Transcription Promotes the Interaction of the FAcilitates Chromatin Transactions (FACT) Complex with Nucleosomes in *Saccharomyces cerevisiae*

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ABSTRACT The FACT (FAcilitates Chromatin Transactions) complex is a conserved complex that maintains chromatin structure on transcriptionally active genes. Consistent with this, FACT is enriched on highly expressed genes, but how it is targeted to these regions is unknown. *In vitro*, FACT binds destabilized nucleosomes, supporting the hypothesis that FACT is targeted to transcribed chromatin through recognition of RNA polymerase (RNAP)-disrupted nucleosomes. In this study, we used high-resolution analysis of FACT occupancy in *Saccharomyces cerevisiae* to test this hypothesis. We demonstrate that FACT interacts with nucleosomes *in vivo* and that its interaction with chromatin is dependent on transcription by any of the three RNAPs. Deep sequencing of micrococcal nuclease-resistant fragments shows that FACT-bound nucleosomes exhibit differing nuclease sensitivity compared to bulk chromatin, consistent with a modified nucleosome structure being the preferred ligand for this complex. Interestingly, a subset of FACT-bound nucleosomes may be "overlapping dinucleosomes," in which one histone octamer invades the ~147-bp territory normally occupied by the adjacent nucleosome. While the differing nuclease sensitivity of FACT-bound nucleosomes could also be explained by the demonstrated ability of FACT to alter nucleosome structure, transcription inhibition restores nuclease resistance, suggesting that it is not due to FACT interaction alone. Collectively, these results are consistent with a model in which FACT is targeted to transcribed genes through preferential interaction with RNAP-disrupted nucleosomes.

KEYWORDS FACT; Spt16; Pob3; nucleosome; chromatin; transcription

FACT (FAcilitates Chromatin Transactions) is an abundant and conserved complex that promotes DNA-dependent processes, such as transcription and DNA replication. In animals and plants, FACT comprises the subunits Spt16 and SSRP1, while in *Saccharomyces cerevisiae*, the role of SSRP1 is performed by two proteins, Pob3 and Nhp6 (Orphanides *et al.* 1999; Brewster *et al.* 2001; Formosa *et al.* 2001). FACT was originally identified through its ability to promote transcription elongation on a chromatin template *in vitro* (Orphanides *et al.* 1998). It was later shown to enhance TBP binding at nucleosomal sites and promote histone displacement from the promoters of inducible genes (Mason and Struhl 2003; Biswas *et al.* 2005). Consistent with these observations, yeast FACT (yFACT) destabilizes nucleosomes *in vitro* (Formosa *et al.* 2001; Rhoades *et al.* 2004; Xin *et al.* 2009; Valieva *et al.* 2016). However, contrary to these data, FACT has also been implicated in the stabilization of chromatin. In yeast, mutation of FACT subunits results in transcription initiation from cryptic sites within gene bodies, enhanced histone turnover, and a failure to reestablish chromatin following transcriptional repression (Kaplan *et al.* 2003; Mason and Struhl 2003; Jamai *et al.* 2009; Hainer *et al.* 2012; Voth *et al.* 2014; Hainer and Martens 2016). The seemingly opposing functions of FACT in both disrupting and stabilizing chromatin have been reconciled in a model in which FACT binds nucleosomes and maintains an altered nucleosome structure that allows RNA polymerase (RNAP) II passage without histone loss (Winkler and Luger 2011, Formosa 2012).

FACT shows increased occupancy on highly expressed genes (Mason and Struhl 2003; Mayer *et al.* 2010; Feng *et al.* 2016; Pathak *et al.* 2018), and is targeted with RNAPII

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upon gene induction in both flies and budding yeast (Mason and Struhl 2003; Saunders *et al.* 2003; Duina *et al.* 2007; Nguyen *et al.* 2013; Vinayachandran *et al.* 2018). However, an unresolved question is how FACT is targeted to these regions. FACT interacts with multiple components of the transcription machinery, including RNAPs, the Paf1 complex, the chromatin remodeler Chd1, and Cet1, a subunit of the capping enzyme, but whether any of these factors are required for global FACT occupancy has not been tested (Krogan *et al.* 2002; Squazzo *et al.* 2002; Simic *et al.* 2003; Tardiff *et al.* 2007; Sen *et al.* 2017). Moreover, various lines of evidence suggest that the recruitment of FACT may not be through direct interaction with the RNAPII transcriptional machinery. First, the timing of RNAPII and FACT recruitment to induced genes during heat shock differs (Vinayachandran *et al.* 2018). Second, FACT is also found at regions transcribed by RNAPI and III (Birch *et al.* 2009; Tessarz *et al.* 2014; Cakiroglu *et al.* 2018). Finally, histone mutants cause delocalization of FACT independently of RNAPII and other elongation factors (Duina *et al.* 2007; Lloyd *et al.* 2009; Nguyen *et al.* 2013; Pathak *et al.* 2018). Together, these data suggest that FACT is directed to transcribed regions through an indirect mechanism.

Several studies have demonstrated the interaction of FACT with nucleosomes *in vitro*, but only in the presence of a destabilizing stress. First, the HMG box protein, Nhp6, promotes the interaction of both human and yFACT with nucleosomes (Formosa *et al.* 2001; Ruone *et al.* 2003; Rhoades *et al.* 2004; Zheng *et al.* 2014; Valieva *et al.* 2016; McCullough *et al.* 2018). Like other HMG box proteins, Nhp6 weakens histone–DNA contacts, potentially "priming" nucleosomes for FACT binding (Travers 2003; Hepp *et al.* 2017). Second, the introduction of a DNA double-strand break promotes FACT binding to nucleosomes *in vitro* (Tsunaka *et al.* 2016). Third, curaxins, a class of drugs that promote the unwrapping of nucleosomal DNA *in vitro*, "trap" FACT on chromatin (Gasparian *et al.* 2011; Safina *et al.* 2017; Nesher *et al.* 2018). Fourth, FACT binds hexasomes, made up of DNA, a H3–H3 tetramer, and a single H2A–H2B dimer, but not nucleosomes *in vitro* (Wang *et al.* 2018). Finally, FACT is enriched in regions experiencing torsional stress (Safina *et al.* 2017), a force disruptive to nucleosomes (Teves and Henikoff 2014). These data, along with the disruptive effect of transcription on nucleosome structure (Kireeva *et al.* 2002; Schwabish and Struhl 2004; Kulaeva *et al.* 2010; Sheinin *et al.* 2013; Chang *et al.* 2014), support the intriguing hypothesis that FACT is targeted through preferential interaction with RNAP-destabilized nucleosomes (Winkler and Luger 2011; Formosa 2012; Hondele and Ladurner 2013). In this study, we tested this hypothesis using high-resolution analysis of FACT occupancy in *S. cerevisiae*. We demonstrate that the interaction of FACT with chromatin is dependent on transcription by any of the three RNAPs. Further, we show that FACT binds nucleosomes *in vivo*, preferentially interacting with genes with high histone turnover. FACT-associated nucleosomes show altered nuclease sensitivity compared to bulk chromatin, which is consistent with FACT's ability to modulate nucleosome structure; however, the enhanced nuclease sensitivity is dependent on transcription, suggesting that it is not due to FACT binding alone. Instead, these data support the model that FACT is targeted to transcribed regions through preferential interaction with RNAP-destabilized nucleosomes.

