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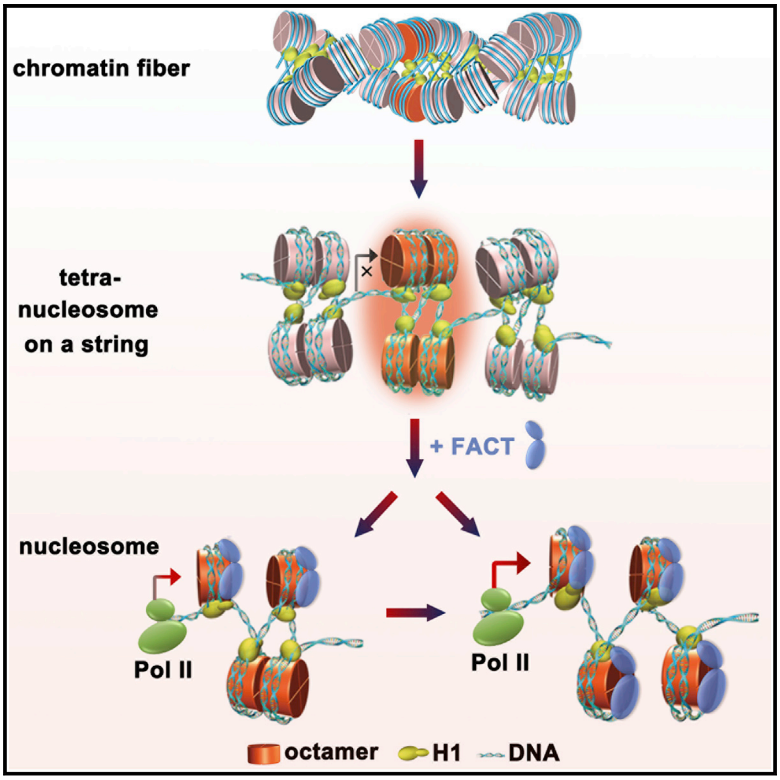
FACT Remodels the Tetranucleosomal Unit of Chromatin Fibers for Gene Transcription

Graphical Abstract



Highlights

- Tetranucleosomes-on-a-string is a distinct intermediate structure of chromatin
- Tetranucleosome is a stable secondary structural unit of chromatin fibers
- FACT negatively regulates the stability of tetranucleosomal unit for transcription
- H1 facilitates the folding and unfolding kinetics of the outer nucleosomal wrap

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In Brief

Li et al. reveal that the tetranucleosomes-on-a-string is a distinct intermediate structure in the hierarchical organization of chromatin fibers and regulated by histone chaperone FACT for transcription by using single-molecule force spectroscopy.

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FACT Remodels the Tetranucleosomal Unit of Chromatin Fibers for Gene Transcription

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SUMMARY

In eukaryotes, the packaging of genomic DNA into chromatin plays a critical role in gene regulation. However, the dynamic organization of chromatin fibers and its regulatory mechanisms remain poorly understood. Using single-molecule force spectroscopy, we reveal that the tetranucleosomes-on-a-string appears as a stable secondary structure during hierarchical organization of chromatin fibers. The stability of the tetranucleosomal unit is attenuated by histone chaperone FACT (facilitates chromatin transcription) in vitro. Consistent with in vitro observations, our genome-wide analysis further shows that FACT facilitates gene transcription by destabilizing the tetranucleosomal unit of chromatin fibers in yeast. Additionally, we found that the linker histone H1 not only enhances the stability but also facilitates the folding and unfolding kinetics of the outer nucleosomal wrap. Our study demonstrates that the tetranucleosome is a regulatory structural unit of chromatin fibers beyond the nucleosome and provides crucial mechanistic insights into the structure and dynamics of chromatin fibers during gene transcription.

INTRODUCTION

Eukaryotic DNA is hierarchically packaged into chromatin, in which the dynamics of chromatin structures play a central role in DNA-related biological processes, such as transcription, DNA replication, recombination, and repair. Nucleosome, the basic unit of chromatin, consists of 147 bp of DNA wrapped around a histone octamer (two copies each of H2A, H2B, H3, and H4) approximately 1.7 times in a left-handed manner, the structure of which has been determined by X-ray crystallography at 1.9–2.8 Å resolution (Davey et al., 2002; Luger et al., 1997). Nucleosomes are connected by linker DNA in a "beads-on-a-string" array and compact further by linker histones into the 30-nm chromatin fiber, which is regarded as the second level of DNA compaction (Olins and Olins, 2003).

On the basis of the measurements of electron microscopy (EM) and analytical ultracentrifugation (AUC), two basic classes of models, namely, the one-start solenoid model and the two-start crosslinker model, have been proposed for chromatin fibers (Dorigo et al., 2004; Robinson et al., 2006). The X-ray structure analysis of tetranucleosomes revealed a zigzag conformation with two stacks of two nucleosomes, supporting the two-start crosslinker model (Schalch et al., 2005). Linker histones have been shown to play critical roles in the formation of the 30-nm chromatin fiber (Woodcock et al., 2006). We determined the 3D cryo-EM structures of 30-nm chromatin fibers containing linker histone H1, which revealed a left-handed double helical structure twisted with tetranucleosomal units (Song et al., 2014). This observation is now supported by two recent findings showing that nucleosomes are organized into discrete "nucleosome clutches" or "tetranucleosomal folding motifs" along the chromatin fiber in vivo (Hsieh et al., 2015; Ricci et al., 2015).

A critical function of the 30-nm chromatin fiber is to regulate the accessibility of transacting factors via dynamic unfolding and folding transitions between the compact 30-nm chromatin fiber and the more accessible nucleosomal array (Li et al., 2010; Li and Reinberg, 2011). Therefore, it is of great importance to understand how the dynamic organization of the 30-nm chromatin fiber is regulated during DNA-related biological processes. However, the highly dynamic and heterogeneous properties of chromatin fibers impose various technical challenges to probe the detailed structure and dynamics of chromatin fibers using traditional biochemical assays. Single-molecule methods offer

powerful tools to investigate the dynamic organization of chromatin fibers by tracing the real-time folding and unfolding of individual nucleosome and chromatin fiber (Bintu et al., 2012; Brower-Toland et al., 2002; Hall et al., 2009; Kruithof et al., 2009; Meng et al., 2015; Yan et al., 2007). Despite many key insights into the stability of linear nucleosomal array obtained from these single-molecule studies, it is still a puzzle whether any distinct intermediates exist between these two states (i.e., the compact 30-nm chromatin fiber and the more accessible nucleosomal array).

In this study, we demonstrate that mechanical unfolding of a 30-nm fiber is a multi-step process and the tetranucleosomes-on-a-string is a distinct intermediate of chromatin. In addition, we find that the stability of the tetranucleosomal unit is negatively regulated by chaperone FACT to facilitate gene transcription. Finally, we find that linker histone H1 enhances the stability of nucleosome and promotes the dynamics of folding and unfolding of the outer turn of individual nucleosome. Together, our results reveal that the tetranucleosome is a regulatory structural unit of chromatin, which provides another level of basis for gene regulation in addition to the nucleosome.

RESULTS

Force Spectroscopy of the 30-nm Chromatin Fiber Reveals Three Major Distinct Structure Transitions

The compact chromatin fiber must unfold to provide access to the genomic DNA for transcription factors and RNA polymerases during eukaryotic gene activation in vivo. To decipher the chromatin fibers dynamics, we used magnetic tweezers to investigate the dynamics of chromatin fibers reconstituted in vitro using purified proteins (Figure S1A) on the basis of a DNA containing 24 tandem repeats of 177-bp Widom 601 nucleosome positioning sequence (24 × 177 bp; Figure 1A, top). The reconstituted fibers were first characterized by AUC, EM, and cryo-EM analysis, as previously described (Song et al., 2014). Without H1, nucleosomal arrays adopted an extended "beads-on-a-string" conformation (Figure 1A, bottom left). With H1, nucleosomal arrays were further condensed into compact chromatin fibers (Figure 1A, bottom right).

