic Acids Res* **32**: 6540–6547
- Whitehouse I, Flaus A, Cairns BR, White MF, Workman JL, Owen-Hughes T (1999) Nucleosome mobilization catalysed by the yeast SWI/SNF complex. *Nature* **400**: 784–787
- Yang JG, Madrid TS, Sevastopoulos E, Narlikar GJ (2006) The chromatin-remodeling enzyme ACF is an ATP-dependent DNA length sensor that regulates nucleosome spacing. *Nat Struct Mol Biol* **13**: 1078–1083
- Yang XF, Zaurin R, Beato M, Peterson CL (2007) Swi3p controls SWI/SNF assembly and ATP-dependent H2A-H2B displacement. *Nat Struct Mol Biol* **14**: 540–547
- Zhang YL, Smith CL, Saha A, Grill SW, Mihadja S, Smith SB, Cairns BR, Peterson CL, Bustamante C (2006) DNA translocation and loop formation mechanism of chromatin remodeling by SWI/SNF and RSC. *Mol Cell* **24**: 559–568
- Zofall M, Persinger J, Kassabov SR, Bartholomew B (2006) Chromatin remodeling by ISW2 and SWI/SNF requires DNA translocation inside the nucleosome. *Nat Struct Mol Biol* **13**: 339–346