Materials and Methods

Yeast strains and antibodies

All strains used in this study were isogenic to S288C. The Pob3TAP and Spt16HA_6 strains were derived from FY602 (*MAT***a** *his3*Δ*200 lys2-128*δ *leu2*Δ*1 trp1*Δ*63 ura3-52*), a generous gift of Fred Winston. Yeast culture, genetic manipulations, and strain verifications were performed using standard protocols. Antibodies used were from Roche [11 583 816 001 (HA)], Biolegend [665004 (Rpb3)], Active Motif [39237 (histone H2B)], and Millipore [PP64 (IgG)].

Spt16 chromatin immunoprecipitation sequencing

Yeast cells were arrested in G1 by treatment with 10 μM α factor for 3 hr (synchronization confirmed by microscopic inspection of cell morphology). Transcription inhibition was performed by treatment with 400 μg/ml 1,10-phenanthroline monohydrate (1,10-pt) for 15 min. Cross-linking was performed with 1% (vol/vol) formaldehyde for 15 min at room temperature and quenched with 125 mM glycine for 15 min.

Sonicated chromatin immunoprecipitation (ChIP) sequencing (ChIP-seq) was performed essentially as described previously (Lawrence *et al.* 2017). Briefly, cells were disrupted by bead beating, chromatin was sonicated to an average DNA length of 250 bp (Biorupter; Diagenode), and parallel immunoprecipitations were performed using α-HA or α-Rpb3 antibodies. Exogenous DNA "spike-ins" were added to ChIP-seq eluates to allow quantification of global changes in ChIP yields. Based on the DNA spike-ins, the yields of DNA relative to input were 0.05% for the Rpb3 ChIP, and 0.06 and 0.04% for the HA ChIP from Spt16-HA_6-expressing and untagged cells, respectively.

Micrococcal nuclease (MNase) ChIP-seq was performed as described previously (Maltby *et al.* 2012). Briefly, cells were disrupted by bead beating, chromatin was digested to mononucleosomes with MNase, and ChIPs were performed using α-HA antibody. Exogenous DNA spike-ins were added to ChIP-seq eluates to quantify global changes in ChIP yields. The yields of DNA relative to input were 1.97 and 0.34% from Spt16-HA_6-expressing and untagged cells, respectively.

Sequencing libraries were prepared as described previously (Maltby *et al.* 2012; Martin *et al.* 2017). Briefly, 2 ng of ChIP or input material were end-repaired, A-tailed, and adapters ligated, before PCR amplification with indexed primers. We performed 10 and 11 rounds of PCR amplification for MNase and sonication ChIP-seq experiments, respectively.

All DNA purification steps used Solid Phase Reversible Immobilization magnetic beads. Samples were pooled, gel-purified (50–600-bp region), and sequenced on an Illumina HiSeq 2500.

Data analysis

Sequenced reads were trimmed for adapter sequences using cutadapt (http://cutadapt.readthedocs.io/en/stable/). Trimmed reads were then aligned to the Saccer3 genome using bowtie2 (Langmead and Salzberg 2012) and filtered for paired reads with a mapping quality of at least 5 using SAMtools (Li *et al.* 2009), with the exception of the data in Supplemental Material, Figure S8, for which a mapping quality score of 1 was used. Fragments mapping to synthetic spiked-in sequences were used to scale ChIP-seq data sets following 1,10-pt transcription inhibition. Reads per genome coverage bigwig tracks were generated using deeptools (Ramírez *et al.* 2014, 2016). Size-selected counts per million fragments per kilobase tracks were generated using a custom bash script using AWK, BEDtools (Quinlan and Hall 2010), and the University of California, Santa Cruz (UCSC) bedgraphtobigwig function (Kent *et al.* 2010). Genome browser shots were generated using the UCSC genome browser (Kent *et al.* 2002).

Genome-wide coverage in specific windows for ChIP-seq data were generated using deeptools, and heatmaps and scatter plots were plotted in R using pheatmap and smoothscatter functions, respectively. For gene body comparisons of Rpb3 and Spt16, gene bodies were defined as +73 bp downstream of the +1 nucleosome core particle (+1 NCP) dyad, to avoid initiation effects, to the TTS (transcription termination site). To avoid gene-length effects, only genes (+1 NCP dyad to the TTS) longer than 500 bp were analyzed, but similar results were seen across all gene lengths.

For heatmaps and average plots relative to the transcription start site (TSS) or +1 NCP dyad, feature-aligned matrixes were constructed using deeptools. Heatmaps were generated using deeptools, while average plots were generated in R and only include data until the TTS to avoid effects of varying gene lengths, with the fraction of genes plotted at a given position indicated by a gray line. The MNase two-dimensional heatmaps were generated using AWK and BEDtools, with the final heatmaps plotted in R using pheatmap.

For histone turnover data (Dion *et al.* 2007), average values over gene bodies were calculated using the java genomics toolkit (https://github.com/timpalpant/java-genomics-toolkit). Partial correlations were calculated using the ppcor function in R (Kim 2015). For gene windows, genes were ordered by Rpb3 levels in a 500-gene sliding window. For each window, the top and bottom 100 genes for Spt16 − Rpb3 were selected and the mean value plotted. The 95% C.I. was bootstrapped and plotted as the shaded area in the graph. Color schemes were generated using the RColorBrewer Package in R.

Fragment length histograms were plotted in R, and fragments overlapping specific genomic regions were selected for using BEDtools.

Published data sets

For histone turnover data (Dion *et al.* 2007), probe intensity values were converted to Saccer3 using the UCSC liftover tool and converted to wig format, with linear interpolation to a maximum of 500 bp, and average values over gene bodies were calculated using the java genomics toolkit (https://github.com/timpalpant/java-genomics-toolkit). Spt16 ChIP-seq data sets were downloaded from Sequence Read Archive (SRA) projects SRP055441 (Feng *et al.* 2016), SRP018874 (Foltman *et al.* 2013), SRP073244 (True *et al.* 2016), and SRP036647 (Wong *et al.* 2014). The Spt16 ChIP-exo data set was downloaded from SRA project SRP106497 (Vinayachandran *et al.* 2018). Spt5 and Rpb1 ChIP-seq data sets (Baejen *et al.* 2017) were downloaded from SRP071780. Sequenced reads were processed as described above.

TSSs, +1 NCPs, and TTSs are from Chereji *et al.* (2018), and total nucleosome dyad positions are from Weiner *et al.* (2015). Coordinates for rDNA and tRNA genes were from http://www.yeastgenome.org/.

FACT purification

Pob3TAP and its associated proteins were purified from 1 liter of yeast cells as described (Lambert *et al.* 2009).

Data availability

Strains and plasmids are available upon request. The ChIP-seq data generated for this study, accessed February 24, 2018, have been deposited in the Gene Expression Omnibus database under accession numbers GSE111426 and GSE110286, available at https://downloads.yeastgenome.org/curation/chromosomal_feature/SGD_features.tab. Twelve supplemental figures have been uploaded to FigShare: https://doi.org/10.25386/genetics.7026278.

