FACT Facilitates Transcription-Dependent Nucleosome Alteration

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The FACT (facilitates chromatin transcription) complex is required for transcript elongation through nucleosomes by RNA polymerase II (Pol II) in vitro. Here, we show that FACT facilitates Pol II–driven transcription by destabilizing nucleosomal structure so that one histone H2A-H2B dimer is removed during enzyme passage. We also demonstrate that FACT possesses intrinsic histone chaperone activity and can deposit core histones onto DNA. Importantly, FACT activity requires both of its constituent subunits and is dependent on the highly acidic C terminus of its larger subunit, Spt16. These findings define the mechanism by which Pol II can transcribe through chromatin without disrupting its epigenetic status.

In eukaryotic cells, all cellular machineries that use DNA as a substrate must overcome the repressive properties of chromatin. Transcription by RNA polymerase II (Pol II) is accompanied by the alteration of chromatin structure in transcribed regions of the genome (1, 2). Even though nucleosomes present a strong barrier for the transcription machinery (2–4), they remain associated with DNA templates during transcription by Pol II in vitro (5). Thus, a crucial issue that remains poorly understood is how Pol II traverses nucleosomes.

Previous biochemical and genetic studies of transcription have identified FACT as a chromatin-specific factor required for transcription elongation on chromatin templates (6, 7), as well as for DNA replication (8–10) and DNA repair (11, 12). In addition, it was shown recently that, in response to ultraviolet-mediated DNA damage, the FACT complex modifies the specificity of casein kinase 2 (CK2) in such a way that CK2 phosphorylates and activates p53 (13, 14). FACT is an abundant nuclear complex composed of two proteins that are evolutionarily conserved in all eukaryotes. The large subunit, p140h/Spt16, is a mammalian homolog of the yeast Spt16/Cdc68 protein, whereas the smaller subunit, structure-specific recognition protein 1 (SSRP1), is a high mobility group (HMG)–like protein with an N terminus homologous to the yeast protein Pob3.

Recently, the yeast FACT subunits were shown to copurify with various transcription elongation factors (15, 16). We have strong indications that FACT is associated predominantly with coding regions in yeast cells and that this association is dependent on transcription (17). In addition, FACT is associated with actively transcribed class II genes in Drosophila polytene chromosomes and displays recruitment kinetics in vivo similar to that of Pol II (18). Collectively, these studies establish that FACT functions in vivo as an elongation factor. A model has been proposed for the mechanism of FACT action whereby, upon transcription, FACT binds to nucleosomes in transcribed chromatin regions and displaces either one or both histone H2A-H2B dimers (7). Here, we provide direct evidence in support of this model.

Human FACT complex was reconstituted from baculovirus-expressed recombinant FLAG-hSpt16 and 6His-SSRP1 proteins (fig. S1). The use of recombinant FACT and its subunits, each containing a different tag, allowed us to perform a series of co-immunoprecipitation experiments to analyze the interactions of FACT subunits with nucleosome components. FLAG-hSpt16 bound to H2A-H2B dimers (Fig. 1A) and to mononucleosomes (Fig. 1C). By contrast, 6His-SSRP1 did not bind to either mononucleosomes or H2A-H2B dimers; however, it did interact with H3-H4 tetramers (Fig. 1, B and C). Therefore, consistent with earlier observations, recombinant FACT interacts with mononucleosomes, and this interaction is mediated through the large subunit, p140h/Spt16 (Fig. 1C). Importantly, Spt16 alone displays reduced interaction with nucleosomes. In support of the protein interaction studies described above and consistent with previous work (19), we also observed that FACT binds to nucleosomes in gel mobility shift assays (17).

