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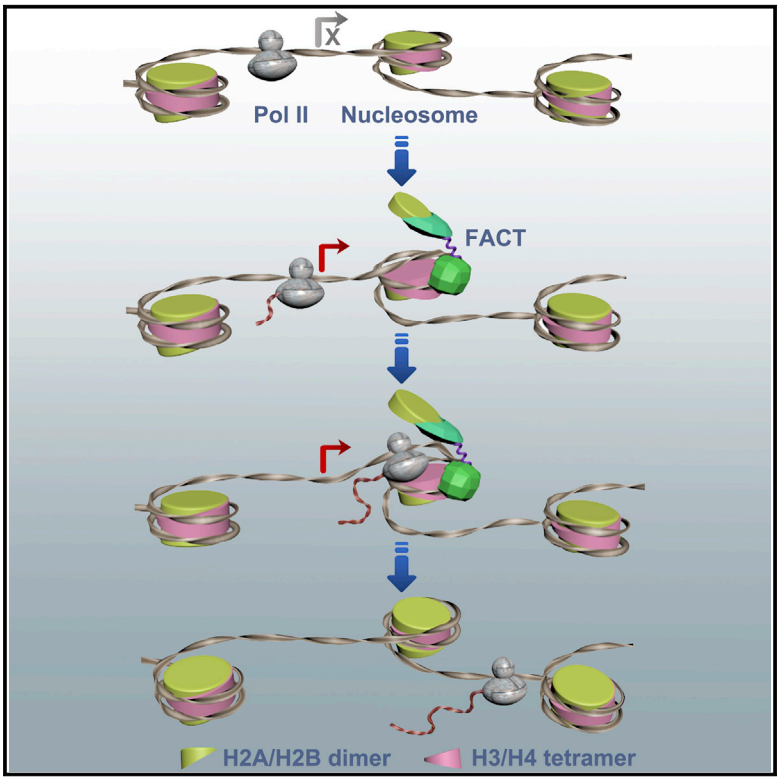
Molecular Cell

Functions of FACT in Breaking the Nucleosome and Maintaining Its Integrity at the Single-Nucleosome Level

Graphical Abstract



Highlights

- FACT has dual functions in breaking the nucleosome and maintaining its integrity
- SSRP1's HMG domain maintains the nucleosome inner wrap by holding the H3/H4 tetramer on DNA
- SSRP1 promotes the deposition of H2A/H2B dimers to form the intact nucleosome
- SPT16 displaces H2A/H2B dimers to disrupt the outer nucleosomal wrap

Authors

Ping Chen, Liping Dong, Mingli Hu, ..., Ming Li, Wei Li, Guohong Li

Correspondence

mingli@iphy.ac.cn (M.L.), weili007@iphy.ac.cn (W.L.), liguohong@ibp.ac.cn (G.L.)

In Brief

Chen et al. reveal that the two subunits of FACT, SSRP1 and SPT16, function distinctly but coordinately on nucleosomes to fulfill its functions in nucleosome disassembly and nucleosome integrity during DNA replication and transcription.

Chen et al., 2018, Molecular Cell *71*, 284–293
July 19, 2018
https://doi.org/10.1016/j.molcel.2018.06.020

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Functions of FACT in Breaking the Nucleosome and Maintaining Its Integrity at the Single-Nucleosome Level

Ping Chen,[1,7] Liping Dong,[1,3,7] Mingli Hu,[1,3] Yi-Zhou Wang,[2,3] Xue Xiao,[2,3] Zhongliang Zhao,[1] Jie Yan,[4,5] Peng-Ye Wang,[2,3] Danny Reinberg,[6] Ming Li,[2,3,*] Wei Li,[2,*] and Guohong Li[1,3,8,*]

[1]National Laboratory of Biomacromolecules, CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China
[2]National Laboratory for Condensed Matter Physics and Key Laboratory of Soft Matter Physics, Institute of Physics, Chinese Academy of Sciences, Beijing, China
[3]University of Chinese Academy of Sciences, Beijing, China
[4]Department of Physics, National University of Singapore, Singapore 117542, Singapore
[5]Mechanobiology Institute, National University of Singapore, Singapore 117411, Singapore
[6]Howard Hughes Medical Institute/Department of Biochemistry and Molecular Pharmacology, New York University Langone School of Medicine, New York, NY 10016, USA
[7]These authors contributed equally
[8]Lead Contact
*Correspondence: mingli@iphy.ac.cn (M.L.), weili007@iphy.ac.cn (W.L.), liguohong@ibp.ac.cn (G.L.)
https://doi.org/10.1016/j.molcel.2018.06.020

SUMMARY

The human FACT (facilitates chromatin transcription) complex, composed of two subunits SPT16 (Suppressor of Ty 16) and SSRP1 (Structure-specific recognition protein-1), plays essential roles in nucleosome remodeling. However, the molecular mechanism of FACT reorganizing the nucleosome still remains elusive. In this study, we demonstrate that FACT displays dual functions in destabilizing the nucleosome and maintaining the original histones and nucleosome integrity at the single-nucleosome level. We found that the subunit SSRP1 is responsible for maintenance of nucleosome integrity by holding the H3/H4 tetramer on DNA and promoting the deposition of the H2A/H2B dimer onto the nucleosome. In contrast, the large subunit SPT16 destabilizes the nucleosome structure by displacing the H2A/H2B dimers. Our findings provide mechanistic insights by which the two subunits of FACT coordinate with each other to fulfill its functions and suggest that FACT may play essential roles in preserving the original histones with epigenetic identity during transcription or DNA replication.

INTRODUCTION

In eukaryotes, genomic DNA is packaged into chromatin by histones. All DNA-related processes, including transcription and DNA replication and repair, occur in the context of chromatin, where the barrier nucleosome must be temporarily removed first and rapidly restored afterward. The original epigenetic identity on the nucleosome needs to be preserved after the DNA replication or transcription (Alabert and Groth, 2012; Kaufman and Rando, 2010). FACT (Facilitates chromatin transcription), named for its ability to facilitate the elongation of RNA Pol II on chromatin template (Belotserkovskaya et al., 2003; Orphanides et al., 1998), is highly conserved among eukaryotes and essential for cell viability (Orphanides et al., 1999; Reinberg and Sims, 2006). FACT, initially identified as an H2A/H2B histone chaperone (Belotserkovskaya et al., 2003), has been characterized to play a pivotal role in almost all chromatin-related processes, including transcription, replication, and repair (Heo et al., 2008; Yang et al., 2016). Previous studies have found that FACT not only facilitates the progression of DNA and RNA polymerases on chromatin, but also maintains the genome-wide integrity of chromatin structure through its chaperone activity (Formosa et al., 2002; Hainer et al., 2011; Jamai et al., 2009; Kaplan et al., 2003; Lejeune et al., 2007; Mason and Struhl, 2003; Wittmeyer et al., 1999). Interestingly, the deficiency of FACT *in vivo* would increase the turnover rates for both H2A/H2B dimers and H3/H4 tetramers, and inappropriately activate repressed genes (Hainer et al., 2011; Jamai et al., 2009; Kaplan et al., 2003), indicating that FACT may also function in maintaining nucleosome integrity. However, the mechanisms of how FACT accomplishes these crucial and seemingly contradictory functions still remain poorly understood (Formosa, 2012).

The human FACT is a large heterodimeric complex composed of two subunits, SPT16 (Suppressor of Ty 16) and SSRP1 (Structure-specific recognition protein-1). The interactions between several distinct domains of the subunits and histones or DNA have been elucidated separately by structural and biochemical investigations. Briefly, for the subunit SPT16, the C-terminal region, especially the highly acidic segment, has been found to interact with the H2B N-terminal basic region; the middle domain and the N-terminal region bind to the H3/H4 tetramer (Stuwe et al., 2008; Tsunaka et al., 2016). The subunit SSRP1 contains

a well-characterized DNA-binding domain HMG-1 at the C terminus, which enables the protein to bind DNA as it enters and exits the nucleosome (Bustin and Reeves, 1996; Yarnell et al., 2001). The middle domain of SSRP1 has been shown to interact with H3/H4 histones and DNA, while the N-terminal domain is required for the interaction of SSRP1 with SPT16 (Keller and Lu, 2002; VanDemark et al., 2006; Zhang et al., 2015). In spite of these results, the structure of the intact heterodimer alone or in complex with the nucleosome is still elusive. It also remains poorly understood how SPT16 and SSRP1 coordinate with each other to accomplish the biological functions of FACT.