Results

Transcription promotes the interaction of FACT with chromatin

To identify pathways for targeting FACT to transcribed regions, we first sought to confirm colocalization of FACT with RNAPII. To this end, we performed ChIP-seq of carboxy-terminal HA_6-tagged Spt16 and the RNAPII subunit Rpb3 from the same whole-cell extracts with two independent biological replicates. As FACT undergoes a dramatic redistribution during S phase (Foltman *et al.* 2013), we synchronized cells in G1 with α-factor. Our Spt16 ChIP-seq data agreed well with previously published FACT ChIP-seq experiments (Figure S1), and we observed a specific and striking colocalization of Spt16, but not an untagged control, with Rpb3 at individual loci (Figure 1A) and genome-wide (Figure 1B). In agreement with previous reports (Mason and Struhl 2003; Mayer *et al.* 2010; Feng *et al.* 2016; Pathak *et al.* 2018), we found FACT enriched over the bodies of protein-coding genes (Figure 1C), with binding primarily occurring downstream of the TSS.

To determine whether transcription promotes FACT binding genome-wide, we first analyzed previously published data

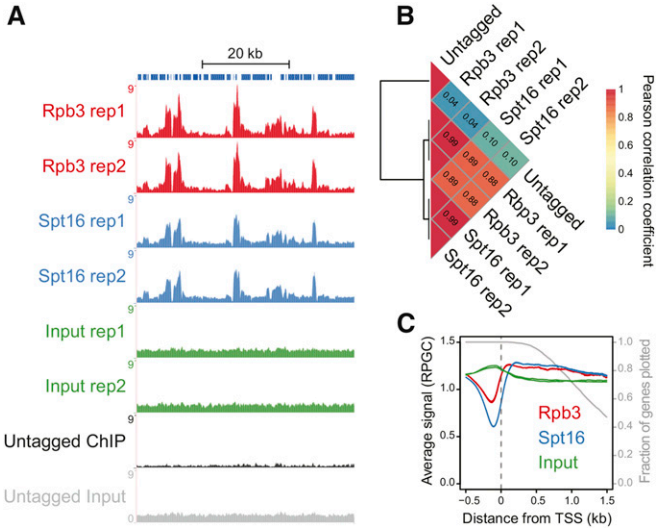

Figure 1 FACT and RNAPII colocalize. (A) Genome browser tracks of sequence coverage (RPGC) from Rpb3 and Spt16-HA$_6$ ChIP (two independent replicates, labeled "rep1" and "rep2") from sonicated chromatin (Input) and an untagged control over chromosome IV 300,000–350,000. The untagged ChIP was scaled using spike-in exogenous DNA (see *Materials and Methods*). The blue bars indicate the position of protein-coding genes. (B) Pearson correlation matrix for sequence coverage of Spt16-HA$_6$, Rpb3, and untagged ChIPs across genome-wide 250-bp bins. (C) The average Rpb3, Spt16-HA$_6$, and sonicated input sequence coverage (two replicates shown) relative to the TSS of 5502 genes. RPGC is the reads normalized to 1× sequencing depth with sequencing depth, defined as mapped reads × fragment length/effective genome size. ChIP, chromatin immunoprecipitation; FACT, FAcilitates Chromatin Transactions complex; RNAP, RNA polymerase; RPGC, reads per genomic coverage; TSS, transcription start site.

mapping RNAPII and Spt16 following depletion of Kin28, a kinase that facilitates promoter escape, using the rapamycin-mediated "anchor away" technique (Wong *et al.* 2014). It should be noted that this analysis was conducted in cells grown in minimal media, which results in the previously observed, albeit unexplained, shift of RNAPII occupancy from the TSS to midgene when compared to cells grown in rich media (Warfield *et al.* 2017). Kin28 depletion results in a 5′ shift of both Rpb3 and Spt16, as well as loss of both proteins over gene bodies (Figure 2A and Figure S2, A and B), consistent with targeting of FACT by transcription. This shift of FACT localization was not commented on previously, perhaps because the authors focused on a small subset of genes (Wong *et al.* 2014), but the 5′ shift of FACT upon Kin28 depletion appears as a general effect across the 5502 genes analyzed here.

To more directly test whether FACT requires RNAPII for binding to transcribed genes, we next treated cells with the general transcription inhibitor 1,10-pt (Grigull *et al.* 2004), and analyzed Spt16 and RNAPII occupancy by ChIP-seq. Importantly, spike-in DNA controls were added immediately following DNA elution to normalize the number of DNA fragments recovered from treated to untreated cells (Chen *et al.* 2015). Although the mechanism of transcription inhibition by 1,10-pt, a zinc chelator, is not fully understood (Johnston and Singer 1978; Lauinger *et al.* 2017), short treatment (15 min) resulted in depletion of RNAPII across gene bodies, with the greatest effect over the promoters and 5′ regions (Figure 2, B and C, Figure S3A, and Figure S4), suggesting that 1,10-pt inhibits transcription initiation. Additionally, as 15 min should have been sufficient time for polymerase clearance from most genes, residual RNAPII at downstream regions indicates that 1,10-pt also impairs elongation.

Similar to RNAPII, treatment of cells with 1,10-pt also depleted Spt16 from gene bodies, and shifted Spt16 occupancy from 5′ to 3′ genic regions at both highly and poorly transcribed genes (Figure 2, B–D, Figure S3, A–F, Figure S4, and Figure S5), indicating that the interaction of FACT with DNA requires transcription. Supporting a strong dependence of FACT targeting on RNAPII, the changes in Spt16 and Rpb3 occupancy upon transcription inhibition were highly correlated genome-wide (Figure S6). Collectively, these results demonstrate that FACT targeting to transcribed regions occurs as a consequence of RNAP activity.

While the above data are consistent with a model in which FACT is targeted through physical interaction with the RNAPII transcriptional machinery, several contradictions to this model exist. First, although FACT and RNAPII levels are tightly correlated across gene bodies, poorly expressed genes exhibited higher Spt16 occupancy relative to RNAPII than highly expressed genes (Figure 3A). This observation is better visualized in Figure 3B, which compares the ratio of Spt16 to Rpb3 with Rpb3 levels. Such enrichment at poorly expressed genes was not observed for Spt5, an elongation factor that interacts directly with RNAPII (Figure 3, C and D). Second, growth of cells in minimal media resulted in a shift of RNAPII occupancy downstream of the TSS, but FACT occupancy remained largely unchanged (Figure 2, compare A and B). Third, following transcription inhibition, the magnitude of Spt16 depletion was less than for Rpb3, even at 5′ genic regions where Rpb3 depletion is most evident (Figure 2B, Figure S3, A and B, and Figure S4), suggesting that FACT remains transiently associated with chromatin following RNAPII passage. Fourth, the length of DNA fragments coprecipitating with Spt16 and Rpb3 differed significantly (Figure S7, A and B), with Rpb3-associated DNA being smaller than that associated with Spt16. Notably, the differences in fragment sizes were observed at both lowly and highly transcribed regions of the genome (Figure S7, C and D), confirming that the differences in fragment size occurred at regions of colocalization, at least as observed across a population of cells. The differences in fragment sizes are unlikely due to technical variation in sample treatment as these ChIPs were performed from the same cell extracts, the DNA purifications and sequencing libraries were prepared in parallel, and the libraries were indexed, pooled, and sequenced together. Instead, the differences in DNA fragment size likely indicate that the majority of Rpb3 and Spt16 do not purify as a single nucleoprotein complex. Finally, transcription inhibition also resulted in depletion of FACT from the 5′ ends of

