Identification of Mutant Versions of the Spt16 Histone Chaperone That Are Defective for Transcription-Coupled Nucleosome Occupancy in *Saccharomyces cerevisiae*

Sarah J. Hainer, Brittany A. Charsar, Shayna B. Cohen, and Joseph A. Martens

Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

**ABSTRACT** The highly conserved FACT (Facilitates Chromatin Transactions) complex performs essential functions in eukaryotic cells through the reorganization of nucleosomes. During transcription, FACT reorganizes nucleosomes to allow passage of RNA Polymerase II and then assists in restoring these nucleosomes after RNA Polymerase II has passed. We have previously shown, consistent with this function, that Spt16 facilitates repression of the *Saccharomyces cerevisiae* **SER3** gene by maintaining nucleosome occupancy over the promoter of this gene as a consequence of intergenic transcription of **SRG1** noncoding DNA. In this study, we report the results of a genetic screen to identify mutations in **SPT16** that derepress **SER3**. Twenty-five spt16 mutant alleles were found to derepress **SER3** without causing significant reductions in either **SRG1** RNA levels or Spt16 protein levels. Additional phenotypic assays indicate that these mutants have general transcription defects related to altered chromatin structure. Our analyses of a subset of these spt16 mutants reveal defects in **SRG1** transcription-coupled nucleosome occupancy over the **SER3** promoter. We provide evidence that these mutants broadly impair transcription-coupled nucleosome occupancy at highly transcribed genes but not at lowly transcribed genes. Finally, we show that one consequence shared by these mutations is the reduced binding of mutant Spt16 proteins across **SRG1** and other highly transcribed genes. Taken together, our results highlight an important role for Spt16 in orchestrating transcription-coupled nucleosome assembly at highly transcribed regions of the genome, possibly by facilitating the association of Spt16 during this process.

In eukaryotes, genomic DNA is packaged with proteins to form chromatin, a repeating array of nucleosomes that contain 147 bp of DNA wrapped around an octamer of histone proteins (Luger *et al.* 1997). In general, this stable association of DNA and histone proteins poses a significant obstacle to many cellular processes, including transcription, DNA replication, and DNA repair, that rely on proteins being able to interact with DNA [reviewed in Bai and Morozov (2010), Duina (2011), Li *et al.* (2007), and Luger (2006)]. Not surprisingly, eukaryotes express a large group of factors with a range of activities that contribute to the reorganization of chromatin to facilitate these processes. These include chromatin remodelers that use the energy of ATP hydrolysis to reposition or remove histones, posttranslational histone modifiers that covalently add chemical moieties (acetyl, methyl, phosphate, ubiquitin groups) to histone residues that alter their function, and histone chaperones that interact with histones to contribute to the disassembly and reassembly of nucleosomes [reviewed in Cairns (2009), Narlikar *et al.* (2002), and Smith and Shilatifard (2010)].

The highly conserved heterodimer FACT (Facilitates Chromatin Transactions) is a prominent member of the histone chaperone family with reported functions in multiple nuclear processes including DNA replication, DNA repair, transcription initiation, and transcription elongation [reviewed in Duina (2011), Formosa (2008, 2011), and...
Winkler and Luger (2011)). Its role in transcription elongation has been particularly well supported by both genetic and biochemical experiments involving yeast and mammalian systems (Formosa 2011). These include the sensitivity of yeast FACT mutants to the transcription elongation inhibitor 6-azauracil, the genetic interaction of these mutants with other known elongation factors, the colocalization of FACT with RNA Polymerase II (Pol II) across transcribed regions of eukaryotic genomes, the physical association of FACT with other transcription elongation factors, and the requirement of human FACT to allow RNA Pol II to transcribe a nucleosomal DNA template in vitro (Belotserkovskaya et al. 2003; Formosa et al. 2001, 2002; Krogan et al. 2002; Orphanides et al. 1998; Simic et al. 2003; Squazzo et al. 2002). Although the precise molecular functions of FACT in transcription elongation remain under investigation, several studies have strongly implicated FACT in facilitating the nucleosome dynamics that occur during transcription elongation. These studies suggest that FACT associates with a nucleosome in front of RNA Pol II resulting in the reorganization of histones that eventually lead to the displacement of H2A-H2B dimers and the passage of RNA Pol II (Belotserkovskaya et al. 2003; McCullough et al. 2011; Orphanides et al. 1998). Once RNA Pol II has passed, FACT is also required to assist in the reassembly of nucleosomes to protect recently transcribed DNA from spurious transcription from cryptic intragenic promoters (Belotserkovskaya et al. 2003; Formosa et al. 2002; Jamai et al. 2009; Orphanides et al. 1999; Schwabish and Struhl 2004; Stuwe et al. 2008; VanDemark et al. 2008).