To trace the conformation change, we applied a continuously increasing force on a single chromatin fiber (Figure 1B). The force-extension curve of a chromatin fiber containing H1 is shown by the blue curve in Figure 1C, with that of a nucleosomal array as a control (without H1, orange curve). The compact chromatin fiber requires a much higher force than the nucleosomal array does to extend to the same degree, indicating the higher stability of the condensed chromatin fiber. In the low-force region (<8 pN) (Figure 1C, stage I), the curve of the chromatin fiber deviates from that of the nucleosomal array at ~1 pN, and a force plateau spanning a range of ~100 nm near 3 pN is identified (Figure 1C, left inset). Above 8 pN, stepwise unfolding events were observed, which correspond to nucleosome ruptures. Two different step sizes have been fitted by the worm-like chain model (Bustamante et al., 1994), which correspond to two major stages of nucleosome disruption (Figure 1C, stages II and II, and Figure S1B) (Hall et al., 2009; Mihardja et al., 2006). Between 8 and 25 pN, the most probable step size is 21.2 ± 0.4 nm (mean ± SE), corresponding to the rupture of the outer DNA wrap (Figure S1C, left, stage II). Above 25 pN, the most probable step size is 25.6 ± 0.6 nm (mean ± SE), corresponding to the rupture of the inner DNA wrap (Figure S1C, right, stage III). Above 40 pN, the force-extension curve approaches that of naked 1.5-μm DNA (Figure 1C, black dashed curve), indicating that under such conditions, all nucleosomes in the chromatin fiber were irreversibly disrupted.

H1 Facilitates the Folding Rate of the Outer Turn of Nucleosomal DNA

Linker histone H1 has been shown to play a critical role in the stability of nucleosome (Mihardja et al., 2006) and the formation of the 30-nm chromatin fiber (Robinson et al., 2006; Song et al., 2014). However, the exact function of H1 in the 30-nm chromatin fiber remains unclear. The force-extension curves in Figure 1C display a sharp force rise when a chromatin fiber with H1 was stretched to ~0.5 μm (data in blue), while no such sharp force rising was observed in nucleosomal arrays without H1 (data in orange). Because ~0.5 μm of extension marks the left boundary of stage II, where stepwise nucleosome unfolding begins, the result indicates that the stability of the individual nucleosome is increased because of H1 binding.

Furthermore, we measured the equilibrium folding and unfolding transitions of nucleosomes at 9–11 pN for chromatins with H1 and of 5–7 pN for nucleosomal arrays without H1, respectively. In these force ranges, the inner wrap of nucleosome DNA remains folded. Two-step transitions in Figure 2A provide a framework for the free energy calculation of the outer DNA wrap (Liphardt et al., 2001) (Figures S2A and S2B). According to the relationship between the kinetic rate constant and tension (Figure 2B), the free energy cost of outer wrap unfolding at zero tension is 53 ± 3 kJ/mol (mean ± SD) for chromatin fibers with H1 (see details in the Supplemental Information). As a control, similar time traces of extension were observed for nucleosomal arrays without H1 at a lower force region (5–7 pN) (Figure S2C, left). The corresponding free energy cost of outer wrap unfolding was 32 ± 2 kJ/mol (mean ± SD) (Figure S2C, right), which is consistent with the previously reported value of ~30 kJ/mol (Mihardja et al., 2006). Compared with nucleosomal arrays without H1, the additional energy cost of ~20 kJ/mol is determined for those with H1, which is consistent with previous observations that H1 enhances the stability of nucleosome by locking the outer nucleosomal DNA wraps (Brown et al., 2006; Robinson et al., 2006; Song et al., 2014).

We also examined the folding kinetics of outer nucleosomal DNA wrap for nucleosomes with and without H1 (Figure 2C). Near the equilibrium tension F_{eq}, the mean life time τ_0 for folded or unfolded states of the nucleosome containing H1 is 0.7 s, while that for the nucleosome without H1 is 19.9 s, suggesting that H1 not only stabilizes the nucleosome but also enhances the folding rate of the outer DNA wrap. To simplify the system and rule out the possible interference of nucleosome-nucleosome interactions in chromatin fibers, we examined the dynamic folding and unfolding of mononucleosomes with H1 (Figures S2D–S2G), which agrees well with the data in the chromatin fibers (Figure 2).

The inner DNA wrap in either the extended nucleosomal array or the compact chromatin fiber containing H1 was disrupted at force above 25 pN. The most probable step size is ~25 nm

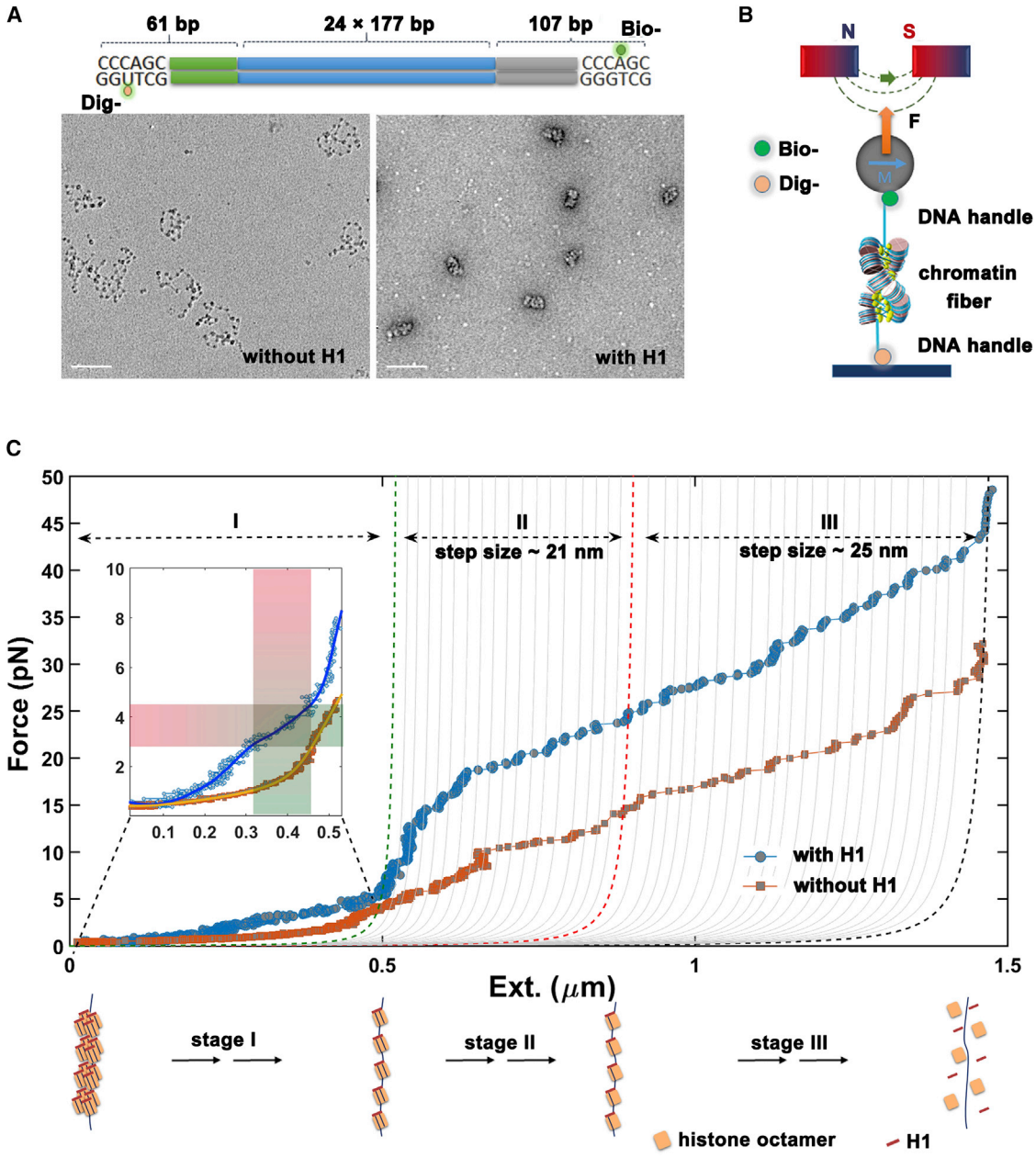


Figure 1. Overall Force-Extension Curves

(A) Top: constructs of the DNA template: a 4,416 bp DNA containing 24 × 177 bp 601 sequence labeled with biotin and digoxigenin respectively. Bottom: EM images of chromatin fibers without (left, metal shadowing) and with H1 (right, negatively stained). Bar length is 100 nm.

(B) Schematic setup of the magnetic tweezers used in our chromatin fiber studies (not to scale).

(C) Comparison of two typical force-extension curves of a chromatin fiber with H1 (blue curve) and without H1 (orange curve) in HE buffer. The chromatin fibers with or without H1 were reconstituted in the same batch of experiment and all the measurements were repeated for ten more samples. Tensions were applied from sub-pN to tens of pN by moving the magnets at a rate of 2 μm/s. Three major distinct stages can be recognized in the force-extension curve of the chromatin fiber with H1 (blue curve). The inset shows the details of stage I at low forces (<8 pN).

See also Figure S1.