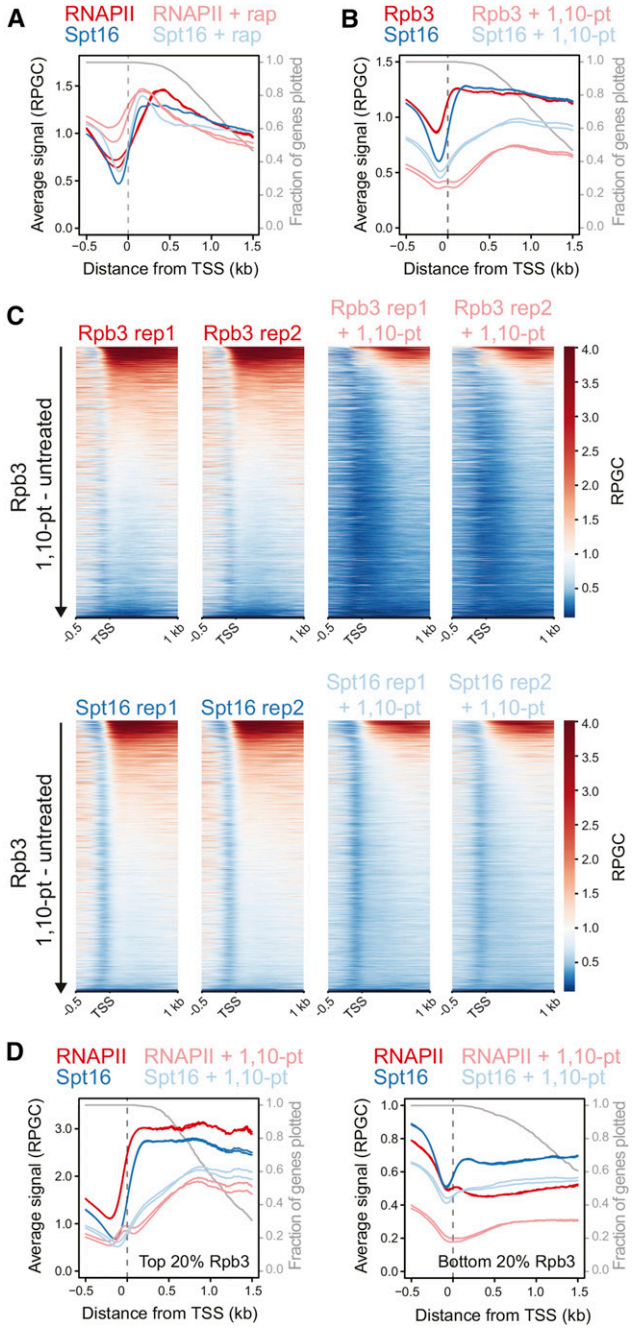

Figure 2 The interaction of FACT with chromatin is dependent on transcription. (A) RNAPII (two replicates shown) and Spt16 sequence coverage (Wong *et al.* 2014, downloaded from SRP036647), from cells expressing Kin28 with an FRB tag that was "anchored away" in the presence of rapamycin (rap), relative to the TSS of 5502 genes. (B and C) Rpb3 and Spt16-HA_6 sequence coverage (two independent replicates, labeled "rep1" and "rep2") relative to the TSS of 5502 genes prior to or following 15-min treatment with 1,10-pt represented as average signal (B) or as a heatmap (C). Fragment coverage was normalized using "spiked-in" control DNA (see *Materials and Methods*). (D) As in (B) but for the top and bottom 20% of transcribed genes (1101 genes), as determined by Rpb3 binding. RPGC is the reads normalized to 1× sequencing depth, with sequencing depth defined as mapped reads × fragment length/effective genome size. 1,10-pt, 1,10-phenanthroline; FACT, FAcilitates Chromatin Transactions complex; RNAP, RNA polymerase; RPGC, reads per genomic coverage; TSS, transcription start site; FRB, FKBP12-rapamycin-binding domain.

RNAPI-transcribed rDNA genes and across RNAPIII-transcribed tRNA genes (Figure S8, A and B and Figure S9A). It should be noted that RNAPI, II, and III share multiple subunits, and thus FACT could conceivably be targeted by multiple polymerases through interaction with these proteins; however, proteomic analysis did not identify these or any other RNAPII subunits as FACT-interacting partners (Bedard *et al.* 2016). Moreover, as seen in Figure S9, A and B, Spt16 accumulates downstream of tRNA genes and thus is unlikely targeted through direct interaction with RNAPIII. Collectively, these data support a model whereby transcription promotes the interaction of FACT with chromatin via an indirect mechanism.

FACT binds RNAP-destabilized nucleosomes in vivo

FACT binds disrupted nucleosomes *in vitro* (Formosa *et al.* 2001; Ruone *et al.* 2003; Zheng *et al.* 2014; Tsunaka *et al.* 2016; McCullough *et al.* 2018; Nesher *et al.* 2018; Wang *et al.* 2018), supporting the hypothesis that it is targeted to transcribed genes through interaction with RNAP-destabilized nucleosomes. To first confirm that FACT binds nucleosomes *in vivo*, we purified FACT via a TAP tag on Pob3. Figure 4A shows that FACT copurified with similar levels of histones H2A, H2B, H3, and H4. Immunoprecipitation of H2B from purified FACT coprecipitated all four core histones (Figure 4B), indicating that FACT interacts with intact histone octamers as opposed to select histone pairs. To verify that these histones were nucleosomal, we repeated Spt16 ChIP-seq from MNase-treated cell extracts. MNase preferentially digests nonnucleosomal DNA and thus, if FACT binds to free DNA as opposed to nucleosomes, the yield of DNA recovered from MNase-treated extracts should have been less than that recovered from sonicated extracts. However, we found the opposite to be true, with MNase treatment increasing the amount of DNA recovered from Spt16 ChIP over 30-fold (see *Materials and Methods*). While these differences could be explained by the impact of sonication on ChIP efficiencies (Pchelintsev *et al.* 2016), the recovery of MNase-resistant fragments associated with FACT, even at highly transcribed genes (see below), strongly suggests that FACT binds nucleosomes *in vivo*.

To determine whether FACT binding alters nucleosome positioning, we subjected the Spt16-associated, MNase-digested DNA to paired-end sequencing. Figure 4, C and D shows that the positioning of FACT-associated DNA closely matched that of input nucleosomes. However, despite similarities, noticeable differences were observed, including increased recovery of sequence fragments from highly expressed genes, depletion and shift of the +1 nucleosome peak, and enrichment of FACT-associated DNA over gene bodies (Figure 4, C and D and Figure S10).