Although a role for FACT in facilitating transcription-dependent nucleosome dynamics has been well documented, less is known concerning the precise contribution of the individual FACT subunits. Yeast FACT is composed of two proteins, Spt16 and Pob3, that are essential for viability and can bind nucleosomes in vitro when aided by a third protein, the HMG box-containing protein Nhp6 (Formosa et al. 2001; Wittmeyer and Formosa 1997). Pob3 consists of three separate domains defined by limited proteolysis: an N-terminal (NT/D) domain that is thought to be involved in dimerization with Spt16, a middle (M) domain that contains a double pleckstrin homology motif, and an acidic C-terminal (C) domain (Liu et al. 2010; VanDemark et al. 2006). The pleckstrin homology motif has been implicated in assisting the interactions between FACT and RPA, an essential protein involved in DNA replication and repair (VanDemark et al. 2006). Spt16 has been characterized as having four distinct domains, referred to as the N-terminal (NTD), dimerization (D), middle (M), and C-terminal (C) domains (VanDemark et al. 2006, 2008). Structures of Spt16-NTD, the one domain that is dispensable for viability, from both S. cerevisiae and S. pombe have recently been solved by X-ray crystallography, revealing a motif that is structurally similar to bacterial aminopeptidases (Stuwe et al. 2008; VanDemark et al. 2008). Although interactions between the Spt16-NTD and histones H2A, H3, and H4 have been reported, the fact that this domain is expendable for Spt16 functions in vivo suggests that there are likely to be other regions of Spt16 that functionally and physically interact with histones (O’Donnell et al. 2004; VanDemark et al. 2008). The Spt16-D domain is thought to interface with the NT/D domain of Pob3 to form the FACT dimer (VanDemark et al. 2006). Although molecular functions of the Spt16-M domain are not known, mutations altering residues within this domain have resulted in phenotypes indicative of transcription initiation and elongation defects, defects in replication, and defects in cell wall integrity, indicating the functional significance of this domain (Myers et al. 2011; O’Donnell et al. 2009; Stevens et al. 2011). Spt16-C is an acidic domain that is essential for viability whose most 3’ end has recently been shown to functionally interact with histone H3 (Belotserkovskaya et al. 2003; Evans et al. 1998). Recent in vitro analysis of the human Spt16-C domain has implicated this domain in the active displacement of nucleosomal DNA during nucleosome reorganization (Winkler et al. 2011).