In this work, we investigated the regulatory functions of the FACT complex, the SPT16 subunit, and the SSRP1 subunit on the nucleosome by use of single-molecule magnetic tweezers, which is a powerful tool to assess the real-time disassembly and re-assembly of single nucleosomes *in vitro*. We revealed that the FACT complex not only destabilizes the nucleosome structure to assist the passage of polymerases, but also enhances the reversibility of nucleosome formation, which may play a central role in preserving the original histones at specific gene loci after the passage of RNA or DNA polymerases during transcription or DNA replication. Furthermore, we found that the two subunits of the FACT complex, SPT16 and SSRP1, function distinctly but coordinately to fulfill the two-face functions of the FACT complex in building, overcoming, and maintaining the nucleosome barrier.

RESULTS

FACT Destabilizes the Nucleosome Structure and Maintains Nucleosome Integrity

The mono-nucleosome with linker histone H1 used in our single-molecule experiments was reconstituted *in vitro* onto a 409-bp DNA fragment containing one repeat of Widom 601 nucleosome positioning sequence and characterized by electron microscopy (EM) imaging (Figure S1A). To trace the structure transition of a mono-nucleosome by magnetic tweezers, we applied a continuously increasing force on the mono-nucleosome by moving the magnets in *z* direction at a rate of 2 μm/s and recorded the real-time end-to-end distance trajectories of the individual nucleosome (Figures 1A and S1B). In the absence of FACT, the nucleosome displayed the well-characterized two-step unfolding dynamics, consistent with previous results of ours (Li et al., 2016) and others (Hall et al., 2009; Mihardja et al., 2006). The first step at low force around 10 pN corresponds to the unraveling of the outer nucleosomal DNA wrap, and the second one at high force around 18 pN is associated with the unraveling of the inner DNA wrap (Figures 1B, purple line, and S1C). Interestingly, in the presence of FACT, the nucleosome was observed to disassemble at force around 5 pN for the outer wrap and was completely disrupted at force around 8 pN for the inner wrap (Figure 1B, green line). The much lower disruption forces indicate that FACT greatly destabilizes the nucleosome and dramatically reduces the magnitude of the nucleosome barrier, which may help to facilitate gene transcription as indicated by previous *in vitro* studies (Belotserkovskaya et al., 2003; Orphanides et al., 1998). In addition, FACT induces a very different unfolding pathway of the nucleosome. In the absence of FACT (Figures 1B, purple line, and S1C), the two-step unfolding process includes one reversible unraveling of the outer wrap with a step size of 21.2 ± 0.4 nm (mean ± SE) and one irreversible unraveling of the inner wrap with a step size of 25.6 ± 0.6 nm (mean ± SE) (Figure 1B, right cartoon). In the presence of FACT, however, the nucleosome is disrupted in a distinct four-step process, with a step size of 10.2 ± 0.5, 10.1 ± 0.3, 12.2 ± 0.5, and 12.1 ± 0.6 nm (mean ± SE), respectively (Figures 1C, green line, and S1D), which might be contributed to two half-outer-wrap and two half-inner-wrap unraveling (Figure 1B, left cartoon). More importantly, all these four transitions are reversible as shown in the time course of length trajectory in Figure S1E.

By performing repeated stretching measurements with magnetic tweezers, we can conveniently monitor the re-assembly of the nucleosome after the original nucleosome structure is fully disrupted (Figures 1C and 1D). In the absence of FACT, we can observe the two-step unfolding pathway of the intact nucleosome in the first cycle, but the nucleosome structure cannot be reconstructed correctly (Figure 1C), indicating that histones may be displaced from the nucleosomal DNA when the nucleosome is fully disrupted (Figure 1C, bottom cartoon). Intriguingly, in the presence of FACT, the nucleosome maintains the four-step unfolding pathway and retains the similar force response in each cycle (Figure 1D). The results strongly indicate that FACT can not only hold the original histone octamer on DNA when the nucleosome is fully unraveled under high tension, but also promote the reassembly of the nucleosome structure when the tension is released (Figure 1D, bottom cartoon). We repeated the stretching measurements up to five times and more details can be found in Figure S1F. Similar results were obtained even when abruptly or long-period (up to 5 min) large force was applied in the repeated force-jump experiments, indicating the strong interaction between FACT and the nucleosome (Figure S1G). In addition, after we removed the free FACT complexes surrounding the nucleosomes in the solution, the similar folding and unfolding dynamics of the nucleosome can be observed, indicating that FACT keeps bound on the nucleosome during the stretching experiments (Figure S1H).

SSRP1 Maintains the H3/H4 Tetramer on Nucleosomal DNA and SPT16 Disrupts the Outer Nucleosomal Wrap by Displacing H2A/H2B Dimers

The human FACT complex is a heterodimer composed of two subunits SPT16 and SSRP1 (Orphanides et al., 1999) (Figure S2A). We investigated the folding and unfolding dynamics of the nucleosome in the presence of the isolated SPT16 and SSRP1 instead of the holo-FACT complex (Figure S2B). The isolated SPT16 and SSRP1 still can destabilize the nucleosome structure and help to maintain the integrity of the nucleosome, but not as efficiently as the holo-FACT complex (Figure S2B). The results indicate that the physical association of the two subunits is important for FACT to fulfill its function efficiently.

To further identify the distinct functions of the two subunits, we studied the folding dynamics of the nucleosome in the presence of either SPT16 or SSRP1, respectively. We first carried out the repeated stretching measurements in the low-force region (0–12 pN) where the inner nucleosomal DNA wrap remains intact, and only the unfolding and refolding dynamics of the outer DNA wrap is observable. For the nucleosome alone, the outer DNA

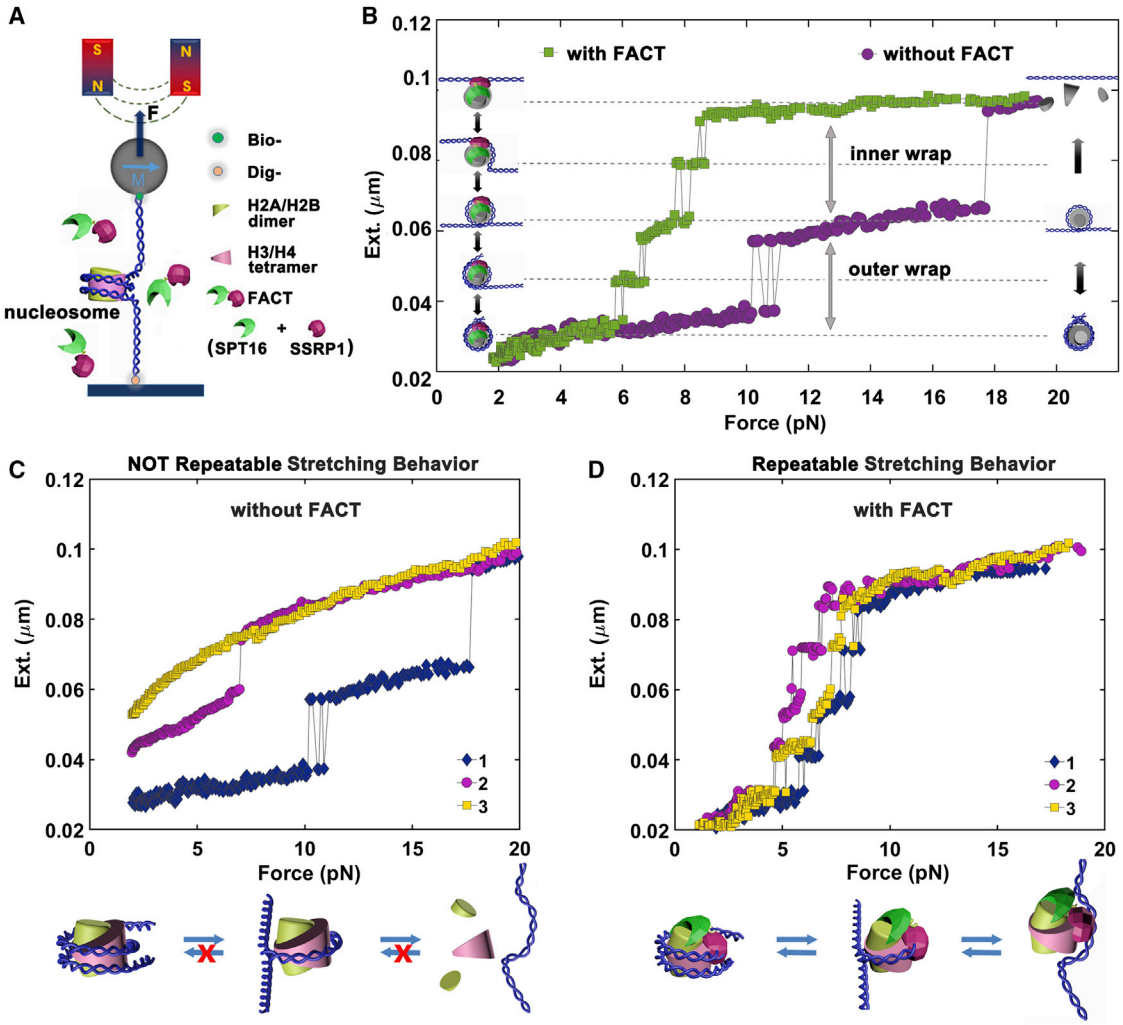

Figure 1. FACT Destabilizes Nucleosome Structure and Maintains Nucleosome Integrity

(A) Schematic setup of the magnetic tweezers used in the study (not to scale) (please find details in STAR Methods).