The reported inability of FACT to bind nucleosomes in the absence of destabilizing stress *in vitro* (Tsunaka *et al.* 2009; Wang *et al.* 2018) suggests that FACT recognizes perturbed nucleosomes *in vivo*. One hallmark of destabilized nucleosomes is increased histone turnover, and indeed, Spt16

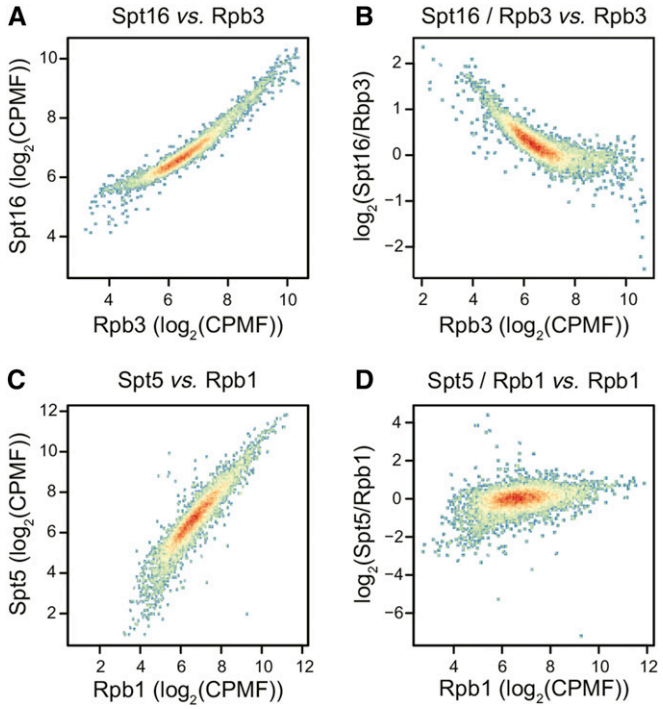

Figure 3 FACT is enriched relative to RNAPII at lowly transcribed genes. (A and B) Smoothed scatter plots of Rpb3 *vs.* Spt16-HA_6 (A) and Spt16-HA_6/Rpb3 (B) across gene bodies. Genes were defined as +73 bp downstream of the +1 nucleosome dyad, to avoid initiation effects, to the transcription termination site. To avoid gene-length effects, only genes longer than 500 bp were analyzed, but similar results were seen across all gene lengths. (C and D) As in (A and B), but for Spt5 and Rpb1 (Baejen *et al.* 2017, downloaded from SRP071780). CPMF, counts per million fragments per kilobase. FACT, FAcilitates Chromatin Transactions complex; RNAP, RNA polymerase.

occupancy correlated with replication-independent (RI) histone turnover (Figure 5A, Spearman rank correlation coefficient of 0.47) (Dion *et al.* 2007). Histone loss is also associated with high levels of transcription, so to test if FACT correlated with histone turnover independently of RNAPII levels, we employed a technique called partial correlation, a statistical method that estimates the direct interaction between two factors while controlling for a confounding variable. Partial correlation analysis revealed that RI histone turnover over gene bodies directly correlated with Spt16 and not Rpb3 (Figure 5A), demonstrating that FACT colocalized with RI histone turnover independently of transcription. To assess this relationship another way, we ordered genes by Rpb3 occupancy and, in a sliding window of 500 genes, identified the 100 genes with the highest or lowest Spt16 occupancy relative to Rpb3 (Figure 5B). Although the differences in Spt16 occupancy were modest, when the sliding windows defined with one data set were used to plot the data from the independent replicate, the differences remained, indicating that they were reproducible (Figure S11). When RI histone turnover was measured over the same sliding windows, we found RI histone turnover was increased at genes with higher Spt16 relative to Rpb3 occupancy (Figure 5C). Although we cannot rule out the possibility that the link between Spt16 occupancy and histone turnover was due to FACT promoting RI histone turnover, the demonstrated ability of FACT to suppress histone turnover *in vivo* argues that this was not the case (Jamai *et al.* 2009). Thus, the link between FACT occupancy and histone turnover supports a model whereby FACT preferentially binds to regions of destabilized nucleosomes.

The use of paired-end sequencing in our analyses afforded us the opportunity to examine the nuclease sensitivity, and thus potential reorganization, of FACT-bound nucleosomes. While FACT-associated DNA exhibited resistance to MNase, consistent with the binding of nucleosomes, specific alterations from bulk chromatin were observed (Figure 6A). FACT-bound DNA fragments, but not those from an untagged control, exhibited increased levels of shorter (100–130 bp) and longer (200–260 bp) than mononucleosome-sized (140–160 bp) fragments. These differences were statistically significant (Figure S12, A–C), and are unlikely to have arisen from technical variation in sample processing as the ChIPs and inputs were processed in parallel, indexed, and pooled together for sequencing. *In vitro*, FACT promotes the loss of an H2A/H2B dimer from nucleosomes (Belotserkovskaya *et al.* 2003) and FACT can form a complex with hexasomes, but not nucleosomes, *in vitro* (Wang *et al.* 2018). Thus, the subnucleosome-sized DNA fragments associated with FACT could be due to disruption of the histone octamer. However, hexasomes are reported to protect only ~110 bp from MNase (Arimura *et al.* 2012), and as few reads of this size were recovered from FACT ChIPs (Figure 6A), it is likely that the majority of FACT-bound nucleosomes contained a complete histone octamer. MNase preferentially digests linker DNA, but transient unwrapping of DNA from the octamer surface can result in "chewing" of the DNA ends. Thus, these smaller DNA fragments are consistent with enhanced unwrapping of DNA from the surface of the histone octamer in nucleosomes associated with FACT.

To determine where the altered, FACT-bound nucleosomes were located, we used two-dimensional occupancy plots to visualize the boundaries of MNase-resistant fragments associated with FACT or with bulk chromatin, relative to the dyad of the +1 NCP of RNAPII-transcribed genes. Shown in Figure 6C, these plots simultaneously display DNA sequencing data as: (1) a heatmap of the total counts of the left (left panels) and right (right panels) boundaries of MNase protection (as designated in Figure 6B), (2) the total length of the MNase-resistant fragments (*y*-axis), and (3) the position of the boundary relative to the dyad of the +1 NCP of 5521 genes (*x*-axis). Analysis of bulk chromatin showed peaks of left boundary signal at ~−75 bp and right boundary signal at ~+75 bp relative to the +1 NCP dyad on the *x*-axis, and ~150 bp on the *y*-axis, consistent with well-positioned, +1 mononucleosomes protecting ~150 bp of DNA (Figure 6C, top panels). Also evident, albeit fainter, were ~−75-bp left boundary and ~+250-bp right boundary signals at ~315 bp on the *y*-axis, indicative of dinucleosomes containing both the +1 and +2 NCP. Of additional note, signals were also observed below 150 bp on the *y*-axis that sloped toward