We have recently provided evidence that FACT contributes to a new mechanism of gene regulation operating at the S. cerevisiae SER3 gene based on its ability to promote transcription-coupled nucleosome dynamics (Hainer et al. 2011; Martens et al. 2004). In the presence of serine, transcription of intergenic SRG1 DNA initiates S’ of the adjacent SER3 gene, which encodes an enzyme for serine biosynthesis (Martens et al. 2004, 2005). As a consequence of SRG1 transcription across the SER3 promoter, FACT assists in the assembly and maintenance of nucleosomes over this region that is otherwise depleted of nucleosomes (Hainer et al. 2011). The presence of these nucleosomes at the SER3 promoter inhibits the binding of transcription factors required to induce SER3 transcription. In this report, we present the results of an unbiased genetic screen to identify mutations of SPT16 that derepress SER3 transcription. Our analyses of these mutants indicate that the integrity of both the Spt16-D and Spt16-M domains not only are required for SRG1 transcription-dependent nucleosome assembly and SER3 repression but also are more broadly required for transcription-coupled nucleosome occupancy at highly transcribed genes. We provide evidence suggesting a possible role for the Spt16-D and Spt16-M domains in promoting the association of FACT to genes being actively transcribed.

MATERIALS AND METHODS

Strains and media

All S. cerevisiae strains used in this study (supporting information, Table S1) are isogenic to a GAL2+ derivative of S288c (Winston et al. 1995). All strains were constructed by transformation or by genetic crosses (Ausubel et al. 1991). YJ920 and YADP50 have been previously described (Hainer et al. 2011; Myers et al. 2011). Strains Y1089-Y1092 were derived from YJ920. The spt16Δ::KanMX and lys2-1286 alleles have been previously described (Clark-Adams et al. 1988; Myers et al. 2011). The ypt1Δ::SER3pr::HIS3 allele was generated by replacing the URA3 open reading frame (ORF) in ypt1Δ::SER3pr::URA3 (Hainer and Martens 2011) with a PCR product containing the HIS3 open reading frame that was amplified from pRS403 (Sikorowski and Hieter 1989). pAO01 and pSPT16-URA3 are centromeric plasmids marked with LEU2 and URA3, respectively, that contain wild-type SPT16 (kindly provided by A. Duina) (Myers et al. 2011). Derivatives of pAO01 containing spt16-G132D and spt16-T828I/859S alleles were generated by standard cloning methods and verified by sequencing. All other spt16 mutants characterized in this study are expressed from plasmids derived from pAO01. Yeast extract-peptone-dextrose (YPD), synthetic complete (SC), omission (SC-), 5-fluoroorotic acid (5-FOA), and galactose media have been previously described (Rose 1991). YPD was supplemented with 5 μg/mL cyclohexamide (CHX) or 200 mM hydroxyurea (HU) as indicated. 3-amino-1,2,4-triazole (3-AT; Sigma) was added to SC medium lacking leucine and histidine at the indicated concentrations.

Screen for spt16 mutants that derepress SER3

Using a previously described strategy (Myers et al. 2011), two regions of SPT16, from +764 to +2044 (region B) and from +1430 to +3521 (region C), were amplified from pAO01 plasmid (gift from A. Duina) using GoTaq polymerase (Invitrogen) and standard PCR conditions. Amplified DNA was cotransformed into YJ1089 with pAO01 plasmid that had been digested with either Eag1 and SnaB1 (region B) or...
SnaB1 and XbaI (region C). Transformants containing gap-repaired plasmids were selected on SC medium lacking leucine and then replica-plated onto medium containing 5-FOA to select for cells that lost the URA3-marked plasmid carrying a wild-type copy of SPT16 (pSPT16-URA3). The resulting colonies were then replica-plated to SC medium lacking histidine and leucine that was supplemented with 5 mM 3-AT. Candidate plasmids were recovered from strains resistant to 5 mM 3-AT, retransformed into Y1089, and restested for their ability to confer 3-AT resistance. The region of SPT16 that was subjected to PCR mutagenesis was first subcloned into a new copy of pAO01 before retransformation. For each plasmid that retested for 3-AT resistance, both strands of the entire SPT16 gene were sequenced and compared with the wild-type gene.

Northern analysis
Cells were grown to approximately 2 × 10⁷ cells/ml in YPD at 30°. Total RNA isolation and Northern analysis was performed as previously described (Collart and Oliviero 2001). Radiolabeled DNA probes to SRG1 (−454 to −123 relative to SER3 ATG), SER3 (+111 to +1342), and SCR1 (−163 to +284) were generated by random-primed labeling of PCR fragments amplified from genomic DNA. RNA levels were quantified using a PhosphorImager (FLA-5000) and ImageJ software.