Human p140h/Spt16 is a 120-kD protein with 36% identity to its Saccharomyces cerevisiae homolog Spt16/Cdc68, which is suggested to have a role in modulating chromatin structure to affect gene expression both positively and negatively (20–22). The protein has a highly acidic C terminus, a property characteristic of
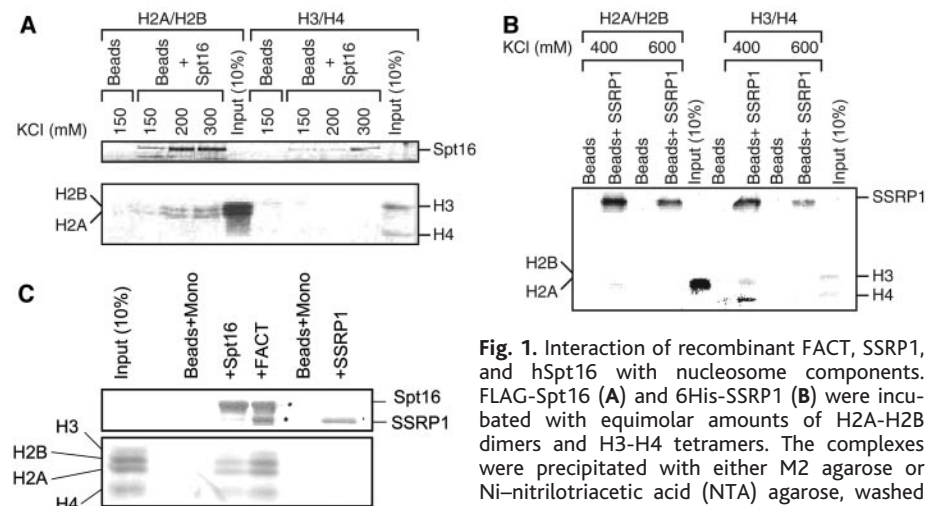
Fig. 1. Interaction of recombinant FACT, SSRP1, and hSpt16 with nucleosome components. FLAG-Spt16 (**A**) and 6His-SSRP1 (**B**) were incubated with equimolar amounts of H2A-H2B dimers and H3-H4 tetramers. The complexes were precipitated with either M2 agarose or Ni–nitrilotriacetic acid (NTA) agarose, washed with the buffer containing the indicated salt concentration, and eluted with either FLAG peptide (A) or 250 mM imidazole (B), respectively. Proteins were visualized by silver staining. (**C**) FACT interacts with mononucleosomes via p140. rFACT, SSRP1, or Spt16 (5 μg of each) were incubated with 5 μg of native HeLa mononucleosomes, followed by immunoprecipitation with either M2 agarose (FACT and Spt16) or Ni-NTA agarose (SSRP1). Bound material was eluted from the resin and resolved on a 15% SDS–polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were visualized by silver staining.

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numerous proteins with histone chaperone activity (*23*). In order to test whether the acidic C terminus of p140/Spt16 is important for FACT function, we generated a truncated protein (FLAG-Spt16ΔC) that lacked ~230 amino acids from the C terminus (fig. S2). Recombinant FLAG-Spt16ΔC was expressed in and purified from Sf9 cells (Fig. 2A, left and middle). When incubated with 6His-SSRP1, FLAG-Spt16ΔC formed a complex, which we termed FACTΔC (Fig. 2A, right). Unlike wild-type FACT and Spt16, neither FACTΔC nor Spt16ΔC were able to interact with mononucleosomes (Fig. 2B). Moreover, FACTΔC did not facilitate Pol II transcription on chromatin templates (Fig. 2C). The acidic C terminus of Spt16 is thus essential for FACT function in transcription.

In order to test the model of FACT action, we first determined its ability to destabilize nucleosome structure (Fig. 3A). Immobilized dinucleosomes containing fluorescently labeled H2A-H2B dimers and H3-H4 tetramers were incubated with FACT and washed with a buffer containing 350 mM KCl. The ratio of dimers to tetramers bound to the beads was determined. The dimer/tetramer ratio of intact nucleosomes not subjected to the 350-mM-KCl washes was set at 1. Nucleosomes that had not been incubated with FACT, but which were subject to the washes, lost about 20% of their H2A-H2B dimers (Fig. 3A). Incubation of the nucleosomes with increasing amounts of FACT followed by 350 mM KCl washes resulted in a further loss of dimers, reaching 45 to 50% at a three- to ninefold molar excess of FACT relative to nucleosomes (Fig. 3A). In contrast, FACTΔC did not have the same effect on nucleosome stability (Fig. 3A). We thus conclude that FACT weakens interactions between H2A-H2B dimers and H3-H4 tetramers and that this property of FACT is dependent on the acidic C terminus of p140h/Spt16.