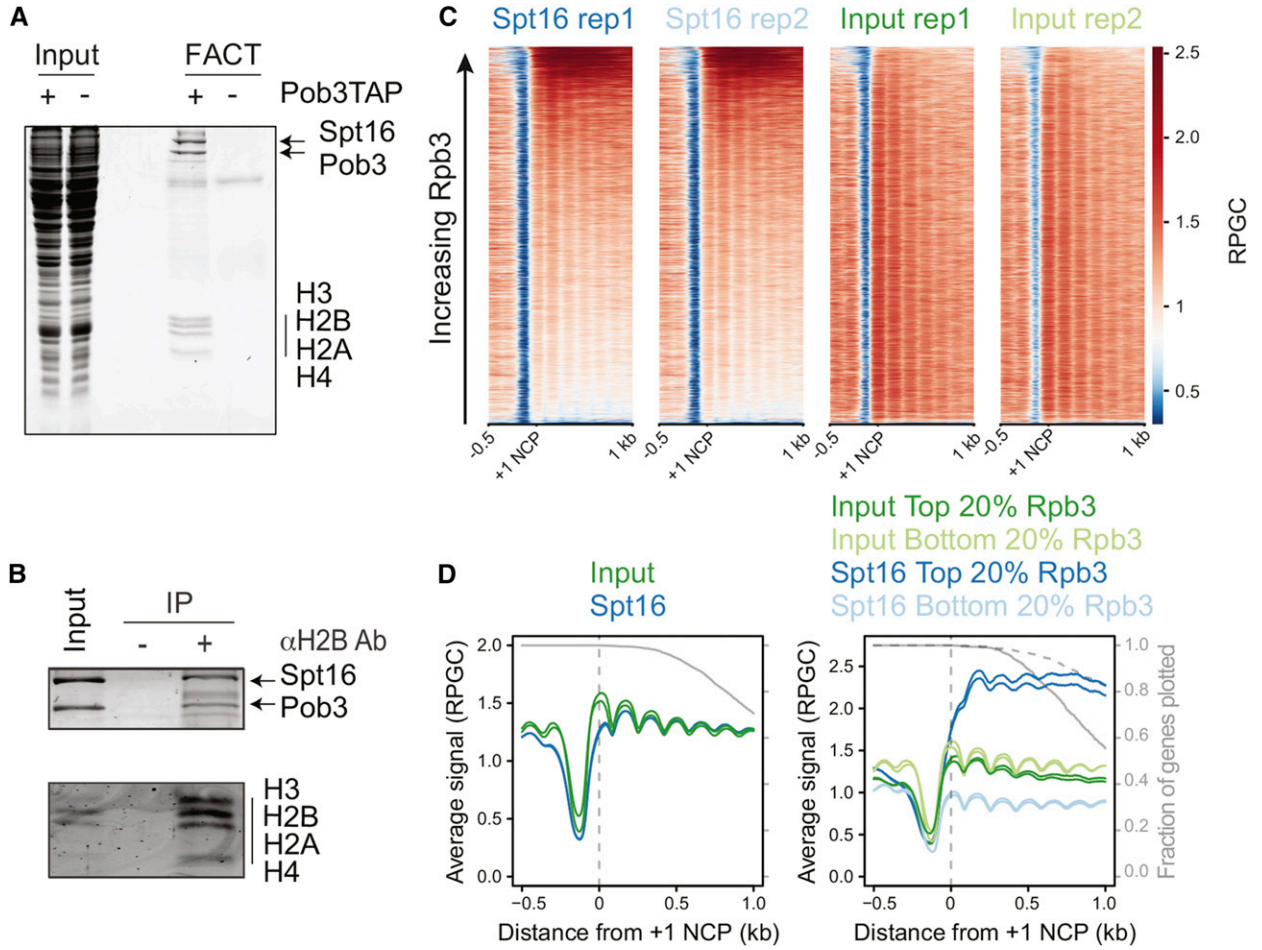

Figure 4 FACT binds nucleosomes *in vivo*. (A) FACT, purified from a strain expressing TAP-tagged Pob3 (+), was subjected to SDS-PAGE analysis with Coomassie staining. An untagged strain (−) was used as a negative control. (B) FACT and associated histones were purified from whole-cell extracts via a TAP tag on Pob3. Purified FACT was eluted, by cleavage of the TAP tag with TEV protease, and subjected to immunoprecipitation with antibodies specific for H2B. The proteins in input and immunoprecipitated fractions were visualized by Coomassie staining of an SDS-PAGE gel. (C) Sequence coverage from Input and Spt16-HA_6 ChIP (two independent replicates, labeled "rep1" and "rep2") from MNase-treated extracts relative to the dyads of 5521 +1 NCPs represented by heatmap. (D) As in (C), but data represented as average coverage for all genes (left panel), and the top and bottom 20% of Rpb3-bound genes (1105 genes each) (right panel). RPGC is the reads normalized to 1× sequencing depth, with sequencing depth defined as mapped reads × fragment length/effective genome size. ChIP, chromatin immunoprecipitation; FACT, FAcilitates Chromatin Transactions complex; MNase, micrococcal nuclease; NCP, nucleosome core particle; RPGC, reads per genomic coverage; TAP, tandem affinity purification; TEV, Tobacco Etch Virus.

the nucleosome dyads. As discussed above, these lower signals likely represent MNase trimming of DNA transiently dissociated from the surface of the histone octamer.

Two-dimensional analysis of fragment boundaries of Spt16 ChIP DNA revealed several interesting features of FACT-bound nucleosomes (Figure 6C, lower panels). First, similar to input, equivalent MNase trimming of Spt16-bound fragments was observed on the promoter-proximal (left boundary) and promoter-distal (right boundary) edges of nucleosomes, suggesting that the direction of transcription had little impact on the increased MNase sensitivity of FACT-bound nucleosomes. Second, discrete signals at ~315 bp, representative of dinucleosomes, were less evident in FACT-associated DNA. Instead, a continuum of fragment sizes from mono- to dinucleosomes was observed, suggesting that FACT can alter typical spacing between nucleosomes. Indeed, DNA fragments in the 200–260-bp range could be indicative of overlapping dinucleosomes, in which one histone octamer invades the ~147-bp territory normally occupied by the adjacent nucleosome. Such structures have been demonstrated *in vitro* by many laboratories (Ulyanova and Schnitzler 2005; Engeholm *et al.* 2009; Kato *et al.* 2017). Indeed, two studies reported overlapping dinucleosomes to be composed of DNA wrapped around a histone hexamer and octamer (Engeholm *et al.* 2009; Kato *et al.* 2017), and thus, the susceptibility of FACT-associated nucleosomes to lose an H2A/H2B dimer (Belotserkovskaya *et al.* 2003) may make these nucleosomes more prone to forming such structures. In contrast, the particles characterized by Ulyanova and Schnitzler (2005), which were the product of remodeling by SWI/SNF, contained two histone octamers on DNA fragments ranging from 170 to 260 bp, which is similar to both the size of DNA and the equal levels of H2A/H2B to