Western analysis
Whole cell extracts (WCE) were prepared from cells grown in YPD at 30° to approximately 3 × 10⁷ cells/ml using trichloroacetic acid as previously described (Cox et al. 1997; Zheng et al. 2010). Equal amounts of WCE were separated by 10% acrylamide SDS-PAGE, transferred to Protein nitrocellulose (Whatman), and assayed by immunoblotting. The antibodies used to detect Spt16, Pob3, and G6PDH were as follows: anti-Spt16 (1:500; gift from T. Formosa), anti-Pob3 (1:2000; gift from T. Formosa), anti-G6PDH (1:50,000; Sigma). After incubation with HRP-conjugated IgG secondary antibody (1:5000; GE Healthcare) for 2 hr at 4°. Dilutions of input DNA and immunoprecipitated DNA were analyzed by qPCR reactions. Primer sets that amplify the following regions were used for qPCR: SER3-41 (−921 to −828, relative to +1 ATG of SER3); SER3-25 (−338 to −289, relative to +1 ATG of SER3); SER3-22 (−300 to −200, relative to +1 ATG of SER3); SER3-7 (+195 to +295); PFK1 (5’: +62 to +164, 3’: +1173 to +1279); PMI1 (5’: +691 to +794, 3’: +1689 to +1791); ADH1 (+845 to +943); CYC1 (+122 to +217); TUB2 (5’: +105 to +202, 3’: +1083 to +1189); and GALI (5’: +79 to +175, 3’: +1366 to +1487). Histone H3, Spt16, and Rpb3 ChIP signals for each gene were normalized to a No ORF control template, which is located within a region of chromosome V that lacks open reading frames (Komarnitsky et al. 2000).

Identification of spt16 mutants that derepress SER3
We recently described a new mechanism of gene regulation in Saccharomyces cerevisiae whereby transcription of SRG1 ncDNA assembles nucleosomes over the promoter of the adjacent SER3 gene to maintain SER3 repression (Hainer et al. 2011). Furthermore, we provided evidence that the histone chaperones, Spt16 and Spf6, are required to maintain this nucleosome occupancy, and repress SER3, likely through their ability to disassemble and reassemble nucleosomes during active transcription (Belotserkovskaya et al. 2003; Hainer et al. 2011). To investigate the role of Spt16 in this mechanism, we performed an unbiased genetic screen to identify novel mutations in SPT16 that derepress SER3 during SRG1 transcription. A PCR-based strategy that has been previously described [see Materials and Methods; Myers et al. (2011)] was used to target mutagenesis of the ‘3’ half of SPT16 that excludes most of the N-terminal domain (NTD), which is dispensable for SER3 repression (S. J. Hainer, unpublished data). These PCR fragments were cotransformed with a gapped LEU2-marked plasmid that contained homology to the PCR fragments into an spt16Δ his3A strain containing an integrated SOR3-pr-HIS3 reporter (Figure 1A) and expressing a wild-type copy of SPT16 from a URA3-marked plasmid (Y1089). Following gap-repair and loss of the URA3-marked plasmid expressing SPT16, we screened for spt16 mutants that derepress the SER3-pr-HIS3 reporter by their ability to confer growth in the presence of 3-AT, a competitive inhibitor of the HIS3 gene product (Figure 1).