(B) Comparison of two typical force-extension curves of a nucleosome with or without FACT in H&E buffer (please find more details of statistic data in STAR Methods). The cartoon right or left represents the dynamics of the nucleosome without or with FACT, respectively.

(C and D) Repeated stretching measurements of a single nucleosome without (C) and with (D) FACT, with the cartoon below representing the relative dynamic process of the nucleosome, respectively. In the first stretching cycle, the nucleosome was unfolded by slowly increasing force up to 20 pN at a loading rate of 0.1 pN/s, and the dynamic unfolding of the nucleosome was recorded. After that, the tension was released to 0 pN rapidly, and we waited for 5 min and then repeated the stretching cycle on the same nucleosome sample. The above steps were repeated 5 times for each nucleosome sample, with the first 3 repeats shown in the figures.

wrap is unfolded and reversibly refolded at force around 10 pN (Figure 2A). In the presence of SSRP1, the formation of the outer wrap remains reversible (Figure 2B). In contrast, SPT16 not only significantly reduces the stability, but also changes the reversibility of the outer wrap. In the presence of SPT16, the outer wrap is unfolded in two steps at around 6 pN, and it cannot be maintained in the repeated stretching experiments (Figure 2C). As the outer wrap is mainly associated with the H2A/H2B dimers (Luger et al., 1997), the results indicate that SPT16 may actively displace the H2A/H2B dimers from the nucleosome so that the outer wrap cannot correctly restore. Our results are consistent with previous biochemical and structural studies (Stuwe et al., 2008; Tsunaka et al., 2016; Winkler et al., 2011), demonstrating that SPT16 prefers to bind the H2A/H2B dimers and displaces the dimers from the nucleosome.

Surprisingly, during the repeated stretching experiments in the high-force region (0–20 pN), compared with the nucleosome alone whose outer and inner DNA wraps are irreversibly unraveled (Figure 2D), SSRP1 maintains the integrity of the inner wrap, but not the outer wrap (Figure 2E). Because the inner DNA wrap is mainly associated with the H3/H4 tetramer (Luger et al., 1997), our results indicate that SSRP1 holds the H3/H4 tetramer on the nucleosomal DNA and maintains the inner wrap integrity. The irreversible unraveling of the outer wrap suggests that SSRP1 alone cannot hold the H2A/H2B dimers when the whole nucleosome structure is fully disrupted. Previously, several biochemical and structural studies

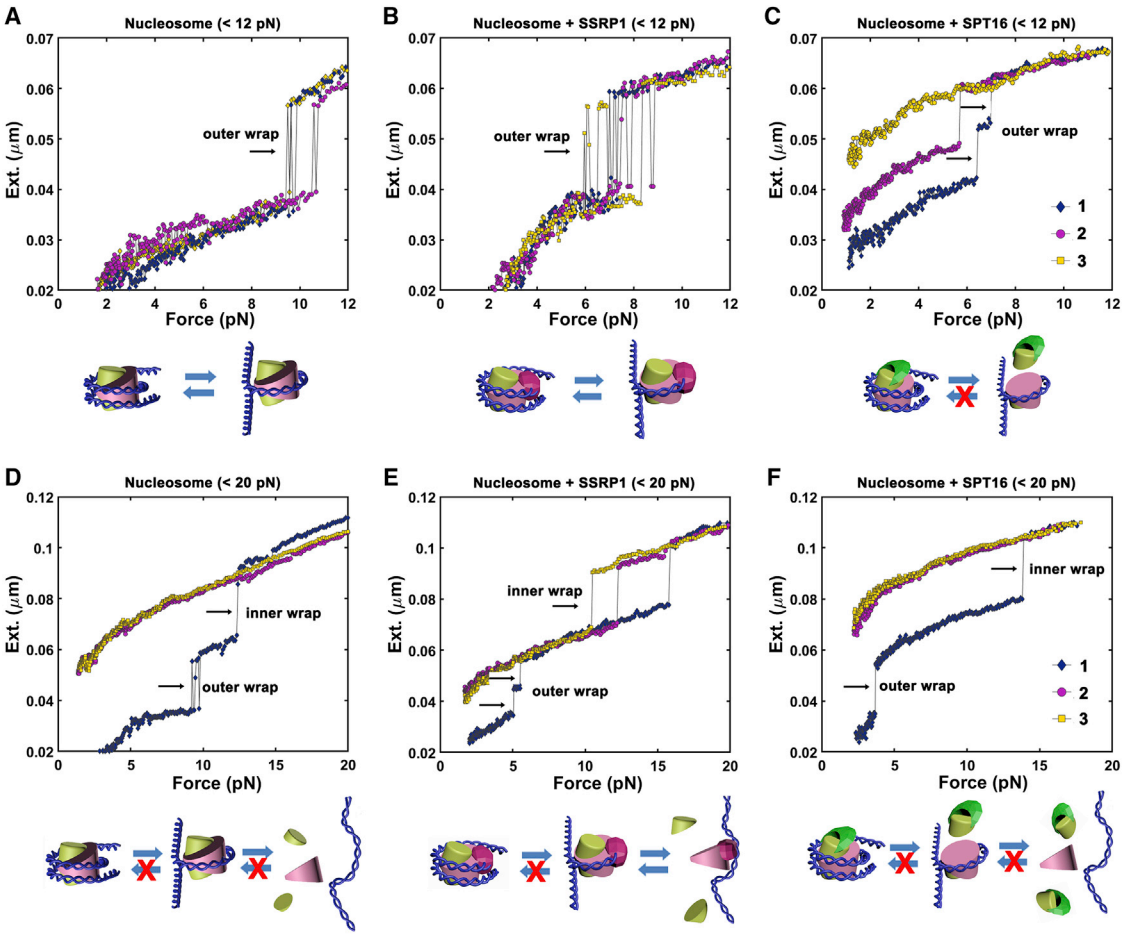

Figure 2. SSRP1 Maintains the H3/H4 Tetramer on the Nucleosome and SPT16 Interacts with the H2A/H2B Dimer to Disrupt the Outer Nucleosomal Wrap

(A–C) Repeated stretching measurements of the same single nucleosome alone (A), with SSRP1 (B), or with SPT16 (C) at the low-force region of 0–12 pN where only the outer nucleosomal DNA wrap is disrupted but the inner wrap keeps intact, with the cartoon below representing the relative dynamic process of the nucleosome, respectively.

(D–F) Repeated stretching measurements of the same single nucleosome alone (D), with SSRP1 (E), or with SPT16 (F) at the high-force region of 0–20 pN where both the outer and inner nucleosomal DNA wrap is disrupted, with the cartoon below representing the relative dynamic process of the nucleosome, respectively. All these curves are the typical stretching behavior chosen from our parallel measurements for independent nucleosome samples (N > 100).

also have shown that SSRP1 prefers to bind nucleosomal DNA and H3/H4 histones (Bustin and Reeves, 1996; Winkler et al., 2011; Yarnell et al., 2001; Zhang et al., 2015), which supports our observations. Comparatively, SPT16 cannot maintain the outer or inner wrap integrity after the nucleosome is fully disrupted under high tension (Figure 2F). In summary, our results showed that, in the nucleosome remodeling process, SPT16 binds to the H2A/H2B dimers and displaces the dimers from the outer wrap, and SSRP1 binds to the H3/H4 tetramer and DNA to maintain the inner wrap integrity.