(Figures S2H and S2I), which is consistent with previous studies (Brower-Toland et al., 2002; Mihardja et al., 2006). These results suggest that H1 has no effect on the folding and unfolding process of the inner DNA wrap. Moreover, in the repeating force-jump measurements between 10 and 35 pN, both the numbers and the sizes of steps decreased successively (Figures S2J and S2K), indicating that the inner DNA wraps may unravel irreversibly from histone octamers.

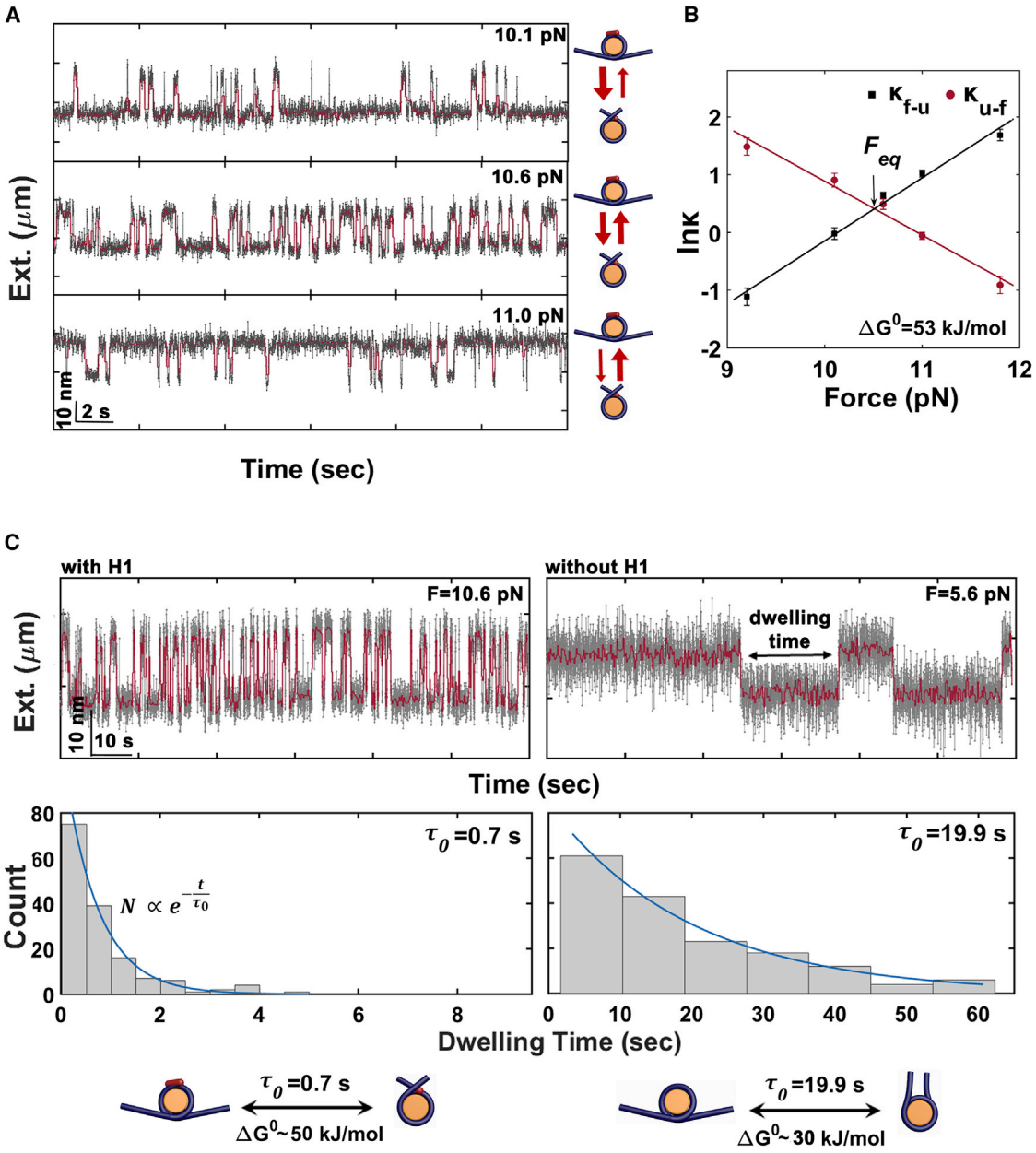


Figure 2. Dynamics of the Outer DNA Wraps of Nucleosomes

(A) Folding and unfolding trajectories of the outer DNA wrap at various tensions.

(B) The relationship between the kinetic rate constant and tension. The equilibrium force F_{eq} was read from the point at which the unfolded rate ($\kappa_{f\text{-}u}$) equals the folded rate ($\kappa_{u\text{-}f}$). The error bar is derived from the SD of repeated measurements.

(C) At F_{eq}, the folding and unfolding kinetics of the nucleosome with H1 are much faster than without H1. The kinetics are represented by the average lifetime τ_0 in the unwrapped state, which is 0.7 s with H1 and 19.9 s without H1.

See also Figure S2.

The Tetranucleosomal Unit Appears as a Distinct Intermediate in the Hierarchical Organization of Chromatin Fibers

The structural transition (Figure 1C, stage I) near 3 pN is attributed to the disruption of nucleosome-nucleosome interactions in higher order chromatins because the nucleosomes remain intact at such low forces (Cui and Bustamante, 2000; Meng et al., 2015). Repeated measurements on different chromatin fibers show a small extent of variation at the force plateau, which indicates the highly homogeneous assembly and structural arrangement of different chromatin fibers (Figure S3A). To gain more insights into its nature, we performed force-clamp measurements of chromatin fibers. Interestingly, repeatable stepwise folding and unfolding dynamics were observed at 3.5 pN

for chromatin fibers with H1 (Figure 3A), while no such structural transitions were observed for nucleosomal arrays without H1 at a range of forces (2.5–4.0 pN) (Figure S3B). By chi-square step-fitting algorithm (Kerssemakers et al., 2006), three critical transition states can be clearly identified, with the most probable step sizes being 7.4 ± 0.4, 13.5 ± 0.6, and 21.5 ± 0.4 nm (mean ± SE), respectively (Figures 3A and 3B). Our previous cryo-EM structures showed a left-handed twist of repeating tetranucleosomal units in chromatin fibers (Song et al., 2014). Within each unit, two sets of nucleosome-nucleosome interfacial interactions are involved, embedding three free linker-DNA segments with a length *L*. As shown in Figure 3C, one linker length (1*L*) would be released if one set of these interfacial interactions were mechanically disrupted, and the subsequent disruption of the other set of interactions would release another two free DNA linkers, contributing a length 2*L*. Alternatively, the two interfacial interactions could be disrupted simultaneously, resulting in a length change of 3*L*. Therefore, the disruption of the tetranucleosomal unit would produce a process of three-state transitions with the step sizes of 1*L*, 2*L*, and 3*L*, respectively (Figure 3D). To confirm this hypothesis, the dynamics of single tetranucleosome with H1 based on a DNA containing four tandem repeats of 177-bp 601 sequence (4 × 177 bp) were also examined (Figures 3E and S3C–S3E), which fully support our conclusions for the tetranucleosomal unit in 24 × 177 bp chromatin samples.

It was shown that H1 binding to nucleosome protects about 20 bp of the linker DNA (Simpson, 1978) and results in the formation of the stem structure of the linker DNAs (Syed et al., 2010). It is reasonable to assume that the binding of H1 to nucleosome results in a contraction of the linker DNAs by several base pairs. According to this model, for the 177-bp nucleosome repeat length (NRL) chromatin fibers containing H1, the average linker length released between the adjacent nucleosomes (*L*) is about 7.4 nm. Therefore, the DNA length contracted by H1 binding (*x*) can be calculated according to the formula $0.34 \times (177 - 146 - x) = 7.4$, where 0.34 is the DNA length (nm) per base pair, 177 the NRL (bp), and 146 the DNA length (bp) protected by the histone octamer. Our results demonstrate that the binding of H1 causes about 9 bp contraction of the linker DNAs.