With this screen, we initially identified 522 mutants that permit growth on medium containing 5 mM 3-AT. Forty SPT16-containing plasmids were then recovered from strains that conferred resistance up to 40 mM 3-AT to enrich for mutations that most strongly derepress SER3. The remaining 482 plasmids have not yet been examined. After retesting for their ability to derepress the SER3-pr-HIS3 reporter, the entire SPT16 gene contained on each of these plasmids was sequenced. Sequencing of the 38 plasmids that successfully
The N-terminal (NTD), dimerization (D), middle (M) domains, and the C-terminal acidic tail region (C). Note that the transcription defect caused by the insertion of a transposon has been previously reported (O’Donnell et al. 2001). Interestingly, one mutant, spt16-G132D, including 3 residues that are within 13 amino acids of each other (Figure 1B). Interestingly, only one of these mutations, spt16-E857K, has been previously reported (O’Donnell et al. 2009; Stevens et al. 2011). In these studies, spt16-E857K was isolated as a dominant suppressor of a transcription defect caused by the insertion of a δ element 5’ of the LYS2 and HIS4 genes (Spt’ phenotype) and was found to genetically interact with mutations in other transcription elongation factors.

**Phenotypic analysis of the spt16 mutants**

To further characterize these mutants, we tested the strains for temperature sensitivities and growth defects on YPD medium supplemented with cycloheximide (CHX), hydroxyurea (HU), mycophenolic acid (MPA), and caffeine. Surprisingly, we found that not one of the spt16 mutants that we isolated confers a growth defect at elevated temperatures (39°C) or in the presence of HU (Figure 2A), phenotypes that have been previously described for other spt16 alleles, including spt16-G132D (Figure 2A, row 2) and spt16-T828I/P859S (Figure 2A, row 3) (Formosa et al. 2001). Interestingly, one mutant, spt16-S715G/D718G, confers cold sensitivity at 15°C, and a number of the mutants cause varying sensitivities to CHX (Figure 2A). No detectable growth defects were observed when strains expressing any one of the isolated spt16 mutants were exposed to MPA or caffeine (S. B. Cohen, unpublished data).

We also tested whether the spt16 mutants that we isolated are dominant for repression of the SER3pr-HIS3 reporter. YJ1090 cells containing wild-type spt16 at its genomic location, SER3pr-HIS3, and a plasmid expressing either wild-type or mutant Spt16 protein, as indicated, were grown to saturation in YPD, diluted to 105 and then spotted in a 10-fold serial dilution series on SC-His-Leu (control) and SC-His-Leu + 10 mM 3-AT plates. Plates were incubated at 30°C for three days. Results were obtained for two independent growth assays in which each plate contained control strains and five to six mutants. Shown are representative dilutions for the control strains and each spt16 mutant strain. Each Spt16 mutant protein is named to describe the location and nature of the amino acid substitution. The locations of the amino acid substitutions in each of these mutants are also indicated (marked by stars) in diagrams of Spt16; gray ovals indicate

**spt16 mutants derepress endogenous SER3**

We next determined the effect of these spt16 mutants on endogenous SER3 and SPT16 RNA levels. We transformed plasmids containing either wild-type SPT16, a previously characterized spt16-G132D mutant (Malone et al. 1991), or one of our newly isolated spt16 mutants into YJ1091 and YJ1092 strains and performed Northern assays on these strains (Figure 3, A and B). For these and subsequent experiments, we limited our analysis to the 12 spt16 mutants having single amino acid substitutions. All of the spt16 mutants tested derepress
SER3 with effects ranging from very strong (30-fold increase for spt16-E857K) to milder (2-fold increase for spt16-Y297H, spt16-N580D, spt16-E671G, and spt16-S765P) that are similar to what we had previously observed for spt16-G132D (Hainer et al. 2011). Although we did observe some variability in SRG1 RNA levels between experiments, average results from four independent experiments indicate that these spt16 mutants do not significantly alter SRG1 RNA levels. Consistent with these Northern data, we found equivalent levels of RNA Pol II localized across the SRG1 transcription unit in strains expressing either wild-type or mutant versions of Spt16 (Figure 6B). Moreover, Western analyses showed that these newly isolated spt16 mutants do not alter the levels of Spt16 or its interacting partner, Pob3 (Figure 3, C and D). Taken together, these data identify amino acids in Spt16 that are critical for SER3 repression.