The FACT Complex Helps to Maintain the Tetrasome Integrity and Facilitates the Deposition of H2A/H2B Dimers into Tetrasomes to Form Nucleosomes by the SSRP1 Subunit

Nucleosome assembly is another important process in maintaining chromatin integrity. Previous studies have shown that the histone H3/H4 tetramer binds to DNA in position first to allow the subsequent correct deposition of H2A/H2B dimers (Li et al., 2012; Oohara and Wada, 1987). Here, we use mono-tetrasomes (the particles made of DNA wrapped around one H3/H4 tetramer) reconstituted *in vitro* with linker histone H1 as substrates to address the functions of FACT on the tetrasome and the H2A/H2B deposition property. The reconstituted mono-tetrasomes were well positioned in the middle of DNA, as characterized by EM imaging (Figure S3A). The obvious one-step unfolding with a step size of 21.2 ± 0.4 nm (mean ± SE) at force around 12 pN, which corresponds to a well-formed inner nucleosomal wrap, cannot be maintained in the repeated stretching measurements (Figure 3A). However, in the presence of FACT or individual SSRP1, the formation of tetrasomes becomes reversible, which indicates that SSRP1, as well as FACT, can hold the original H3/H4 tetramer on the DNA template and promote reconstruction of the tetrasome (Figures 3B and 3C). In

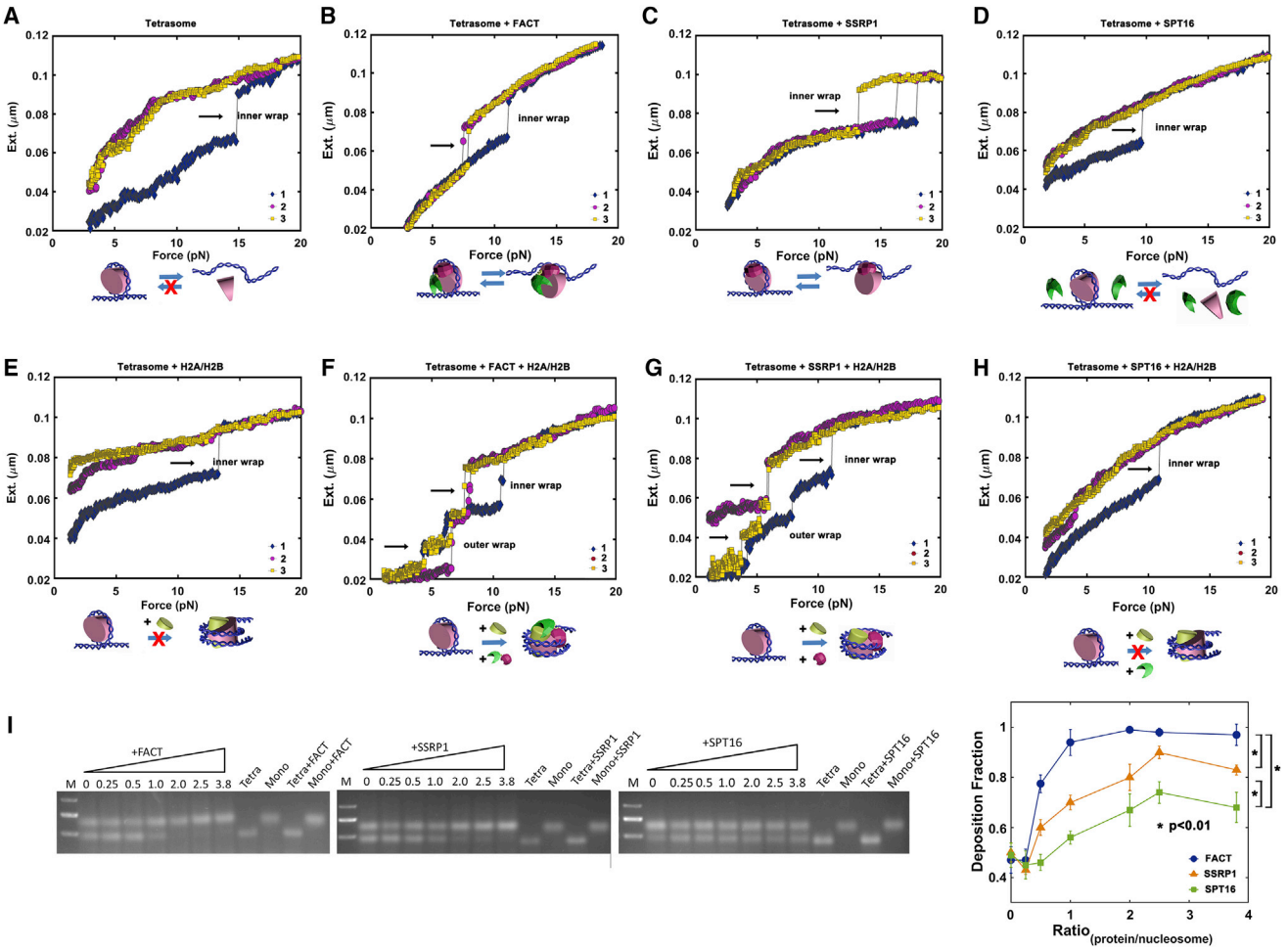

Figure 3. The FACT Complex Helps to Maintain Tetrasome Integrity and Deposit H2A/H2B Dimers into Tetrasomes to Form Intact Nucleosomes

(A–H) The repeated stretching measurements of the same single mono-tetrasome alone (A), with FACT alone (B), with SSRP1 alone (C), with SPT16 alone (D), with H2A/H2B alone (E), with FACT and the H2A/H2B dimer (F), with SSRP1 and the H2A/H2B dimer (G), and with SPT16 and the H2A/H2B dimer (H) (the cartoon below representing the relative dynamic process of the nucleosome, respectively).

(I) A gel shift assay shows the properties of FACT, SSRP1, or SPT16 to promote the deposition of the H2A/H2B dimer into tetrasome to form the nucleosome. The first lane is the marker, and the next 7 lanes show the tetrasome incubated with the H2A/H2B dimer and different concentrations of FACT-related protein (with the molar ratios of the protein/nucleosome indicated). The last 4 lanes are the controls indicating the positions of the tetrasome, nucleosome, tetrasome + protein, and nucleosome + protein. The quantified analyses from 4 independent repeats were shown on the right panel. Error bars indicate SEM. Statistical analysis was performed using one-tailed Student's t test with p value indicated, respectively.

contrast, SPT16 cannot maintain the inner wrap after the tetrasome is totally disrupted (Figure 3D).

We further examined whether FACT can deposit H2A/H2B onto the tetrasome to assemble the nucleosome. Interestingly, in the presence of H2A/H2B and FACT, the unfolding dynamics of the tetrasome becomes very similar to that of intact nucleosomes (Figure 3F), with the four-step unfolding of outer and inner nucleosomal DNA wraps clearly observable. More importantly, the unfolding dynamics of the newly formed nucleosomal complex becomes repeatable in the stretching cycles. These results indicate that FACT can successfully deposit H2A/H2B dimers onto the tetrasome to form an intact nucleosome. At the same time, we monitored the regulatory effect of either SSRP1 or SPT16 on this process. We found that SSRP1 alone still can deposit H2A/H2B dimers onto the tetrasome to form an intact nucleosome with the four-step unfolding process clearly observable (Figure 3G). However, because SSRP1 alone cannot hold H2A/H2B dimers on the nucleosome well as we found in Figure 2E, we always observed the loss of the outer DNA wrap and its regain by H2A/H2B deposition in the repeated stretching measurements (Figures 3G and S3B). As shown in Figure 3G, we can only observe the disruption step of inner DNA wrap in the second repeat of the assay, which indicates that the outer DNA wrap is not correctly formed here. In contrast, SPT16 alone cannot promote the deposition of H2A/H2B (Figure 3H). As revealed in our previous experiments (Figures 2C and 2F), SPT16 prefers to bind the H2A/H2B dimers, and it may keep the H2A/H2B dimers on the nucleosome for helping SSRP1 to

reassemble an intact nucleosome. Our biochemical analysis by gel electrophoresis in Figure 3I shows that, with the addition of increasing amounts of FACT, the band corresponding to tetrasomes shifts quickly to the band corresponding to nucleosomes, confirming that FACT promotes the deposition of H2A/H2B dimers onto the tetrasome to form the nucleosome. As comparisons, the subunit SSRP1 has a weaker effect on the formation of nucleosomes, indicating that SSRP1 may facilitate the deposition of H2A/H2B dimers, but it cannot stably maintain the H2A/H2B dimers on the nucleosome. SPT16 has little effect on the deposition of H2A/H2B onto tetrasomes to form intact nucleosomes. These biochemical results agree very well with our single-molecule experimental results.

The HMG Domain Plays Essential Roles in SSRP1's Function on the Nucleosome

We have shown that SSRP1 can hold the original H3/H4 tetramer on DNA to maintain the inner wrap integrity; meanwhile, SSRP1 can deposit H2A/H2B dimers into the tetrasome to form an intact nucleosome. SSRP1 contains a well-characterized DNA-binding domain HMG-1 at its C terminus (Yarnell et al., 2001), which may enable the protein to bind nucleosomal DNA where it enters and exits the nucleosome (Bustin and Reeves, 1996). Consistent with this hypothesis, we found that deletion of the HMG domain in SSRP1 greatly impairs its DNA binding activity (Figure 4A). In addition, our biochemical analysis by gel electrophoresis showed that the truncated SSRP1 with the HMG domain deleted (SSRP1ΔHMG) exhibits a much lower ability to deposit H2A/H2B dimers onto the tetrasome, as compared with the full-length SSRP1 (Figure 4B). Consistently, our single-molecule investigation showed that, with the HMG domain deleted, SSRP1 cannot maintain the integrity of the inner nucleosomal wrap and the tetrasome and loses its ability to deposit H2A/H2B dimers onto the tetramer as well (Figures 4C–4E). The results reveal that the DNA-binding HMG domain of SSRP1 plays essential roles in maintaining nucleosome integrity by FACT.