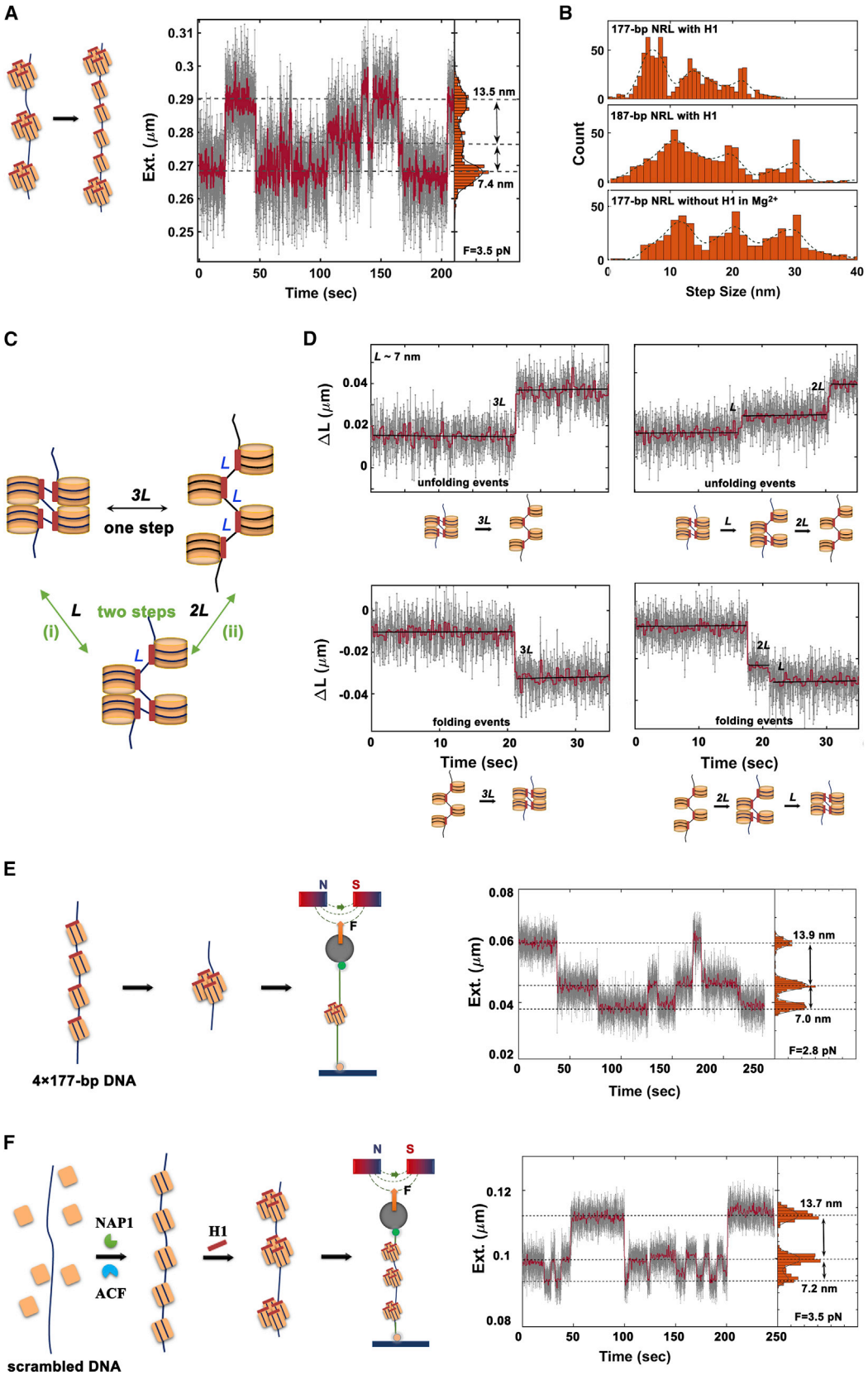
To double-check the proposed dynamics of the tetranucleosomal unit, we carried out the similar measurements on the 187-bp NRL chromatin fiber, whose overall structures are very similar to that of the 177-bp NRL chromatin fiber (Song et al., 2014). As shown in Figures S4B and S4C, the force-extension curves for chromatin with 187-bp NRL are very similar to that with 177-bp NRL. At 3.2 pN, similar folding and unfolding behavior was also recorded (Figure S4D, middle), and the three step sizes derived from the distribution are 10.5 ± 0.4, 19.4 ± 0.6, and 30.2 ± 0.5 nm (mean ± SE), respectively (Figure 3B, middle). Interestingly, the difference in NRL does not change the overall dynamics of the structural transition but increases the average step size from 7.4 nm for chromatin fibers with 177-bp NRL to 10.5 nm for those with 187-bp NRL. The 10-bp increment in NRL leads to an increase of length *L* by 3.4 nm, which is very close to the increase in step size observed in our experiments. More important, for both the chromatin fibers, the jumps with length *L* occur prior to the jumps with length 2*L* in the unfolding process, while the jumps with length *L* follow the jumps with length 2*L* in the refolding process (Figures 3D and S4E). These dynamic processes agree well with our proposed model in Figure 3C. Together, we conclude that the transition at ~3.5 pN represents the folding and unfolding of the tetranucleosomal unit.

Similar measurements were also performed on chromatin fibers assembled with scrambled (non-repetitive 601) DNA sequence (Li et al., 2010) (Figures 3F and S3F–S3H). Stepwise folding and unfolding dynamics with three critical transition states near 3 pN were also clearly identified (Figure 3F). But the most probable step sizes were not as certain as that for chromatins assembled on the 601 DNA sequence. Several distinct step sizes can be observed for chromatin fibers assembled with scrambled DNA sequence (Figures 3F and S3H). Our results suggest that tetranucleosomal units also exist in chromatin fibers assembled with scrambled DNA sequence, but its structure is not as homogeneous as that for chromatins assembled on the 601 DNA sequence.

A recent micro-C analysis of chromosome folding in vivo demonstrated that tetranucleosomal folding motifs with zigzag conformation may widely exist in the yeast genome in which low stoichiometry of the linker histone HHO1 is found with nucleosomes (Hsieh et al., 2015). Therefore, the tetranucleosomal unit might also be a common folding intermediate during chromatin compaction in the absence of linker histones. It has been established that Mg^{2+} promotes folding of nucleosomal arrays, even in the absence of linker histones (Schalch et al., 2005; Shogren-Knaak et al., 2006). We measured the force-extension curve of the 177-bp NRL chromatin fiber in the presence of Mg^{2+} at low forces (Figure S4F). Intriguingly, the force plateaus corresponding to the higher order structural transition were also observed (Figure S4G). Similarly, the stepwise folding and unfolding dynamics in force-clamp measurements at 3.1 pN were identified (Figure S4A, bottom, and Figure S4D, bottom) with step sizes of 11.0 ± 0.4, 20.3 ± 0.5, and 30.0 ± 0.7 nm (mean ± SE) (Figure 3B, bottom). Without the proposed 9-bp contraction of the linker DNA by H1 binding, the average length *L* for the chromatin fiber in Mg^{2+} (~11.0 nm) is about 3.6 nm longer than that with H1 (~7.4 nm). These results demonstrate that the chromatin fiber in Mg^{2+} possesses a similarly stable intermediate structure (i.e., the tetranucleosomal unit) (Figure 3C).

Tetranucleosomes-on-a-String Is a Distinct Secondary Structure during In Situ Chromatin Condensation

Histone chaperone NAP-1 has been shown to correctly deposit histone H1 into nucleosomes to compact chromatins (Li et al., 2010). To examine the H1-induced condensation of nucleosomal arrays into higher order structures in situ, we added the mixture of H1 and NAP-1 into the flow cell in which the 177-bp NRL nucleosomal arrays are tethered. The gradual condensation behavior with several ~20 nm steps at 1.6 pN was traced as shown in Figure 4A. After the condensation, we carried out the force-extension measurement for the in situ condensate, and a force plateau spanning a range of ~100 nm near 3 pN was identified (Figure 4B), indicating that the condensation of chromatin fibers in situ follows a very similar pathway as that of the pre-reconstituted chromatin fibers with H1 (Figure 1C, inset). In addition, force-clamp measurements for in situ condensed