We next used mononucleosomal templates that recapitulate many important features of chromatin transcribed in vivo (*24*) to measure the effect of FACT on the transcription of nucleosomal DNA. The nucleosomal template is a mixed population of two differently positioned, single-nucleosome cores (N1 and N2) (Fig. 3B) present in an ~2:1 (N1:N2) ratio. To transcribe these templates, assembled Pol II elongation complexes were first immobilized on beads through a tag in Pol II and then ligated to the mononucleosomes (*24*). Subsequently, RNA was pulse-labeled by "walking" Pol II, and transcription continued in the presence or absence of a higher concentration of KCl, recombinant FACT (rFACT), or rFACTΔC (Fig. 3C). As expected, at low ionic strength (40 mM), nucleosomes presented a strong barrier to the transcribing Pol II: Only 10% of templates (mostly free DNA present in the nucleosome preparation) were transcribed to completion in both the presence and absence of rFACTΔC (lanes 2 and 3). Increasing the ionic strength in the transcription reaction resulted in destabilization of nucleosomes (*24*). At KCl concentrations of 0.3 M and 1 M, ~50% and 70% of the templates were transcribed to completion, respectively (lanes 5 and 6); the nucleosome-specific arrest was relieved at the majority of the positions along the template. In the presence of rFACT, the nucleosomal barrier was partially relieved even at the lower ionic strength (40% of the templates were fully transcribed; compare lanes 2 and 4), as was observed previously with promoter-initiated Pol II (*6*). However, rFACT affected only a subset of the transcription arrest sites (100- to 145-nucleotide RNA; compare lanes 2 and 5). Similar data were obtained with the use of native FACT (*25*). Thus, in contrast to the general effect of increased salt concentration, FACT affects only the promoter-distal transcriptional ar-

rest. This observation can be explained by either selective destabilization of the N2 nucleosomes or by a selective effect on the promoter-distal part of the arrest pattern on all nucleosomal templates.

Fully transcribed templates are released from the immobilized Pol II, allowing analysis of the fate of nucleosomes during transcription. Previously, it has been shown that nucleosomes transcribed by Pol II at 300 mM KCl become depleted of one H2A-H2B dimer and are converted into hexasomes (*24*). The nucleosomal templates were transcribed in the presence of rFACT or rFACTΔC and at various concentrations of KCl. Labeled templates released into solution were analyzed by native gel electrophoresis (Fig. 3D). As expected, transcription in the presence of 300 mM KCl resulted in the appearance of a new, faster-migrating band in the gel that was previously identified as the hexasome (lane 7). Hexasomes were not formed either during partial transcription (45-nucleotide RNA formation, lane 1), during incubation in the presence of rFACT without nucleotide triphosphates (NTPs) (*25*), or during transcription at 40 mM KCl both with and without rFACTΔC (lanes 3 and 4). Under these conditions, transcription of the nucleosomal templates was incomplete (Fig. 3C). Importantly, the hexasomes were formed during transcription at 40 mM KCl only in the presence of rFACT (lane 5).