DISCUSSION

The FACT complex has been found to play essential roles in nucleosome remodeling during transcription and DNA replication and repair. Although the interactions between several distinct domains of FACT and histones or DNA have been elucidated independently by structural and biochemical studies (Bustin and Reeves, 1996; Keller and Lu, 2002; Stuwe et al., 2008; Tsunaka et al., 2016; Valieva et al., 2017; Yarnell et al., 2001), it still remains poorly understood how FACT reorganizes the nucleosome, and how its two subunits, SPT16 and SSRP1, coordinate with each other to accomplish this process (Formosa, 2012). Here, using magnetic tweezers, we revealed the two-face functions of FACT in destabilizing the nucleosome and maintaining its integrity at the single-nucleosome level (Figure 5). We demonstrated that the intact FACT complex not only destabilizes both the inner and outer nucleosome wraps, but also changes the folding and unfolding kinetics of transitions and enhances the reversibility of nucleosome formation. Previously, it was shown that FACT can bind nucleosomes and promote the global accessibility of nucleosomal DNA to restriction endonuclease, suggesting that the FACT complex reorganizes the nucleosome structure (Xin et al., 2009). Our results indicate that binding of the FACT complex would alter the interaction between DNA and the histone octamer in the nucleosome, not only reorganizing the nucleosome structure but also retaining the nucleosome components (histones and DNA) together in a looser manner (Figure 5). In such a way, the FACT complex could hold the original histones along with the nucleosomal DNA and maintain nucleosome integrity, but it also destabilizes the inner and outer wraps to assist the passage of polymerases through the nucleosome barrier.

Moreover, our investigations show that the two subunits of the FACT complex, SPT16 and SSRP1, function distinctly but coordinately to fulfill the two-face functions of the FACT complex. Compared with the holo-FACT complex, the binding capacity of the two subunits to the nucleosome is reduced. To investigate the binding capacity of FACT and its two subunits on nucleosomes, we measured the dissociation constants K_D for FACT, SPT16, and SSRP1 binding to our 409-bp nucleosome by single-molecule magnetic tweezers (see STAR Methods and Figure S4A). The resulting K_D for FACT, SPT16, and SSRP1 binding to 409-bp nucleosome is 16.2 ± 6.3 (mean ± SE), 62.4 ± 15.9 (mean ± SE), and 44.8 ± 7.7 nM (mean ± SE), respectively, which agrees well with the previous data obtained in bulk assays (Winkler et al., 2011). In addition, the effect of different FACT subunits on the nucleosome dynamics after it binds to the nucleosome has been investigated. We demonstrated for the first time that the subunit SSRP1 functions as a scaffold to "keep things in place" to maintain nucleosome integrity, by preserving the H3/H4 tetramer on DNA to restore the inner nucleosomal DNA wrap through its histone binding domains and DNA binding HMG domain. Consistent with this hypothesis, previous studies have shown that SSRP1 interacts with both the H3/H4 tetramer and nucleosomal DNA (Winkler et al., 2011; Yarnell et al., 2001; Zhang et al., 2015). In addition, we found that SSRP1 can facilitate the deposition of H2A/H2B dimers into the nucleosome but cannot tightly maintain the H2A/H2B dimers on the nucleosome in the absence of the SPT16 subunit. Maintaining H2A/H2B on the nucleosome requires cooperation between SPT16 and SSRP1. The DNA-binding HMG domain of SSRP1 plays an essential role in maintaining nucleosome integrity. The binding capacity of SSRP1ΔHMG to the nucleosome is reduced significantly, with K_D increasing ~10-fold compared with the full-length SSRP1 (see STAR Methods and Figure S4A). With the HMG domain deleted, SSRP1 almost loses its functions on the nucleosome reassembly. We should note that, in yeast and fungi, the SSRP1 subunit is called Pob3, which lacks the HMG DNA binding motif found at the SSRP1, and the DNA-binding activity instead appears to be supplied by the separate protein Nhp6 (Brewster et al., 2001; Wittmeyer and Formosa, 1997). SSPR1 is essential for viability, but yeast cells can grow slowly without Nhp6, and viable strains of fission yeast lacking the Pob3 have also been described (Lejeune et al., 2007; Stillman, 2010). It will be of great interest to investigate how the Pob3 and/or Nhp6 interact with the nucleosomes.

In contrast, we showed that the subunit SPT16 is mainly responsible for displacement of H2A/H2B dimers and unraveling of the outer wrap through its H2A/H2B dimer binding domain as

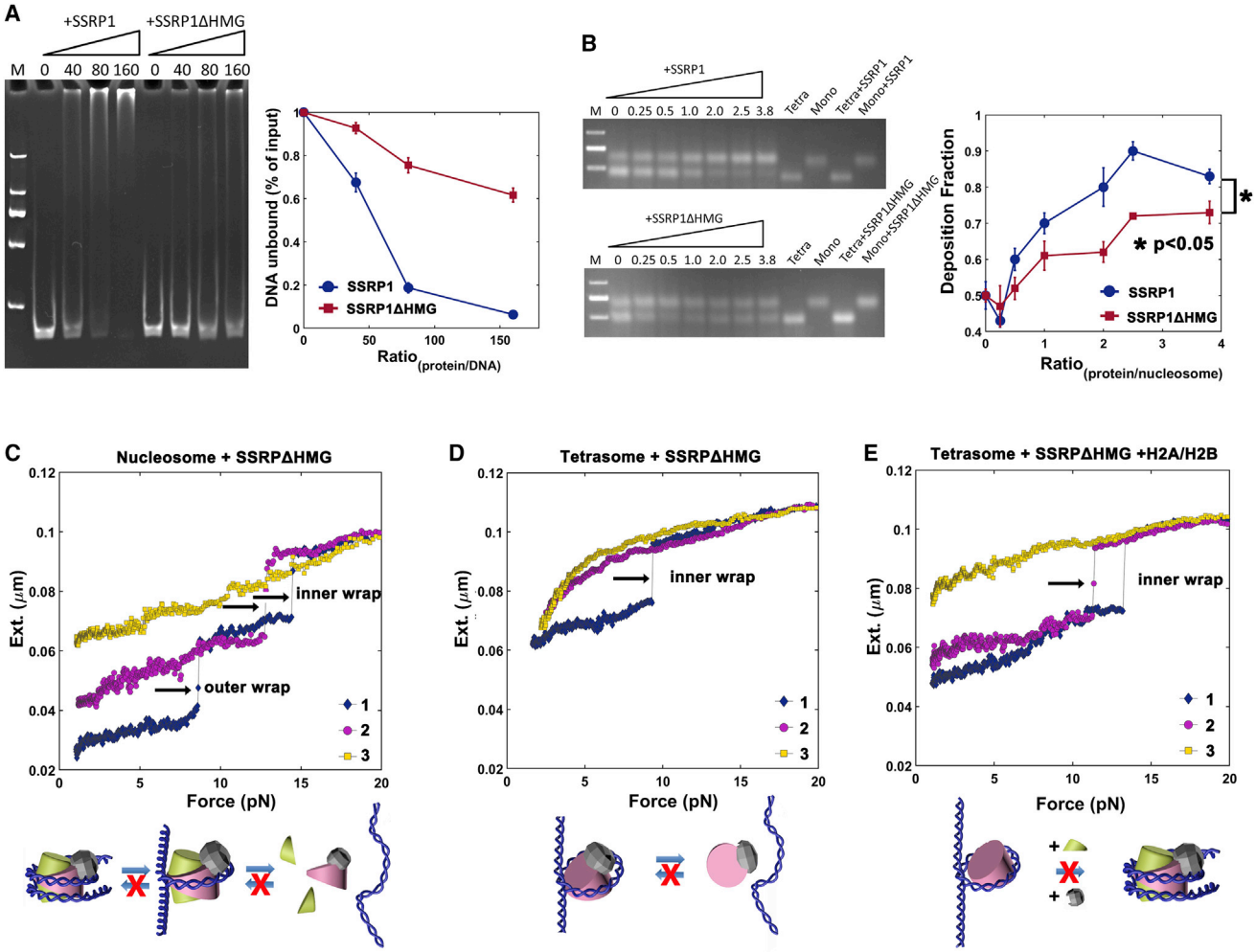

Figure 4. The HMG Domain Plays Essential Roles in SSRP1 Function on the Nucleosome

(A) A gel shift assay shows the DNA binding properties of SSRP1 and SSRP1ΔHMG. The first lane is the marker, and the next lanes show DNA incubated with different concentrations of SSRP1 or SSRP1ΔHMG (with the molar ratios of protein/DNA indicated). The quantified analyses from 4 independent repeats were shown on the right panel. Error bars indicate SEM.