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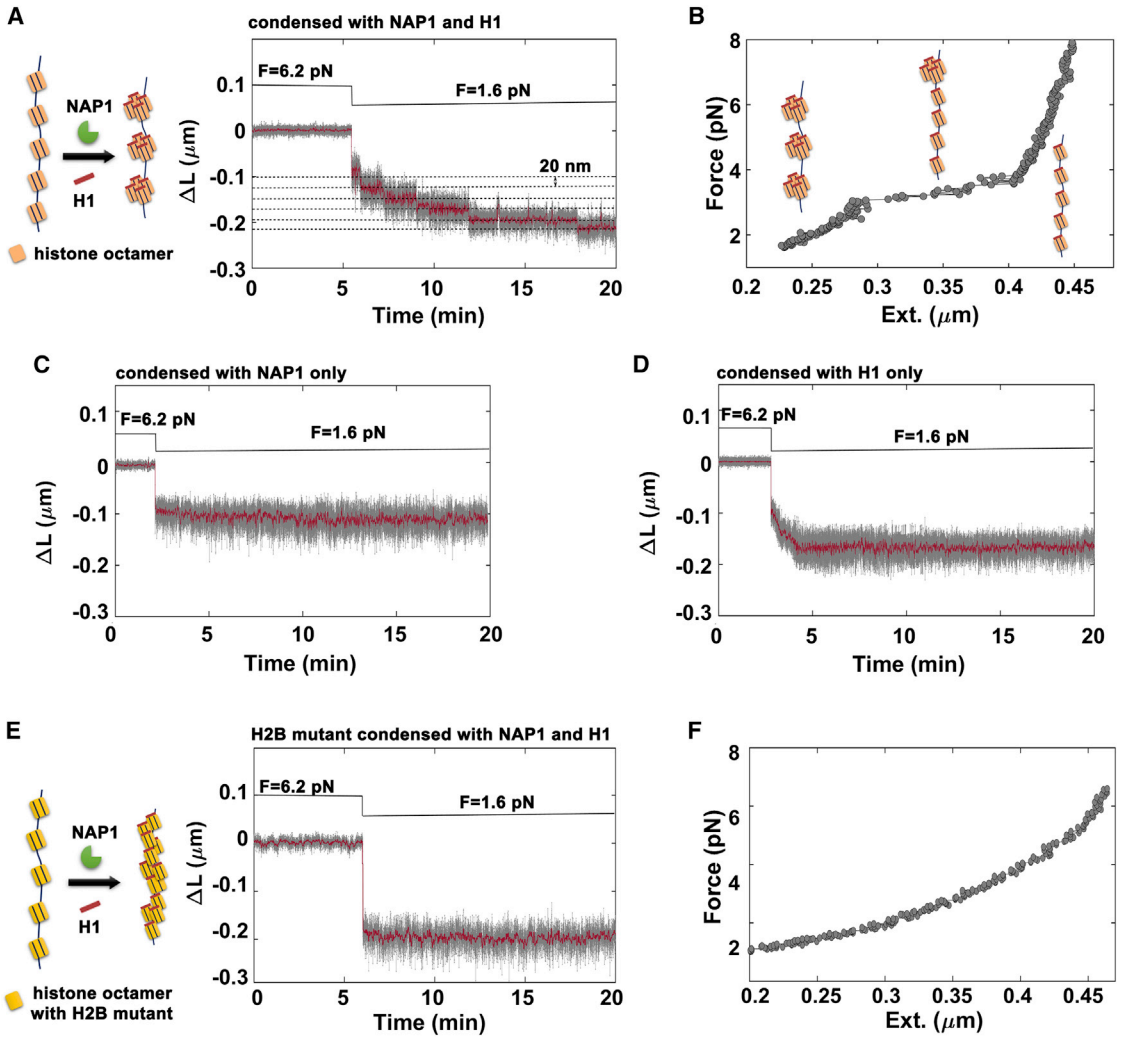

Figure 4. Real-Time Condensation of Single Nucleosomal Array into Chromatin Fiber In Situ

(A) The stepwise condensation process of a nucleosomal array in the presence of H1 and NAP1.

(B) The force-extension measurement reveals the similar structural transition at ~3 pN for the in situ condensed chromatin.

(C and D) No similar stepwise condensation behavior is observed with NAP1 (C) or H1 (D) only.

(E) No similar stepwise condensation behavior is observed for samples with H2B mutant in the same buffer conditions.

(F) No structural transition at ~3 pN is observed by the force-extension measurement on the H2B mutant chromatin.

See also Figure S5.

chromatin fibers at 3.3 pN revealed the similar folding and unfolding dynamics of the tetranucleosome unit as that observed above for reconstituted chromatin fibers with H1 (Figure S5A). Similar step sizes of 7.8 ± 0.3, 13.0 ± 0.4, and 20.8 ± 0.3 nm (mean ± SE) were obtained (Figure S5A). However, no similar structural transition process could be observed for nucleosome arrays condensed only by either NAP-1 or H1 (Figures 4C and 4D). These results demonstrated that tetranucleosomal units

Figure 3. Folding and Unfolding Dynamics of Tetranucleosomal Units

(A) Stepwise folding and unfolding dynamics at 3.5 pN for the 177-bp NRL's chromatin fiber with H1.

(B) Step size distributions derived from ten chromatin fibers with more than 200 steps of the three samples.

(C) Model for the folding and unfolding dynamics of tetranucleosomal units with two alternative pathways.

(D) Examples showing that the jumps with length *L* occur prior to the jumps with length 2*L* in the unfolding process, while the jumps with length *L* follow the jumps with length 2*L* in the refolding process.

(E) Stepwise folding and unfolding dynamics at 2.8 pN for chromatin fiber assembled on four tandem repeats of 177-bp DNA with H1.

(F) Stepwise folding and unfolding dynamics at 3.5 pN for chromatin fiber assembled on scrambled DNA with H1.

See also Figures S3 and S4.

were also formed during in situ chromatin condensation by H1 chaperoned with NAP-1 (Li et al., 2010).

The tetranucleosomal unit of chromatin fibers has been shown to be stabilized by the interactions between the H2B α1/αC helix and the H2A α2 helix of the neighboring octamers in each dinucleosomal stack (Schalch et al., 2005; Song et al., 2014). The interactions within the tetranucleosomal unit might be disrupted by mutations of S109AE110AT112A in histone H2B (H2BS109AE110AT112A). We therefore investigated how these mutations affect in situ chromatin condensation by H1 chaperoned with NAP-1. Our AUC analysis showed that these mutations indeed impair chromatin compaction by H1 (Figure S5B). Similarly, no regular condensation steps were observed after the addition of H1 and NAP-1 in our single-molecule experiment (Figure 4E). In addition, no similar structural transition for the tetranucleosome unit could be observed in force-extension measurements of the in situ condensed chromatin fibers with H2B mutations (Figure 4F). Above results suggest that the formation of the tetranucleosomal unit in situ requires the nucleosome-nucleosome interactions in the dinucleosomal stacks.

Histone Chaperone FACT Regulates the Dynamics of the Tetranucleosomal Unit

As a stable structural intermediate of the chromatin fiber, the tetranucleosomal unit may play an important regulatory function in the genome-associated processes. We and others have previously shown that tetranucleosomal units of chromatin fibers are stabilized by interactions between the H2B α1/αC helix and the H2A α2 helix of the neighboring octamers in each dinucleosomal stack (Schalch et al., 2005; Song et al., 2014). Therefore, any factor that interferes with the interfacial interactions in the nucleosomal stacks could regulate the dynamics of tetranucleosomal units. A recent study revealed that H2B α1 helix is the primary region of the interaction between H2A/H2B and FACT (Hondele et al., 2013), which was originally identified as a histone chaperone specific for H2A/H2B to facilitate chromatin transcription (Belotserkovskaya et al., 2003; Orphanides et al., 1999), implying that FACT may regulate the dynamics of the tetranucleosomal unit (Figure 5A). We performed AUC analysis to monitor the effect of FACT on the compaction of chromatin fibers. It turned out that the addition of FACT does not affect the sedimentation of the nucleosomal arrays without H1, while it reduces the sedimentation coefficient of chromatin fibers containing H1 (Figures S6A and S6B), indicating that FACT indeed alters the compaction of chromatin fibers containing H1.

To further study the effect of FACT on the tetranucleosomal unit, we repeated measuring the force-extension curve of chromatin fibers after the addition of FACT. The force-extension curve was initially similar to that without FACT, featuring a force plateau around 3.5 pN (Figure 5B). Following the addition of FACT, the force plateau gradually lowered to ~1.7 pN in 30 min (Figure 5B), which suggests that FACT can attenuate the tetranucleosomal unit. We then carried out force-clamp measurements for chromatin fibers at 2.0 pN in the absence or presence of FACT (Figure 5C). Interestingly, six successive 20-nm steps in Figure 5C coincide perfectly with six tetranucleosomal units in the 24 × 177 bp chromatin fiber, indicating the sequential disruption of tetranucleosomal units by FACT. Moreover, after the chromatin fiber was incubated with FACT for 2 hr, a stepwise folding and unfolding process was identified at 1.7 pN, with step sizes of 7.2 ± 0.2, 13.8 ± 0.4, and 21.0 ± 0.4 nm (mean ± SE) (Figure 5D), which are similar to those observed for chromatin fibers with H1 in the absence of FACT (Figure 3A). The result indicates that FACT does not completely disrupt the tetranucleosomal unit but attenuates the stability of the tetranucleosomal unit. In addition, six successive ~30 nm step sizes were also observed for disruptions of six tetranucleosomes in the presence of Mg^{2+} (Figure S6C), which is also consistent with the tetranucleosome model for chromatin fibers in Mg^{2+} (Figure 3B).