To confirm the identity of the hexasomes, histones H2A-H2B were added to the templates transcribed in the presence of rFACT or at 300 mM KCl (Fig. 3D). As expected, selective binding of H2A-H2B to the hexasomes resulted in their conversion into complete nucleosomes [compare lanes 5 and 7 and 6 and 8 (*24*)]. In summary, transcription through the nucleosome is greatly stimulated by FACT but not FACTΔC; one H2A-H2B dimer is displaced from the nucleosomes during transcription.
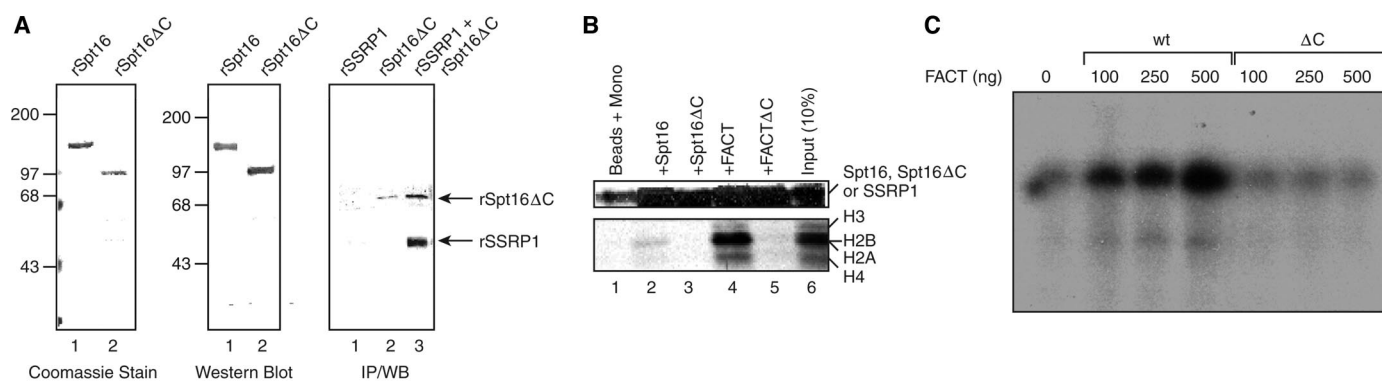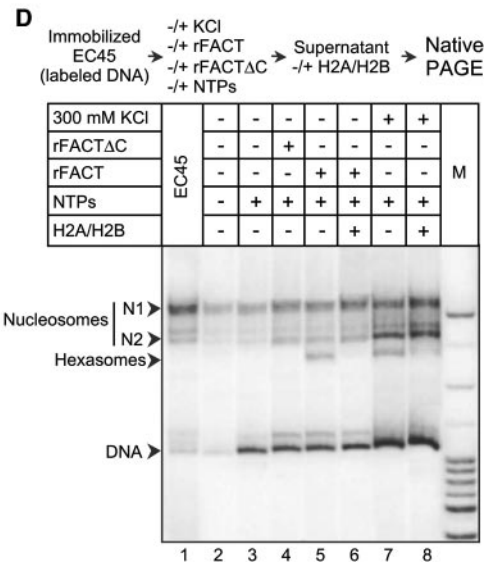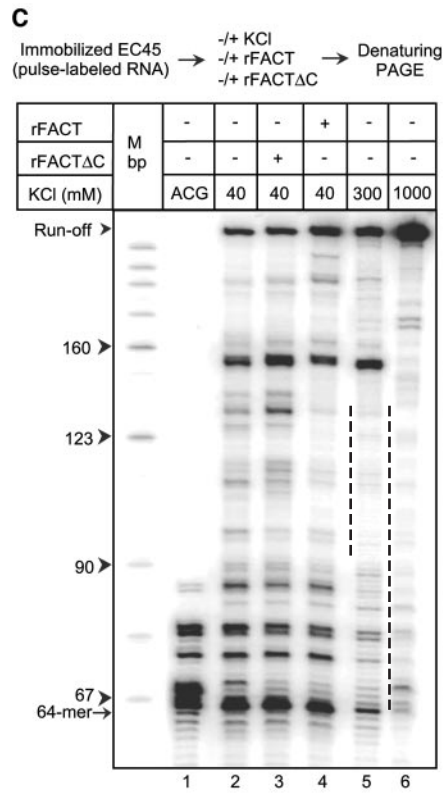