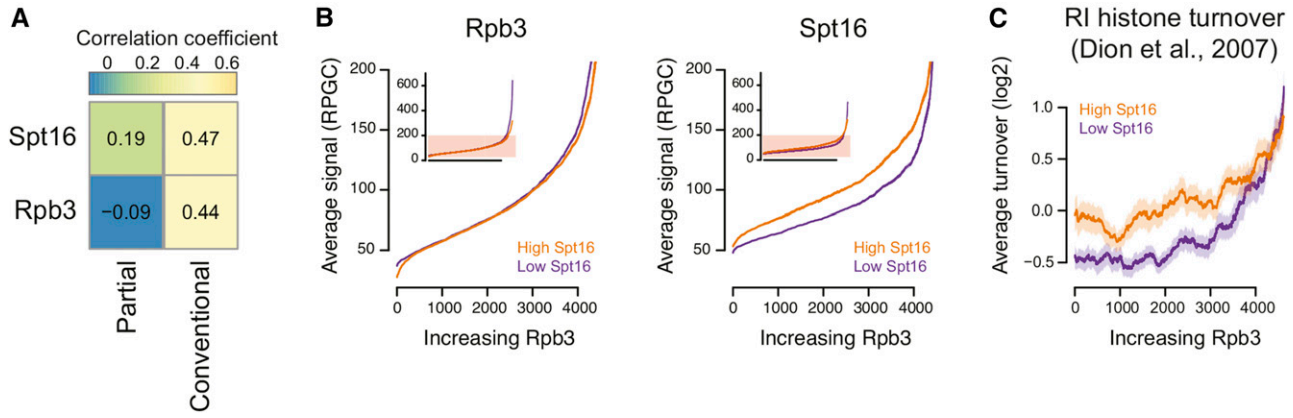

Figure 5 FACT localizes to genes with increased histone turnover independently of RNAPII. (A) Gene-based conventional and partial Spearman correlation coefficients represented by heatmap. Genes were defined as +73 bp downstream of the +1 NCP dyad, to avoid initiation effects, to the transcription termination site. To avoid gene-length effects, only genes longer than 500 bp were analyzed, but similar results were seen across all gene lengths. (B) Genes, as in (A), were ordered by Rpb3 levels, and in 500-gene sliding windows the top and bottom 20% of Spt16-HA_6 − Rpb3 were designated as high- and low-Spt16 groups, respectively. Mean enrichments of Rpb3 and Spt16-HA_6 are plotted with the shaded regions representing the 95% C.I.s. Main plot shows data with *y*-axis truncated at 200 RPGC to better visualize changes in Spt16-HA_6 at moderately transcribed genes. Smaller insert depicts the plot with the full *y*-axis. RPGC is the reads normalized to 1× sequencing depth with sequencing depth defined as mapped reads × fragment length/effective genome size). (C) Replication-independent histone turnover (Dion *et al.* 2007), plotted using the same gene windows as in (B), with the shaded regions representing the 95% C.I.s. FACT, FAcilitates Chromatin Transactions complex; NCP, nucleosome core particle; RNAP, RNA polymerase; RPGC, reads per genomic coverage.

H3/H4 associated with FACT in our analyses (Figure 4, A and B and Figure 6A). Unlike chromatin remodelers, FACT does not require ATP to alter nucleosome structure (Formosa 2012), and thus the possibility that both complexes generate similar chromatin alterations is surprising. However, as such structures make up only a subset of FACT-bound nucleosomes, we were unable to determine the make-up of these particles based on our data alone.

While, the altered nuclease sensitivity of Spt16-bound nucleosomes is consistent with a preference of FACT for transcription-disrupted nucleosomes, yFACT is also reported to alter nucleosome structure *in vitro* (Formosa *et al.* 2001; Rhoades *et al.* 2004; Xin *et al.* 2009; Valieva *et al.* 2016), and thus a modified nucleosome structure could also be a consequence of FACT binding. To differentiate between these two possibilities, we rationalized that, if FACT modifies nucleosome structure on its own, then the altered pattern of digestion should be independent of the destabilizing stress of transcription. To test this, we divided input and Spt16 ChIP DNA fragments into three bins [100–130 bp (purple lines), 140–160 bp (teal lines), and 200–260 bp (orange lines)] and plotted the abundance of each relative to the dyad of the +1 NCP of highly expressed genes (dark lines) and poorly expressed genes (light lines) (Figure 7). Interestingly, larger Spt16-bound DNA fragments were enriched upstream and overlapping the +2 NCP, suggesting that FACT was bound to particles in which the +1 NCP was shifted downstream to create an overlapping dinucleosome with the +2 NCP. This analysis also revealed that Spt16 coprecipitated increased amounts of shorter- (100–130 bp) and longer (200–260 bp)-sized DNA fragments from highly expressed genes compared to poorly expressed genes. The increased levels of alternate fragments at highly expressed genes was diminished upon transcription inhibition. Collectively, these results indicate that altered MNase sensitivity of FACT-bound nucleosomes is dependent on transcription, suggesting that nucleosome disruption is primarily a cause as opposed to a consequence of FACT binding. These data are consistent with a model in which FACT is targeted to active genes through preferential interaction with RNAP-disrupted nucleosomes.

Discussion

In this study, we showed that the FACT complex binds nucleosomes *in vivo* and is recruited to chromatin as a consequence of transcription by any of the three RNAPs. These data, together with extensive evidence that FACT binds destabilized nucleosomes *in vitro*, are consistent with a model in which FACT is targeted to transcribed regions through preferential interaction with RNAP-disrupted nucleosomes. Individual domains of FACT subunits have been shown to bind DNA and all four core histones *in vitro* (Stuwe *et al.* 2008; VanDemark *et al.* 2008; Winkler *et al.* 2011; Hondele *et al.* 2013; Kemble *et al.* 2013, 2015; Hoffmann and Neumann 2015; Tsunaka *et al.* 2016). The unwrapping of nucleosomal DNA by RNAPs could reveal high-affinity binding sites that stabilize the binding of FACT at transcribed genes.

Our results show that Spt16-associated nucleosomes exhibit altered sensitivity to MNase, which is indicative of FACT binding to atypical nucleosome structures, including potentially overlapping dinucleosomes. While the function of overlapping dinucleosomes has not been investigated (Ulyanova and Schnitzler 2005; Engeholm *et al.* 2009; Kato *et al.* 2017), one possibility may be to increase nucleosome mobility on