Next, we examined the effect of FACT-ΔC, a truncated protein of FACT with ~230 amino acids from the C terminus of Spt16 deleted (Figure S6A), on tetranucleosomes (Figure 5E). FACT-ΔC lacks the U-turn motif, the primary interaction region of Spt16 with the H2B α1 helix (Hondele et al., 2013), and cannot interact with mononucleosomes or facilitate transcription on chromatin templates (Belotserkovskaya et al., 2003). Interestingly, with the U-turn motif deleted, FACT-ΔC cannot affect the stability of the tetranucleosomal unit as wild-type FACT (Figure 5E). Further investigation shows that the U-turn motif of Spt16 itself attenuates the tetranucleosomal unit (Figure 5F). By contrast, the U-turn mutant with the conserved residues engaged with H2B (Asn901/Lys904/Ile905/Thr908) mutated to serine, which was shown to fail to interact with H2A/H2B (Hondele et al., 2013), does not show this effect on the tetranucleosomal unit (Figure 5G). Together, these results indicate that FACT, likely the U-turn motif of Spt16, attenuates the stability of the tetranucleosomal unit.

FACT Destabilizes Tetranucleosomal Units and Motifs of Chromatin Fibers to Facilitate Gene Transcription

Recently, a novel Hi-C-based method, "Micro-C," was developed by Rando and colleagues to enable mapping the chromosome folding at nucleosome-resolution in yeast (Hsieh et al., 2015). They demonstrated that the interactions between the N/N+2 nucleosome pairs (interactions between the nucleosomes with one nucleosome's interval) are abundant similarly to that for the N/N+1 nucleosome pairs (interactions between the adjacent nucleosomes) in the whole genome, which corresponds to the existence of tetranucleosomal units and motifs in chromatin fiber folding. We performed chromatin immunoprecipitation sequencing (ChIP-seq) analysis for ySpt16, which is the large subunit of FACT, to monitor the genome-wide distribution of FACT in yeast. To explore the effect of FACT on tetranucleosomal units in vivo, we analyzed the genome-wide correlation between Micro-C interactions (N/N+2) and levels of FACT (ySpt16) in yeast. Our analysis revealed that most of the N/N+2 Micro-C interactions (tetranucleosomal units and motifs) are negatively correlated with the levels of FACT (ySpt16) (see Figure 6A for the whole yeast genome and Figures 6C and S7 for specific gene regions), which support our observation that histone chaperone FACT may remodel the chromatin compaction by destabilizing tetranucleosomal units (Figures 5B and 5C). Previously, it was shown that gene compaction with a high level of tetranucleosomal units and motifs was anti-correlated with transcription activity (Hsieh et al., 2015). We analyzed correlations between transcription activities of genes with the enrichment

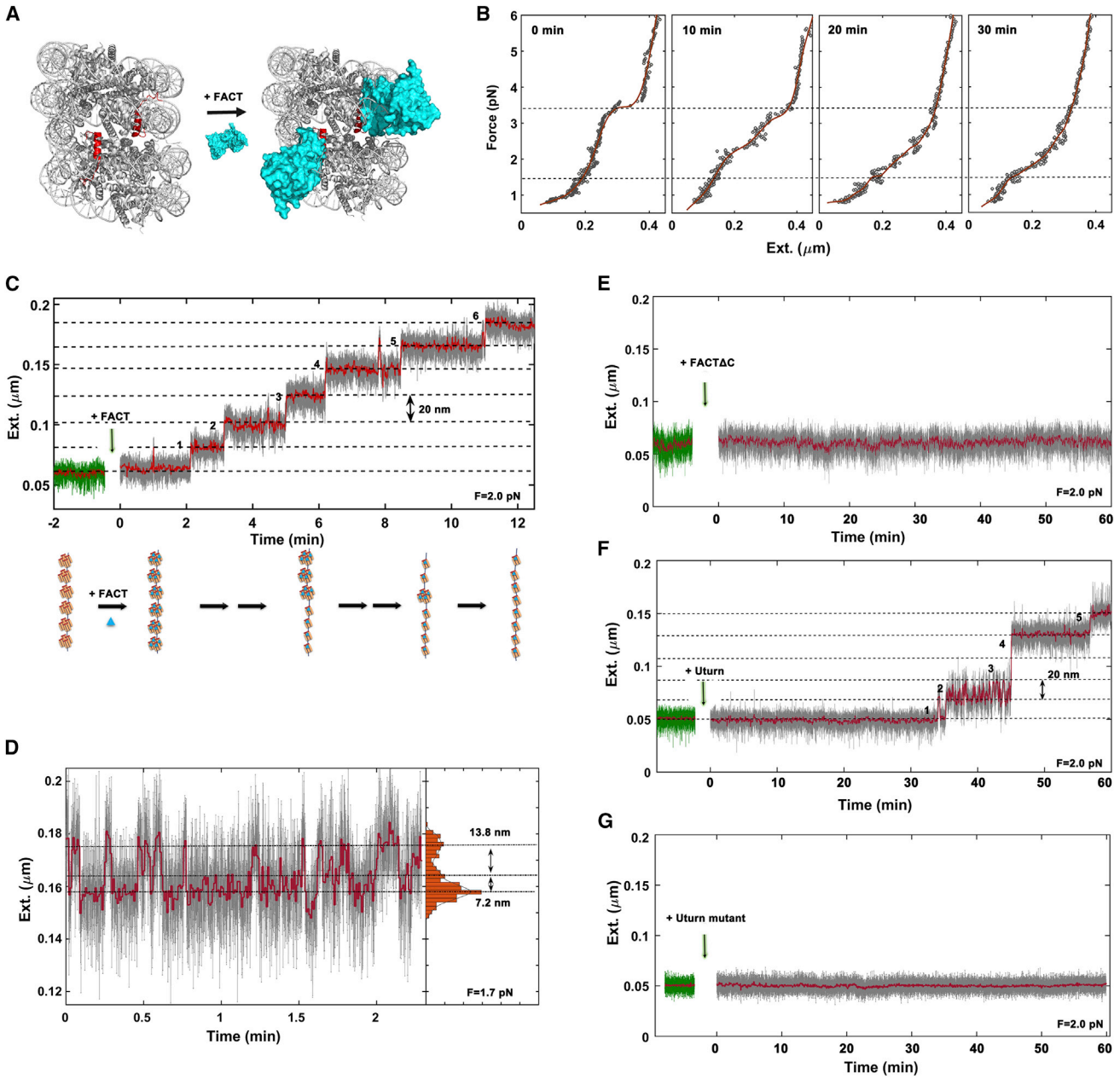

Figure 5. FACT Regulates the Dynamic Behavior of Tetranucleosomal Units

(A) The proposed binding site of FACT on nucleosomes. The dinucleosomal stack (gray) was adopted from the X-ray structure of the tetranucleosome (Protein Data Bank [PDB] accession number 1ZBB) with the H2B α1 helix highlighted in red. FACT (blue) and its binding position was adopted from the X-ray structure of H2A-H2B heterodimer with spt16M domain of Spt16 (PDB accession number 4KHA).

(B) Gradual variation of the force-extension curve of a chromatin fiber with H1 before and after incubation with FACT for 10, 20, or 30 min.

(C) Time course measurement of the extension of chromatin fiber with H1 at 2.0 pN before and after the addition of FACT.

(D) Stepwise folding and unfolding at 1.7 pN after chromatin fibers were incubated with FACT for 30 min.

(E–G) Time course measurement of the extension of chromatin fiber with H1 at 2.0 pN before and after the addition of FACTΔC (E), U-turn motif (F), and the mutant of U-turn motif (G), respectively.