(B) A gel shift assay shows the properties of SSRP1 and SSRP1ΔHMG to promote H2A/H2B dimer deposition onto tetrasome to form the nucleosome. The first lane is the marker, and the next 7 lanes show the tetrasome incubated with the H2A/H2B dimer and different concentrations of SSRP1 or SSRP1ΔHMG (with the molar ratios of the protein/nucleosome indicated). The last 4 lanes are the controls indicating the positions of the tetrasome, nucleosome, tetrasome + protein, and nucleosome + protein. The quantified analyses from 4 independent repeats were shown on the right panel. Error bars indicate SEM. Statistical analysis was performed using one-tailed Student's t test with p value indicated, respectively.

(C–E) Repeated stretching measurements of the same single nucleosome with SSRP1ΔHMG (C), with SSRP1ΔHMG (D), and with SSRP1ΔHMG and the H2A/H2B dimer (E) (the cartoon below representing the relative dynamic process of the nucleosome/tetrasome, respectively).

reported previously (Belotserkovskaya et al., 2003; Tsunaka et al., 2016; Winkler et al., 2011). In addition, SPT16 helps SSRP1 to maintain H2A/H2B on the nucleosome; in the absence of the SPT16 subunit, SSRP1 cannot hold the H2A/H2B dimers on the nucleosome. The C-terminal domain of SPT16 has been shown to play essential roles for FACT-mediated nucleosome reorganization (Belotserkovskaya et al., 2003; Tsunaka et al., 2016; Winkler et al., 2011). In our study, we also found, with the whole C terminus of SPT16 deleted, the FACT functions on nucleosomes very similarly to SSRP1 alone (Figure S4B), suggesting that the main function of SPT16 in the FACT complex is contributed by its C terminus. In addition, recently we have also shown that the SPT16 subunit can destabilize the tetranucleosomal units by its U-turn motif to facilitate gene expression (Li et al., 2016). Our studies reveal that the SPT16 subunit plays essential roles in FACT function to destabilize the chromatin structure, not only by displacing H2A/H2B dimers at the nucleosome level, but also by interrupting the inter-nucleosome interactions within tetranucleosomal units in chromatin fibers.

FACT has been shown to functionally interact with different histone modifications or binding partners in distinct chromatin-related processes. For instance, FACT has been shown to

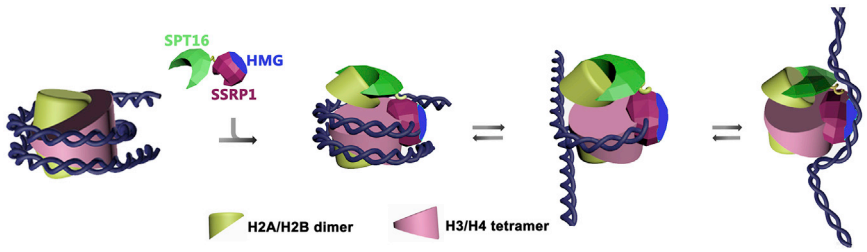

Figure 5. The Mechanism Model Proposed for the Function of FACT on the Nucleosome

FACT's dual function of attenuating and maintaining the nucleosome structure is obtained through three interactions, interaction between SPT16 and the H2A/H2B dimer, interaction between SSRP1 and the H3/H4 tetramer, and the interaction between HMG and DNA.

cooperate with H2B mono-ubiquitination (at K120 in mammals) to promote transcription elongation (Fleming et al., 2008; Pavri et al., 2006). In contrast, it was reported that FACT activity is blocked by H2A mono-ubiquitination (at K119 in mammals) to inhibit transcription elongation (Fleming et al., 2008; Pavri et al., 2006; Zhou et al., 2008). In addition, the interaction of FACT with RPA or MCM2 may help to coupling FACT to the DNA replication process (Tan et al., 2006; VanDemark et al., 2006; Yang et al., 2016), and the association of FACT with CENP-C or CENP-T/-W allows FACT function at centromeres (Prendergast et al., 2016). Our present results define the basic mechanism by which the two subunits of FACT complex coordinate on nucleosomes to fulfill its two-face functions. It will be of great interest to investigate whether or how these different factors regulate these two-face functions of FACT in different biological processes.

In eukaryotes, in addition to that encoded in DNA sequence, organisms and cells can inherit epigenetic information from the parental generation—this is also known as epigenetic inheritance. In such a mechanism, parental histones must re-associate with two newly synthesized daughter DNAs around their original location on the mother DNA (Alabert and Groth, 2012; Kaufman and Rando, 2010). Otherwise, epigenetic inheritance of chromatin states would become conceptually impossible. Thus, the maintenance of nucleosome integrity plays essential roles to preserve the original epigenetic identity during DNA replication and transcription. Many studies using the SV40 replication system have demonstrated that parental nucleosomes do not completely dissociate and old histones remain associated with DNA during the replication process (Bonne-Andrea et al., 1990; Ishimi et al., 1991; Krude and Knippers, 1991; Randall and Kelly, 1992; Sugasawa et al., 1992), although some earlier investigations showed that parental nucleosomes undergo some level of disassembly during the passage of replication fork (Gruss et al., 1993; Jackson, 1990). Most recently, by reconstituting chromatin replication with purified proteins, Kurat et al. showed that parental nucleosomes are efficiently reassembled in the back of the replisome in the presence of FACT, Nhp6, chromatin remodelers, and lysine acetyltransferases (Kurat et al., 2017). In addition, a previous *in vivo* study also demonstrated that FACT, cooperatively with MCM2, helps to preserve chromatin integrity during transcription and DNA replication (Foltman et al., 2013). These studies suggest that FACT may help to preserve chromatin integrity through cooperating with other factors during transcription and DNA replication. In this study, we provide a molecular detail of how the SSRP1 subunit functions as a scaffold to sustain the FACT function in maintaining nucleosome integrity, which suggests that FACT may play essential roles in restoring nucleosomes to preserve the old histones with epigenetic identity at specific gene loci throughout RNA or DNA polymerase passage during transcription and the DNA replication process.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and can be found with this article online at https://doi.org/10.1016/j.molcel.2018.06.020.

ACKNOWLEDGMENTS

This work was supported by grants to G.L. from the Ministry of Science and Technology of China (2017YFA0504200 and 2015CB856200), the National Natural Science Foundation of China (31630041, 31525013, and 31521002), and the Chinese Academy of Sciences (CAS) Strategic Priority Research Program (XDB19040202), to M.L. from the National Natural Science Foundation of China (61275192), to W.L. from the National Natural Science Foundation of China (11474346) and the Key Research Program of Frontier Sciences, CAS (QYZDB-SSW-SLH045), to P.C. from National Natural Science Foundation of China (31471218) and the Youth Innovation Promotion Association CAS (2015071), and to P.-Y.W. from the National Key Research and Development Program (2016YFA0301500). The work was also supported by the Key Research Program on Frontier Science (QYZDY-SSW-SMC020) and the HHMI international research scholar grant (55008737) for G.L. All EM data were collected and processed at the Center for Bio-imaging, Core Facility

for Protein Sciences, Institute of Biophysics, and Chinese Academy of Sciences. We are also indebted to the colleagues whose work could not be cited due to the limitation of space.