Fig. 2. The C-terminal acidic region of Spt16 is important for both FACT interaction with nucleosomes and FACT function in transcription. (**A**) Spt16ΔC forms a complex with SSRP1. Spt16 and Spt16ΔC were expressed in Sf9 cells and affinity purified via the FLAG tag. A Coomassie Blue–stained gel (left) and a Western blot (middle) probed with antibodies against p140hSpt16, respectively. (Right) When incubated together, SSRP1 and Spt16ΔC form a stable complex, FACTΔC, isolated by immunoprecipitation (IP) with the use of M2 agarose. (**B**) Spt16ΔC and FACTΔC do not bind to mononucleosomes. The IP experiment was performed essentially as described in Fig. 1C. (**C**) FACTΔC does not facilitate transcription on chromatin templates. Transcription assays were performed as in fig. S1C. wt, wild type.
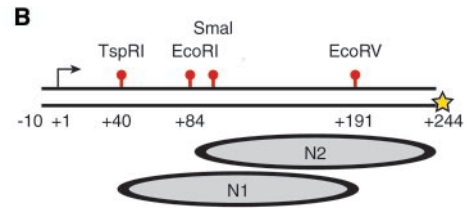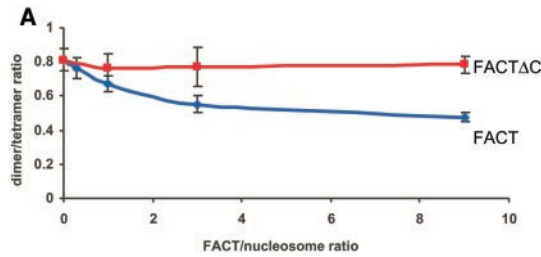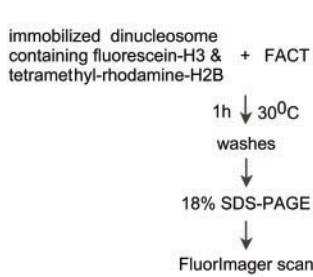
Fig. 3. FACT-facilitated transcription through the nucleosome is accompanied by displacement of one H2A-H2B dimer. (**A**) FACT destabilizes the interaction between H2A-H2B dimers and H3-H4 tetramers in the nucleosome. The experiments were performed as outlined in the schematic (left). After incubation with FACT, dinucleosomes were washed, and proteins were resolved by 18% SDS-PAGE. The gels were quantified on a FluorImager with the use of ImageQuant software (*29*). (B to D) The experiments were performed essentially as described in (*24*). (**B**) Positions of the nucleosomes on a 254-base-pair (bp) template. The position of the label is indicated with a star. Positions of the restriction enzyme sites relative to the 5′ end of the RNA (+1) are indicated. The two clusters of nucleosomal positions (N1 and N2) are shown by ovals. (**C**) FACT can relieve the nucleosomal barrier to Pol II elongation. After preformation of stalled elongation complexes (EC45) containing pulse-labeled RNA (lane 1), the 254-bp nucleosomal templates were incubated with NTPs at the indicated concentrations of KCl in the presence (+) or absence (–) of rFACT or rFACTΔC (lanes 2 to 6). Dashed lines indicate the areas where pausing is relieved in the presence of 0.3 M KCl or rFACT. Arrowheads on the left indicate the lengths of transcripts. (**D**) FACT-facilitated transcription through the nucleosome results in the formation of hexasomes. Immobilized, DNA-end-labeled nucleosomes were transcribed in the presence or absence of rFACT, rFACTΔC, or 300 mM KCl. The beads were pelleted, and the supernatant was analyzed in a native gel. Supernatant contains fully transcribed and some nontranscribed templates (*24*). In a control experiment, EC9 was converted into EC45, and only nontranscribed nucleosomes were released into solution (lane 1) (*24*). The positions of the polymerase-free nucleosomes N1 and N2, hexasomes, and DNA are indicated by arrowheads. Note that differently positioned hexasomes have similar mobilities in the gel (*24*). Higher background of nontranscribed templates at 300 mM KCl (lanes 7 and 8) is because of disruption of the elongation complexes by higher salt.