See also Figure S6.

of N/N+2 Micro-C interactions (tetranucleosomal units and motifs) and levels of FACT (ySpt16). Interestingly, our genomic analysis revealed that the genes with higher level of FACT (ySpt16) and tetranucleosomal units and motifs depleted are significantly more active than the ones with lower level of FACT (ySpt16) and tetranucleosomal units and motifs enriched (see Figure 6B for

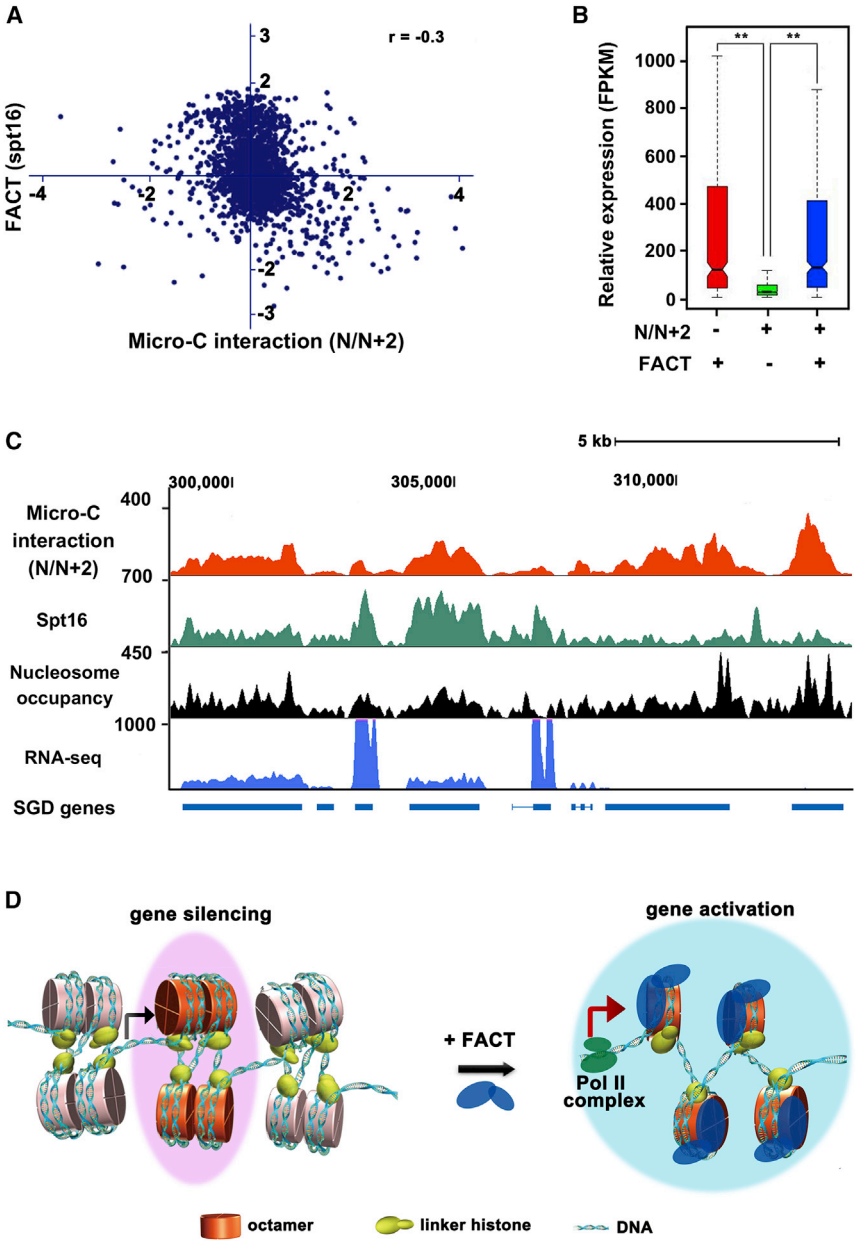

Figure 6. Genome-wide Analysis on the Correlation of FACT with Tetranucleosomal Units in Yeast

(A) Scatterplot for anti-correlated pair of tetranucleosomal units and FACT. The x axis represents the spt16 ChIP data, the y axis shows the interactions between nucleosome N and N+2 based on micro-C data, normalized to nucleosome occupancy and ChIP input data.

(B) Genome-wide analysis on the correlations between transcription activities of genes with the enrichment of N/N+2 Micro-C interactions (tetranucleosomal units) and the levels of FACT (ySpt16). **Significant difference ($p < 0.01$) according to Wilcoxon signed-rank test.

(C) The distribution of tetranucleosomal units and FACT in specific gene examples. The chromatin regions with FACT enriched are correlated with low level of N/N+2 interactions and associated with highly transcribed genes.

(D) The model of FACT facilitating gene transcription by destabilizing the tetranucleosome structural units.

See also Figure S7.

the whole yeast genome and Figures 6C and S7 for specific gene regions). Together, our results indicate that FACT facilitates the transcription of a subset of genes by destabilizing the tetranucleosomal unit and motif of chromatin fibers (Figure 6D), which agree well with our above results from single-molecule analysis in vitro.

In this study, the hierarchical organization of 30-nm chromatin fibers has been revealed by magnetic tweezers (Figure 7). First, the chromatin fiber unfolds to an intermediate "tetranucleosomes-on-a-string" structure by disrupting the interactions between tetranucleosomal units. Second, the tetranucleosomal unit further unfolds to a complete open nucleosomal array by disrupting the nucleosome-nucleosome interactions within the unit. Next, the outer DNA wrap unravels from the histone octamer in a reversible two-state hopping manner. Ultimately, the inner nucleosomal DNA wrap unravels irreversibly from the histone octamer.

DISCUSSION

Function of H1 on the Stability and Kinetics of Nucleosomes

The structure and dynamics of nucleosomes without linker histones have been extensively studied in the past two decades (Bintu et al., 2012; Brower-Toland et al., 2002; Hall et al., 2009), showing that nucleosome unravels in two stages (Brower-Toland et al., 2002; Kruithof et al., 2009; Mihardja et al., 2006; Mochrie et al., 2013; Sheinin et al., 2013). Linker histone H1 has been proposed to play a critical function in gene repression (Brown et al., 2006; Zhou et al., 2013). However, the exact function of H1 still remains to be determined. In this study, we investigated the effect of H1 on the dynamics of the nucleosome in the context of chromatin fiber. We revealed that the binding of H1 results in a free energy increase by about 20 kJ/mol to disrupt the outer DNA wrap, which agrees well with previous proposals that H1 enhances the stability of the nucleosome by locking the outer DNA wraps at the entry and exit point (Syed et al., 2010; van Holde and Zlatanova, 1996). More important, we observed that H1 accelerates greatly the folding rate of the outer wraps of the nucleosome, which may allow H1 act as a key factor to control the accessibilities of transcription factors or polymerase to their target DNA sites hidden in nucleosomes. The dissociation of H1 results in a slower folding and unfolding rate of

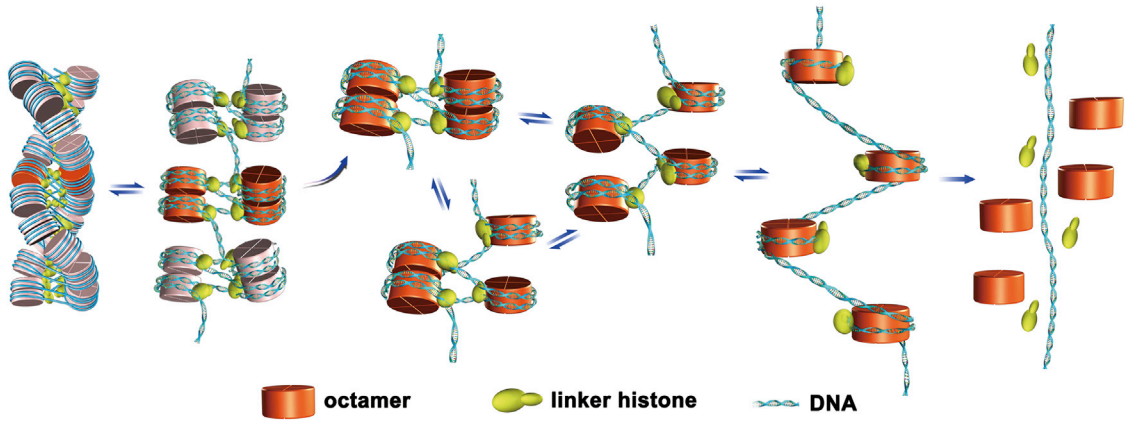

Figure 7. Model of the Dynamic Organization of Chromatin Fibers

The left-handed double helical chromatin fiber unfolds to a "tetranucleosomes-on-a-string" extended structure; then the tetranucleosomal unit further unfolds to a complete open nucleosomal array in one or two steps; finally, the outer nucleosomal DNA wrap unravels in a reversible two-state hopping manner, followed by the irreversible unfold of the inner DNA wrap.

nucleosomes, which may provide a sufficient time window to allow the transcription factor to access and reside at its target sites and the RNA pol II to elongate through the unwrapped nucleosome.

The Tetranucleosomal Unit Is a Stable Intermediate of the Chromatin Fiber

The structural transition between the 30-nm chromatin fiber and the nucleosomal array has been proposed to play critical functions in regulating the accessibility of DNA template for transacting factors during DNA metabolism (Li et al., 2010; Li and Reinberg, 2011). Deciphering the structure and dynamics of the 30-nm chromatin fibers in molecular details is essential for understanding such regulations. Several single-molecule techniques have been applied to investigate in real-time the dynamic condensation of individual chromatin fibers (Cui and Bustamante, 2000; Kruithof et al., 2009; Pope et al., 2005). However, all previous studies failed to reveal the precise structural and dynamic properties of these transitions. To identify the nature of these transitions, we performed force-clamp measurements of 30-nm chromatin fibers with H1, whose high-resolution 3D cryo-EM structures have been resolved recently (Song et al., 2014). Stepwise folding and unfolding dynamics of the tetranucleosomal unit were clearly observed at low forces (3–4 pN). Similar dynamic processes can be observed when nucleosomal arrays condense in situ into 30-nm chromatin fibers by H1 (Figure 4). In addition, the tetranucleosomal unit also exists in the chromatins assembled in the presence of Mg^{2+} only or on scrambled (non-repetitive 601) DNA sequence. These results suggest that the tetranucleosomal unit is a stable structural intermediate of the chromatin fiber.