AUTHOR CONTRIBUTIONS

P.C., W.L., M.L., and G.L. conceived the project, analyzed the data, and wrote the manuscript. W.L. performed the single-molecule magnetic tweezer analysis. P.C. and L.D. performed the *in vitro* chromatin assembly, EM, and biochemical analysis. M.H. and Z.Z. assisted in the preparation of DNA template and proteins. Y.-Z.W. and X.X. assisted in preparation magnetic tweezer analysis. J.Y., P.-Y.W., and D.R. helped to discuss the project.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: January 23, 2018
Revised: May 1, 2018
Accepted: June 13, 2018
Published: July 19, 2018

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and Virus Strains		
DH10Bac Competent Cell	Biomed	BC112
SPT16 Baculovirus	Belotserkovskaya et al., 2003	N/A
SSRP1 Baculovirus	Belotserkovskaya et al., 2003	N/A
SSRP1ΔHMG Baculovirus	This paper	N/A
Chemicals, Peptides, and Recombinant Proteins		
T4 DNA ligase	NEB	M0202L
SpeI-HF	NEB	R3133
XhoI	NEB	R0146
Phusion High-Fidelity DNA Polymerases	NEB	M0530L
2 × Taq Master Mix (Dye)	CWBIO	CW0682
Glutaraldehyde	Sigma-Aldrich	G5882
FLAG Peptide	Sigma-Aldrich	F3290
sf-900 II SFM	GIBCO	10902-088
fetal bovine serum	ExCell Bio	FSS500
Penicillin/Streptomycin	HyClone	SV30010
Cellfectin II reagent	Invitrogen	P/N58760
Critical Commercial Assays		
SanPrep Column Plasmid Mini-Preps Kit	Sangon Biotech	B518191
SanPrep Column PCR Product Purification Kit	Sangon Biotech	B518141
SanPrep Column DNA Gel Extraction Kit	Sangon Biotech	B518131
anti-Flag M2-agarose	Sigma-Aldrich	A2220
Ni Sepharose™ 6 Fast Flow	GE Healthcare	17-5318-02
Superdex 200 10/300 GL	GE Healthcare	17-5175-01
Deposited Data		
Raw Imaging Files	This study, Mendeley Data	https://doi.org/10.17632/9tc87th633.1
Experimental Models: Cell Lines		
Sf9 Cell	ATCC	CRL-1711
Oligonucleotides		
409 DNA F primer: GGAAACAGCTATGACCATG (5′ biotin)	Sangon Biotech	N/A
409 DNA R primer: GTAAAACGACGGCCAGTGAGCG(5′ digoxin)	Sangon Biotech	N/A
SSRP1ΔHMG F primer: GGACTAGTATGCCGCGGGGTTCTCATC	Sangon Biotech	N/A
SSRP1ΔHMG R primer: CCGCTCGAGTTACACCTCCACAGGCTTC	Sangon Biotech	N/A
Recombinant DNA		
pVL1392-SSRP1	Belotserkovskaya et al., 2003	N/A
pFastBac1 baculovirus transfer vector	Invitrogen	100360014
pFastBac1-SSRP1ΔHMG	This paper	N/A
Software and Algorithms		
Gel-Pro Analyzer software	Media Cybernetics	N/A
Other		
Gel DocTM EZ Imager system	Bio-Rad	N/A

CONTACT FOR REGENT AND RESOURCE SHARING

Further information and requests for reagents should be directed to Lead Contact Guohong Li (liguohong@ibp.ac.cn).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cell culture and transfection

Sf9 cells were cultured in sf-900 II SFM (GIBCO) supplemented with 2% fetal bovine serum (FBS, ExCell Bio), 1% Penicillin/Streptomycin (HyClone) at 27°C. His_6-SSRP1ΔHMG (1-538 aa) was subcloned into pFastBac1 baculovirus transfer vector (Invitrogen) at the SpeI and XhoI sites and transformed into DH10Bac cells (Biomed) to generate recombinant bacmid. Purified bacmid was transfected into Sf9 cells using Cellfectin II reagent (Invitrogen) and the resulting baculovirus was amplified to P4.

METHOD DETAILS

Protein purification

Recombinant histones and FACT complexes were cloned and purified as previously described (Belotserkovskaya et al., 2003; Li et al., 2010). The baculovirus-driven expression of Flag-SPT16, His_6-SSRP1 and His_6-SSRP1ΔHMG follows the protocols presented previously (Belotserkovskaya et al., 2003; Li et al., 2010). Sf9 cells (1.5-2 × 10^6/ml) were infected with baculovirus containing Flag-SPT16, or His_6-SSRP1, or His_6-SSRP1ΔHMG and incubated at 27°C for 72 hr. SPT16 and SSRP1 were co-expressed for FACT complex. The infected cells were collected by centrifugation, washed with ice-cold PBS, and lysed in Flag-150 buffer (150mM NaCl, 20mM Tris-HCl, pH 8.0, 0.05% NP-40, 10% glycerol, 1mM PMSF). The recombinant FACT complex and SPT16 were purified in two steps. First, the cell extracts were incubated with anti-Flag M2-agarose (Sigma) for 4 hr at 4°C, with the resin washed by Flag-150 buffer. Bound proteins were eluted in the presence of 0.5mg/ml Flag peptide (Sigma). Second, the proteins were further purified by a Superdex 200 10/300 GL size exclusion column (GE Healthcare). For the purification of SSRP1 and SSRP1ΔHMG, the cell extracts were incubated with Ni-NTA agarose (GE Healthcare) for 4 hr at 4°C. The resins were washed with buffer containing 20mM imidazole, and the bound proteins were eluted with 200mM imidazole. The fractions containing FACT complex or subunits were dialyzed against BC-100 buffer (100mM NaCl, 10mM Tris-HCl, pH 8.0, 0.5mM EDTA, 20% glycerol, 1mM DTT, 1mM PMSF) and stored at −80°C.

Nucleosome reconstitution

For magnetic tweezers' investigation, a 409 bp DNA template containing a single 601 sequence (Figure S1A, top panel) was prepared by PCR from plasmid using a biotin (bio)-labeled forward primer and a digoxigenin (dig)-labeled reverse primer. Respective histone octamers, H3/H4 tetramers and H2A/H2B dimers were reconstituted as previously described (Dyer et al., 2004). Equimolar amounts of individual histones in unfolding buffer (7M guanidinium HCl, 20mM Tris-HCl, pH 7.5, 10mM DTT) were dialyzed into refolding buffer (2M NaCl, 10mM Tris-HCl, pH 7.5, 1mM EDTA, 5mM 2-mercaptoethanol), and purified through a Superdex S200 column. Chromatin samples were assembled using the salt-dialysis method as previously described (Song et al., 2014). The reconstitution reaction mixture with histone octamers/tetramers and 601 based DNA templates in TEN buffers (10mM Tris-HCl, pH 8.0, 1mM EDTA, 2M NaCl) were dialyzed for 16 hr at 4°C in TEN buffer, which was continuously diluted by slowly pumping in TE buffer (10mM Tris-HCl, pH 8.0, 1mM EDTA) to a lower concentration of NaCl from 2 M to 0.6 M. All the nucleosomes used in the paper were assembled with linker histone H1 added. An equal molar amount of histone H1 (relative to mono-nucleosome) was then added and dialyzed in TE buffer with 0.6 M NaCl for 3 hr. Samples were collected after final dialysis in measurement HE buffer (10 mM HEPES, pH 8.0, 1 mM EDTA) for 4 hr. The stoichiometry of histone octamer/tetramer binding to the DNA template was determined by EM and gel shift analysis (Song et al., 2014).

Electron microscopy analysis

Metal shadowing with tungsten for EM study were performed as described previously (Chen et al., 2013). Reconstituted nucleosome or tetrasome samples were prepared using DNA concentrations of 20 μg/mL in HE buffer. The samples were fixed with 0.4% glutaraldehyde (Sigma) in the same buffer on ice for 30 min. 2 mM spermidine was added into the sample solution to enhance the absorption of chromatin to the grid. Samples were applied to glow-discharged carbon-coated EM grids and incubated for 2 min and then blotted. Grids were washed stepwise in 20 mL baths of 0%, 25%, 50%, 75%, and 100% ethanol solution for 4 min, each at room temperature, air-dried and then shadowed with tungsten at an angle of 10° with rotation. Samples were examined using a FEI Tecnai G2 Spirit 120 kV transmission electron microscope.

Histone deposition assays

Tetrasomes and nucleosomes for histone deposition assays were reconstituted on 187 bp 601 DNA fragments using the salt-dialysis method as described above. Different molar ratios of FACT complexes were titrated and first incubated with H2A/H2B dimer at 4°C for 30 min, and then mixed with the constant reconstituted tetrasomes in reaction buffer (10 mM HEPES, pH 8.0, 1 mM EDTA, 60 mM NaCl). The samples were incubated at 30°C for 1 hr prior to electrophoresis on 1.5% agarose gels in 1 × TAE buffer

(40 mM Tris, pH 8.0, 40 mM acetic acid, 1 mM EDTA) for 1.5 hr at 80 V. The gels stained with ethidium bromide were scanned by a Gel Doc™ EZ Imager system (Bio-Rad), with the band intensities quantified by Gel-Pro Analyzer software (Media Cybernetics).

DNA binding assay

0.1 μg 187 bp 601 DNA fragments were incubated with increasing concentrations of FACT proteins in DNA binding buffer (20 mM Tris, pH 7.5,75 mM NaCl,1 mM EDTA,15% glycerol) at 25°C for 1 hr. The reaction products were separated by 5% native PAGE electrophoresis in 0.25 × TBE buffer (22.5 mM Tris, pH 8.0, 22.5 mM boric acid, 0.5 mM EDTA) for 1.5 hr at 80V. The gels were stained with ethidium bromide and scanned on a Gel Doc™ EZ Imager system (Bio-Rad). The DNA intensity of each band was quantified with Gel-Pro Analyzer software (Media Cybernetics) and used to calculate the percentage of unbound DNA.