Our results are consistent with a model in which FACT facilitates Pol II–driven transcription by helping to displace one H2A-H2B dimer from the nucleosome. However, nucleosomes carry important epigenetic information, and disruption of nucleosomal structure may have severe consequences for diverse cellular functions. Thus, mechanisms should exist to allow nucleosomes to remain intact after transcription. The larger subunit of FACT, Spt16, contains a highly acidic C terminus, which could potentially have histone chaperone activity. It is possible that FACT first helps to disrupt the nucleosome and then, with the use of its putative chaperone activity, ushers the H2A-H2B dimer back into place after transcription has occurred. Therefore, we tested whether FACT could facilitate histone deposition onto DNA (Fig. 4). DNA was incubated with core histones in either the absence or the presence of intact FACT, individual FACT subunits, or FACTΔC. Complex formation between histones and DNA was monitored by electrophoresis on a native polyacrylamide gel. When core histones were mixed with DNA at physiological salt concentrations, they formed aggregates that did not enter the gel (Fig. 4A, lane 2). In contrast, in the presence of either native or recombinant wild-type FACT, a slower-migrating band appeared (lanes 3, 4, 6, and 7). However, this band was not detected when DNA and core histones were incubated with FACTΔC (lanes 9, 10). To confirm that the slower-migrating band corresponded to DNA-histone complexes and to analyze which core histones were deposited onto DNA in the presence of FACT, we performed the deposition assays with the use of histone octamers containing fluorescein-H3 and tetramethyl-rodamine (TMR)–H2B. The DNA band shift was visualized on an ethidium bromide–stained gel (Fig. 4B, right). A FluorImager (Molecular Dynamics, Sunnyvale, CA) scan of the same gel demonstrated that both fluorescein-labeled H3-H4 tetramers and TMR-labeled H2A-H2B dimers were present in the shifted band [Fig.
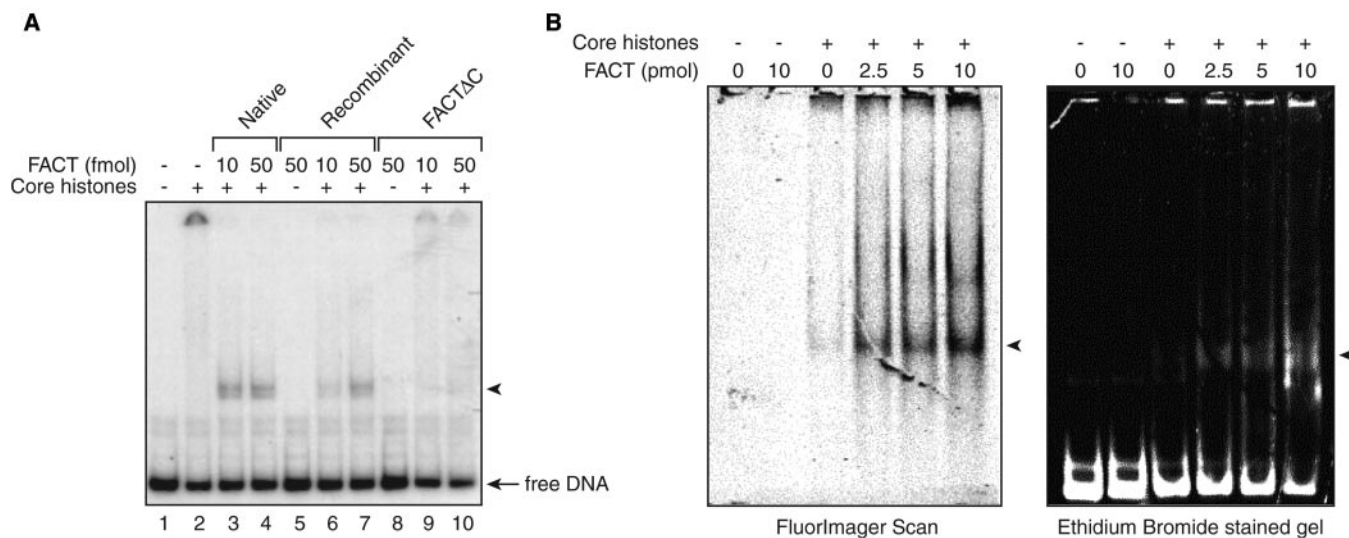
Fig. 4. FACT possesses histone chaperone activity. (**A**) FACT, but not FACTΔC, can promote core histone deposition onto DNA. The DNA-histone complexes marked with arrowheads appeared in the presence of wild-type FACT (lanes 3, 4, 6, and 7) but not in the presence of FACTΔC (lanes 9 and 10). (**B**) All four core histones are deposited onto DNA in the presence of FACT (arrowheads). Fluorescently labeled core histones and 190-bp 5S DNA