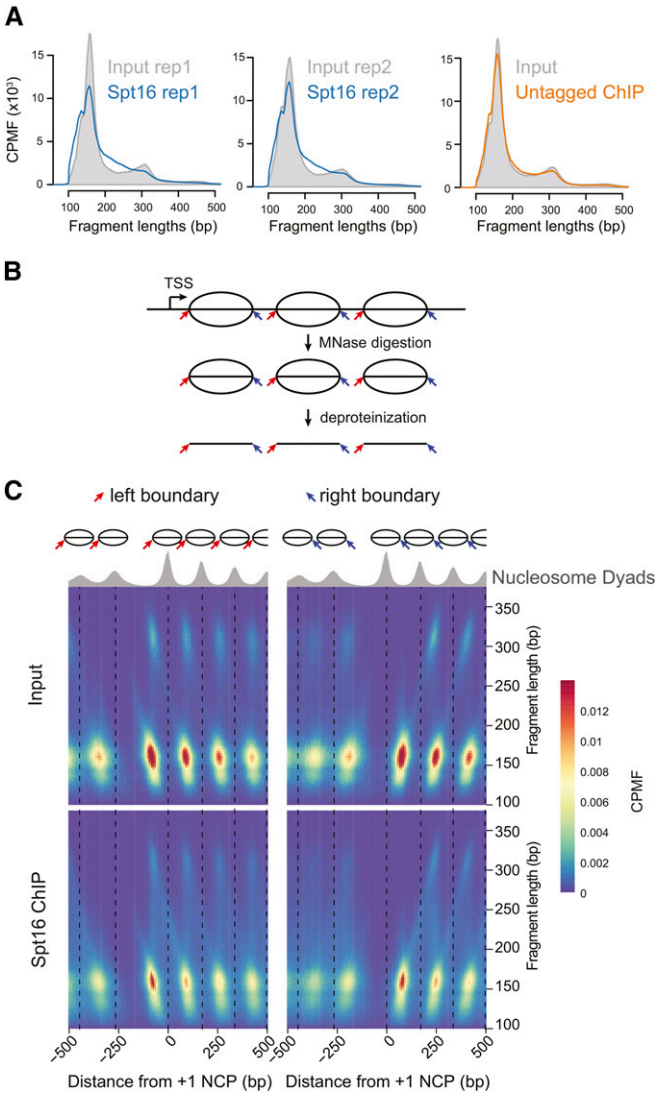

Figure 6 FACT-bound nucleosomes exhibit altered MNase sensitivity. (A) Histograms depicting DNA fragment lengths recovered from input and αHA ChIP from MNase-digested chromatin from Spt16-HA_6 (two independent replicates, labeled "rep1" and "rep2") and untagged strains. (B) Designation of left (red arrows) and right (blue arrows) nucleosome fragment boundaries identified through sequencing of MNase-digested chromatin. (C) Two-dimensional plots of positions of nucleosome boundaries in input and Spt16-HA_6 ChIP from MNase-digested chromatin, relative to the +1 NCP dyad of 5521 annotated genes. The sum of fragment boundary counts is indicated as a heatmap, the sequence fragment lengths (split into 3-bp bins) is plotted on the *y*-axes, and the position of nucleosome boundaries relative to the +1 NCP dyad is plotted on the *x*-axes. As guides for nucleosome positions, sequence coverage of midpoints (3 bp) of 100–200-bp input fragments is indicated on the top of the heatmap in gray and average nucleosome dyad positions are indicated as vertical, dashed lines. ChIP, chromatin immunoprecipitation; CPMF, count per million fragments per kilobase; FACT, FAcilitates Chromatin Transactions complex; MNase, micrococcal nuclease; NCP, nucleosome core particle; TSS, transcription start site.

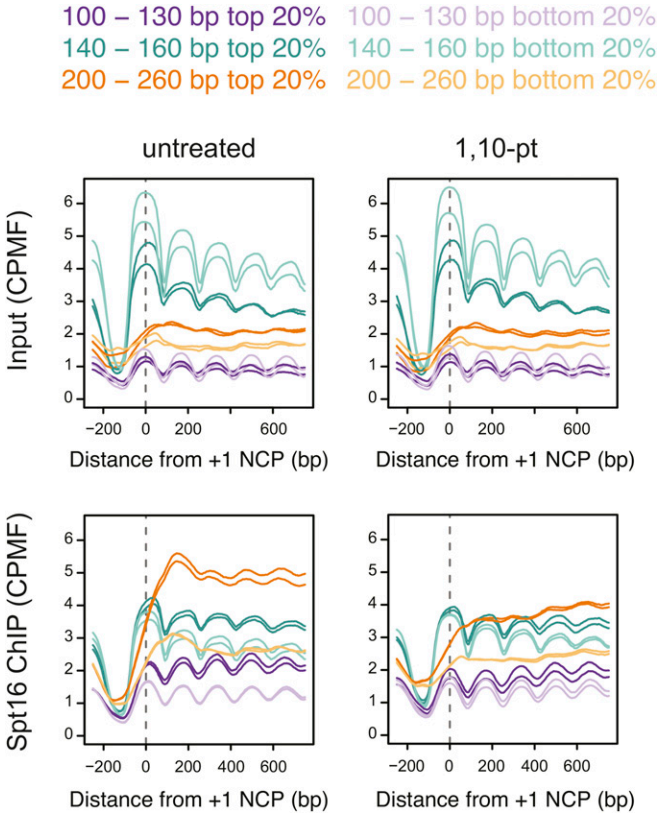

Figure 7 Transcription alters the nuclease sensitivity of FACT-bound nucleosomes. MNase input and Spt16-HA_6 ChIP sequence coverage (two replicates shown) from cells with and without treatment with 1,10-pt, relative to the +1 NCP dyad of the top and bottom 20% of transcribed genes (1105 genes each). Sequence fragments were selected and CPMF of all sizes is plotted. 1,10-pt, 1,10-phenanthroline; ChIP, chromatin immunoprecipitation; CPMF, count per million fragments per kilobase; FACT, FAcilitates Chromatin Transactions complex; MNase, micrococcal nuclease; NCP, nucleosome core particle.

transcribed genes. Nucleosomes restrict the movement of their neighbors (Kornberg and Stryer 1988; Zhang *et al.* 2011), but facilitating the formation of overlapping nucleosomes would increase flexibility of movement without the need for octamer eviction, which coincides with the ability of FACT to both promote transcription while stabilizing chromatin structure. The altered sensitivity of FACT-associated nucleosomes is largely dependent on active transcription, which is consistent with it being a cause, as opposed to a consequence of, FACT binding. However, it should be noted that multiple studies have shown that yFACT can disrupt nucleosome structure *in vitro* (Formosa *et al.* 2001; Rhoades *et al.* 2004; Xin *et al.* 2009; Valieva *et al.* 2016), and thus we cannot rule out the possibility that FACT further alters chromatin structure once bound.

In addition to functioning in transcription, FACT plays an essential role in DNA replication. During S phase, FACT relocates to newly replicated chromatin (Foltman *et al.* 2013; Alabert *et al.* 2014), where it interacts with multiple components of the replication machinery (Wittmeyer and Formosa 1997; Gambus *et al.* 2006; VanDemark *et al.* 2006). Moreover, mutation of FACT results in sensitivity to hydroxyurea, delayed S phase progression (Schlesinger and Formosa 2000; Herrera-Moyano *et al.* 2014), and defects

in chromatin assembly on nascent DNA (Yang *et al.* 2016). Finally, mutation of FACT results in dependence on the S phase checkpoint for viability (Schlesinger and Formosa 2000). However, despite extensive data supporting a requirement for FACT in DNA replication, its actual function in this process is unclear. Part of this mystery is rooted in confusion over the molecular function of FACT. While FACT was originally proposed to be a histone chaperone that deposits free histones on DNA (Belotserkovskaya *et al.* 2003), other data suggest that FACT instead binds intact, but destabilized, nucleosomes (Ruone *et al.* 2003; Tsunaka *et al.* 2009, 2016; Xin *et al.* 2009; Winkler *et al.* 2011; Valieva *et al.* 2016, 2017). Chromatin assembled on newly replicated DNA undergoes a maturation step shortly following DNA replication (Fennessy and Owen-Hughes 2016; Vasseur *et al.* 2016), raising the possibility that FACT recognizes newly formed, unstable nucleosomes on nascent DNA and, akin to its role in transcription, stabilizes these nucleosomes until maturity.

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