Single-molecule magnetic tweezers analysis

The basic principle of magnetic tweezers was described as previous studies (Gosse and Croquette, 2002; Meglio et al., 2009). The experimental setup of our home-made single-molecule magnetic tweezers includes five major parts as shown in Figure S1B (not to scale): (1) LED lighting source (625nm, red, Thorlabs); (2) two small NdFeB magnets with a distance of 0.5 mm; (3) the flow cell formed with a 130-μm-thick double-sided tape (cut with a channel (5 × 50 mm^2)) sandwiched between a functioned coverslip (24 × 60 mm, 0.13 mm, CITOGLAS, China) and a Mylar film; (4) 60 × oil immersion objective (UPLSAPO60XO, NA 1.35, Olympus) ; (5) 1280 × 1024 CCD (MC1362, Mikrotron).

To anchor the nucleosome sample, the surface of coverslip needs to functionalize. The coverslips were cleaned in the plasma cleaner for 60 s. The polystyrene beads (1 μm, QDSpher) were pipetted on the coverslip and put on a hot plate at 90°C for 20 s to melt on the coverslip. These firmly bound beads served as reference beads for drift correction. Then, the coverslip was coated with collodion treated with 70% collodion for 15 mins. To coat the coverslip with anti-digoxigenin, the flow cell was incubated with 100 μL anti-digoxigenin (0.1mg/ml) at 37°C for 2 hr. To avoid the non-specific bond between samples and the coverslip, the flow cell was incubated in passivation buffer (10 mg/ml BSA, 1 mM EDTA, 10 mM phosphate buffer, pH 7.4, 10 mg/ml Pluronic F127 surfactant (Sigma-Aldrich), 3 mM NaN_3) overnight at 37°C. The flow cell was ready for measurement.

The nucleosome sample was anchored to a super-paramagnetic bead and the coverslip in the flow cell in two steps. First, the nucleosome and beads (M280 Invitrogen Norway) were mixed on Hula Mixer (ThermoFisher) at 1 turns/min for 15 mins. Thus, the bio-labeled end of nucleosomal DNA was anchored to the streptavidin-coated beads via the biochemical reactions between biotin and streptavidin (Smith et al., 1992). Then, we injected the mixture into the flow cell and incubated for 15 mins to anchor the nucleosome sample to the surface of coverslip. The other dig-labeled end of the nucleosomal DNA was bound to the anti-digoxigenin-coated coverslip via the bonds between digoxigenin and anti-digoxigenin. The nucleosome samples were tethered between the beads and coverslip as shown in Figure S1B.

The applied tensions on the super paramagnetic beads arise from the strong magnetic field gradient of the two magnets. The tensions are tuned by adjusting the magnets position in z direction. Based on the geometry of a pendulum, the stretching force on the nucleosome in z direction was calculated as $F = k_B Tl/ < \delta x >^2$ according to the equipartition theorem, where l is the extension of DNA and δx is the fluctuation of the bead in x direction (Gosse and Croquette, 2002; Yan et al., 2004). In LabView software, the improved force calibration based on the power-spectral-density (PSD) analysis by considering both the translational and the rotational motions of the beads is achieved (Daldrop et al., 2015). To ensure the single nucleosomes are tethered between beads and coverslip, the magnets were rotated for 50 turns before the real measurements. The beads with more than one sample anchored between the beads and coverslip will decrease to the surface of the coverslip. Those beads with single nucleosome tether rotate freely and are chosen for measurements.

The bead image was projected onto the CCD camera through the microscope objective. The real-time position (x, y, z) of the bead at various forces was recorded by comparing the diffraction pattern of the bead with calibration images at various distances from the focal point of the objective. In our LabView software, the quadrant-interpolation (QI) algorithm were applied to trace the three-dimensional position of the beads in the flow cell (van Loenhout et al., 2012). The QI algorithm enables highly parallel single molecule experiments and reduces the pixel bias. In our measurements, we can trace about 40 samples simultaneously (as shown in Figure S1B). These parallel measurements help us to trace the dynamics of folding and unfolding at high-throughput level. We can perform the effective statistic based on the abounding measurements of the different samples. All measurements were carried out at 25°C. In the repeated stretching cycle, the nucleosome was unfolded by increasing force up to 20 pN at the loading rate of 0.1 pN/s, the dynamic unfolding of the nucleosome was recorded. After that, force was reduced to 0 pN rapidly and waited for 5 mins, and then repeated the stretching cycle on the same nucleosome sample.

Force-extension measurement of mono-nucleosomes

To trace the conformation transition of nucleosome, the force-extension curve was measured (Figure 1B). To reveal the detail of the structural transition, we tuned the magnets position in z direction by moving the magnets at 2 μm/s continuously and the corresponding force on the nucleosome changed from ~0.5 pN to ~20.0 pN. More than 100 measurements were carried out for nucleosomes with linker histone H1. To calibrate the force in the force-extension measurement, the force calculation for a 1-μm DNA tethered between the bead (M280) and the coverslip was carried out. At each magnet position, the x position of the bead was recorded in 5 minutes and the corresponding force was calibrated by power-spectral-density (PSD) analysis. We repeated force measurements for

10 independent DNA tethers. The relationship between force and magnet position can be fitted well with a single exponential function (Gosse and Croquette, 2002). Forces in force-extension measurements of nucleosomes were derived from the fitted exponential function with magnets positions.

Force-jump measurement of mono-nucleosome with FACT

To check the effect of large force on the interaction between FACT and nucleosome, we performed the force-jump experiments. We anchored the nucleosome samples into the flow cell and injected 100 μL FACT (40 nM) into the flow cell and incubated for 15 min. At the beginning, we exerted 1.9 pN for 2 min and then 12.0 pN for 1 min. We repeated the measurement for three times (Figure S1G, left). After the fore-jump measurement, we carried out the force-extension measurement for the same sample (Figure S1G, left). The force-extension measurement revealed that the high force did not affect the interaction between FACT and nucleosome. To further confirm these results, we performed the force-jump experiments at a larger force and for a longer time. We exerted 20 pN in the force-jump measurements and hold for 5 min (Figure S1G, right). The force-extension measurement revealed that the larger force did not affect the interaction between FACT and nucleosome (Figure S1G, right). These results indicate that the tension exerted on nucleosomal DNA does not affect the interaction between FACT and nucleosome and the mechanism of FACT regulating nucleosome structure.

QUANTIFICATION AND STATISTICAL ANALYSIS

The dissociation constant K_d measured by single-molecule magnetic tweezers

We performed the K_d measurement for FACT-related proteins binding to the 409 bp nucleosome by single-molecule magnetic tweezers.

In the case: nucleosome + FACT $\rightleftharpoons$ complex, the value *Kd* is defined as:

$$k_d = \frac{[nucleosome][FACT]}{[complex]} = \frac{[nucleoosme]}{[complex]}[FACT] = \frac{N_{nucleosome}}{N_{complex}}[FACT]$$

In the flow cell of magnetic tweezers, the number of FACT anchored to nucleosome on the surface of coverslip is much lower than the number of FACT in the flow cell. We can ignore the change of concentration of FACT in the flow cell caused by nucleosome binding. The K_d value can be obtained by calculate the ratio of the number of nucleosome alone and the number of complex at a certain concentration of FACT. The force-extension measurement has revealed that the outer wrap of nucleosome with linker histone H1 is disrupted at tension around 10 pN in the absence of FACT, and at tension around 6 pN in the presence of FACT (Figure 1B). If we exert 8 pN on the nucleosome sample, nucleosome without FACT will not be disrupted and nucleosome with FACT will be disrupted (Figure S4A). The different information of extensions of nucleosome at 8 pN can help us to distinguish whether FACT binds to nucleosome. For the precise measurement, we performed the extension measurement for hundreds of independent nucleosome samples and counted the number of nucleosome alone ($N_{nucleosome}$) and the number of complex ($N_{complex}$) at 40 nM FACT concentration. The calculated K_d between FACT and nucleosome is 16.2 ± 6.3 nM (mean ± SE, N = 507). We performed the similar measurements of the K_d values for SPT16, SSRP1 and SSRP1ΔHMG respectively. The calculated K_d between SPT16 and nucleosome is 62.4 ± 15.9 nM (mean ± SE, N = 613), K_d between SSRP1 and nucleosome 44.8 ± 7.7 nM (mean ± SE, N = 521) and K_d between SSRP1ΔHMG 432.3 ± 158.1 nM (mean ± SE, N = 637).

DATA AND SOFTWARE AVAILABILITY

Raw image files are deposited on Mendeley Data (https://doi.org/10.17632/9tc87th633.1).