fragments were incubated for 30 min at 30°C in the absence or presence of the FACT complex. The reaction products were resolved on a 5% native polyacrylamide gel. The gels were scanned on a FluorImager to detect labeled histones (left) and stained with ethidium bromide to visualize DNA (right). Both H2A-H2B dimers (not shown) and H3-H4 tetramers were present in the band coincident with the shifted DNA band (right).

4B, left, and (*17*)]. Thus, in the presence of FACT, all four core histones are deposited onto DNA. Interestingly, neither SSRP1 nor hSpt16 alone are able to mediate histone deposition (*17*). Thus, we conclude that FACT possesses a histone chaperone activity that requires the intact complex and that the acidic C terminus of Spt16 is critical for this function of FACT.

Our data add an additional level of complexity to the model for the mechanism of FACT activity that was proposed earlier (*7*). We show that the interaction between FACT and nucleosomes is mediated by the highly acidic C terminus of Spt16. Although both FACT and Spt16 can bind to nucleosomes and H2A-H2B dimers, SSRP1 can only bind to H3-H4 tetramers but not to intact nucleosomes. We hypothesize that, upon FACT binding to the nucleosome in the transcribed region, Spt16 facilitates the H2A-H2B displacement, which promotes the interaction between SSRP1 and the "altered" nucleosome. We demonstrate that FACT facilitates Pol II–driven transcription through chromatin by destabilizing nucleosomal structure so that one of the H2A-H2B dimers is removed upon Pol II passage. The loss of an H2A-H2B dimer during transcription was previously observed when the reaction was conducted in vitro under conditions of high ionic strength (*24*). Such conditions are known to affect nucleosome structure (*26*). In the presence of FACT, a similar outcome is achieved at much lower salt concentrations. Thus, FACT facilitates destabilization of the dimer-tetramer interactions during transcription. This hypothesis is consistent with previous findings

that yeast carrying mutations in the histone genes that alter the interactions between H2A-H2B dimers and H3-H4 tetramers exhibit phenotypes identical to those caused by mutations in the *SPT16* gene (*27*). A recent genetic study has suggested that nucleosome integrity is disrupted during transcription in vivo (*28*). Most importantly, the latter study also implied a role for yeast FACT in nucleosome reassembly after Pol II passage, because mutations in *SPT16* and *POB3* resulted in the dependence of yeast cells on the Hir/Hpc nucleosome assembly pathway (*28*). In this report, we show that FACT does indeed possess histone chaperone activity (that is, it can promote core histone deposition onto DNA in vitro) and thus it could also be involved in maintaining nucleosome integrity after Pol II passage.

Finally, facilitation of transcription by Pol II is only one of the many physiological processes in which FACT is involved. The dual role of FACT in both nucleosome destabilization and reassembly might explain the severe effects that mutations in FACT components have on cell viability.

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