Although the existence of the 30-nm fiber in vivo is still under debate, several studies have suggested that a common tetranucleosomal folding unit and motif with zigzag conformation may widely exist in yeast and HeLa cells (Hsieh et al., 2015). Cryo-EM studies have shown that a 30-nm fiber is indeed the most predominant form of starfish sperm and nucleated chicken erythrocyte chromatin, and observed to be arranged in a zigzag two-start helix conformation (Horowitz et al., 1994; Scheffer et al., 2011; Woodcock, 1994). By using the Hi-C-based Micro-C technique, Rando and colleagues recently found that the N/N+2 nucleosome pairs and the N/N+1 nucleosome pairs are similarly abundant in the whole yeast genome, indicating the existence of tri- or tetra-nucleosomal folding motif in yeast genome (Hsieh et al., 2015). Similarly, using electron micro-assisted nucleosome interaction capture crosslinking assays, a recent study further supported the existence of tri- or tetra-nucleosomal folding motif with zigzag features in nuclear chromatin fiber of interphase HeLa cells (Grigoryev et al., 2016). The common existence of tetranucleosomal folding unit and motif broadly supports our in vitro findings that the tetranucleosomal unit appears as a stable structural intermediate of the chromatin fiber.

FACT Facilitates Gene Transcription by Remodeling the Tetranucleosomal Unit of Chromatin Fibers

The unfolding and folding dynamics of the tetranucleosomal unit may play an important regulatory function in the genome-associated processes. FACT, a conserved histone chaperone for H2A-H2B dimers, has been shown to play essential roles in nucleosome remodeling during DNA transcription, replication and repair (Belotserkovskaya et al., 2003; Formosa, 2013; Fujimoto et al., 2012; McCullough et al., 2015; Orphanides et al., 1998, 1999; Saunders et al., 2003). In this study, we have revealed that FACT can attenuate the stability of the tetranucleosomal unit. In addition, we find that the peptide of U-turn motif of Spt16 alone, which might weaken the nucleosome-nucleosome interactions within tetranucleosomal units by interacting with the N-terminal α1 helix of H2B (Hondele et al., 2013), can achieve this function of FACT, although the structure of the peptide may differ from that in the native FACT complex. Extensive structural and biochemical investigations in vitro have been performed on interactions between FACT and histones or nucleosomes (Belotserkovskaya et al., 2003; Orphanides et al., 1999; Tsunaka et al., 2016; Winkler and Luger, 2011), implying that

distinct domains of FACT could be responsible for its different activities in remodeling the nucleosome or the tetranucleosome. Therefore, it will be of great interest to investigate how distinct domains of FACT, including the Spt16M or Spt16C module, work on nucleosome or higher order chromatin structure by using the single-molecule techniques.

In addition, genome-wide analyses showed that FACT destabilizes the N/N+2 Micro-C interactions (tetranucleosomal units and motifs) to facilitate the transcription of a subset of genes in yeast. Accordingly, it is reasonable to speculate that in vivo, the tetranucleosomal unit can also be remodeled by protein factors that can interfere with interfacial interactions in the nucleosomal stacks. Apart from FACT discussed here, several other chromatin remodelers, such as INO80, which recognizes the H2B αC helix (Tosi et al., 2013), and certain repressive protein factors, such as PRC1, HP1, and MeCP2, may also fulfill their functions in the DNA-related biological processes by modulating the stability of tetranucleosomal units. Therefore, it is of great interest to identify these factors and decipher their regulatory interactions with tetranucleosomal units. Together, our results reveal that the tetranucleosomal unit provides an additional level of gene regulation beyond the nucleosome.

Moreover, our cryo-EM structures demonstrated that tetranucleosomal units are bridged and twisted by H1-H1 interactions and two inter-nucleosomal interactions between histone H4 N terminus and H2A/H2B acidic patches on the neighboring nucleosomes (Song et al., 2014). It is reasonable to assume that prior to the "tetranucleosomes-on-a-string" structure, a structural transition should occur by disrupting the interactions between the tetranucleosomal units. In our study, a small force plateau was often observed in force-extension curves at very low force region (~1.0 pN), which may result from the disruption of the inter-nucleosomal interactions between tetranucleosomal units. Unfortunately, in our magnetic tweezers assay, thermal fluctuations of the magnetic beads at such low-force region smeared out the signals of the transitions. The energy required to disrupt the interactions between the two tetranucleosomal units can be estimated as 1.0 pN × 7.4 nm = 1.8 k_BT, which is comparable to the thermal fluctuations, suggesting that chromatin fibers may undergo spontaneously rapid folding and unfolding dynamics between a compact regular 30-nm chromatin fiber and an extended "tetranucleosomes-on-a-string" at physiological conditions in vivo. Considering that the apparent gaps between tetranucleosomal units may provide a platform for epigenetic regulation either by histone modifications, histone variants, or other architectural proteins, it will be of great interest to decipher the dynamic stacking and unstacking process of tetranucleosomal units and the factors involved in regulating this dynamic process.

EXPERIMENTAL PROCEDURES

For additional details, see Supplemental Experimental Procedures.

Chromatin Reconstitution

Chromatin reconstitution was performed as described previously (Song et al., 2014). For the scrambled (non-repetitive 601) DNA sequence, the plasmid pBluescript II SK (~2.9kb) was digested by BseYI and labeled with either dUTP-digoxigenin or dATP-biotin by Klenow reaction at the end. Human FACT and FACT-ΔC were cloned and purified as previously described (Belotserkovskaya et al., 2003). The peptides for the U-turn motif and its mutant were chemically synthesized (Scilight Biotechnology) with the sequence listed as follows: U-turn VQSLNWTKIMKTIVDDPEGFFEQGGWSFLEPE; U-turn mutant VQSLSWTSSMKSIVDDPEGFFEQGGWSFLEPE. Metal shadowing with tungsten and negative staining for EM study and AUC analysis were performed as described previously (Chen et al., 2013). Details can be found in Supplemental Experimental Procedures.

Genome-wide Data Analysis

The single-end spt16 ChIP-seq and input data were downloaded from Gene Expression Omnibus (GEO: GSE66215) (Feng et al., 2016) and mapped to the *S. cerevisiae* sacCer3 assembly using bowtie2 (Langmead and Salzberg, 2012) with the default parameters, and the only uniquely mapped reads were used for further analysis. Micro-C interaction data analyzed as previously described (Hsieh et al., 2015) and nucleosome positioning data obtained from (Hall et al., 2009). The pair-end RNA sequencing (RNA-seq) data were downloaded from the National Center for Biotechnology Information with the accession number GSE67149 to analyze the genes' transcription level using TopHat with the default parameters, and retaining the uniquely mapped tags (Trapnell et al., 2009).

Magnetic Tweezers Analysis

In magnetic tweezers, single chromatin fibers were bound to a coverslip and a bead (M280/T1; Invitrogen). The bead image was projected onto a JAI Giga-Ethernet charge-coupled device (CCD) camera at 60 Hz through a microscope objective (Olympus 100 × 1.2, oil immersion). In the force-extension measurements, we tuned the magnets' position in the *z* direction by moving the magnets at 2 μm/s continuously, and the force on the chromatin fiber changed from ~0.5 pN to ~40.0 pN. To investigate the condensation of nucleosomal array with H1 and NAP1, we injected the mixture of NAP1 and H1 into the flow cell and adjusted the magnets to hold nucleosomal arrays at 6.2 pN. The length of nucleosomal array was traced in real time at 1.6 pN. More experimental details can be found in Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and seven figures and can be found with this article online at http://dx.doi.org/10.1016/j.molcel.2016.08.024.

AUTHOR CONTRIBUTIONS

W.L. performed the single-molecule magnetic tweezers analysis. P.C. performed the in vitro chromatin assembly and AUC and EM analysis. J.Y., J.F., Q.L., and Z.Z. performed and assisted in genome-wide analysis. D.L. and L.D. assisted in the preparation of DNA template and proteins. J.Y. and P.-Y.W. helped discuss the project. W.L., P.C., M.L., and G.L. conceived the project, analyzed the data, and wrote the manuscript.

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