**Effect of spt16 mutants on nucleosome occupancy over the SER3 promoter**

To examine the effect of a subset of the spt16 mutants on nucleosome occupancy at SER3, we performed nucleosome-scanning assays on six of the single amino acid substitutions that most strongly derepress SER3 (Figure 4). As previously described (Hainer et al. 2011), MNase protection across SER3 was normalized to the protection of a well-studied, nucleosome-bound region of the GAL1 promoter whose digestion by MNase is unaffected by these spt16 mutants (S. J. Hainer, unpublished data; see Materials and Methods for details). Compared with strains containing wild-type control plasmids, protection from MNase digestion was reduced across the SRG1-transcribed region in all the spt16 mutants examined to degrees approximately equal to or exceeding that of spt16-G132D (Figure 4), which we had previously shown to decrease nucleosome occupancy across the SER3 locus (Hainer et al. 2011). MNase protection across the SER3 promoter region was most dramatically reduced in the spt16-E857K mutant (Figure 4H), which is consistent with the strong derepression of SER3 that is observed in this mutant. The other six mutants that displayed more modest defects in SER3 repression also had more modest reductions in the MNase protection across the SER3 promoter. However, we did observe subtle differences in the MNase protection patterns between these mutants. Two of the spt16 mutants resulted in greater sensitivity to MNase toward the 5′ of SRG1 relative to the 3′ of SRG1 (spt16-K579E and spt16-L669S), compared with the other mutants that had increases in MNase sensitivity that were more evenly distributed across the SRG1 transcription unit (Figure 4, compare −400 and −200 regions in panels D and F with panels E and G).

To confirm that the changes in MNase protection across the SRG1 transcription unit caused by these spt16 mutants reflect changes in nucleosome occupancy, we measured histone occupancy across this region by ChIP. For the most part, histone H3 occupancy across the SRG1 transcription unit was reduced in the spt16 mutants to degrees that correlate with the results of our MNase experiments (Figure 4I). Taken together, these data identify Spt16 residues whose integrity is required to maintain SER3 repression by facilitating SRG1 transcription-dependent nucleosome occupancy across the SER3 promoter.

**Effect of spt16 mutations on phenotypes associated with defects in transcription and chromatin structure**

Having shown a role for at least six of the spt16 single mutants in regulating chromatin structure at SER3, we tested whether all 12 single mutants confer other phenotypes indicative of chromatin-related transcriptional defects. We first determined whether these spt16 mutants can confer an Spt− phenotype (suppressor of Ty 8 element insertion),
which is caused by defects in chromatin and aberrant transcription initiation (Clark-Adams et al. 1988). *spt16Δ* strains containing the *lys2-128d* allele were transformed with plasmids containing either wild-type *SPT16* or mutant *spt16* alleles and assayed for their ability to grow on medium lacking lysine (Figure 5A). As a control, we also introduced a plasmid expressing the *spt16-G132D* allele, which has been previously shown to have an Spt2 phenotype (Evans et al. 1998).

Compared with the cells expressing wild-type *SPT16*, most of the *spt16* mutants grew robustly in the absence of lysine, similar to what was observed for the *spt16-G132D* control, indicating that these mutants confer a strong Spt- phenotype (Figure 5A). In contrast, the two *spt16* mutants that most weakly derepress *SER3*, *spt16-E671G* and *spt16-E679G*, had no detectable Spt- phenotype.

Next, we tested whether these *spt16* mutants permit the production of aberrant intragenic transcripts, a phenotype that has been associated with defects in transcription-coupled nucleosome reassembly (Carrozza et al. 2005; Kaplan et al. 2003). For these experiments, we employed a previously described GALpr-*FLO8-HIS3* reporter gene in which *HIS3* gene expression is dependent on transcription initiation from a cryptic promoter within the *FLO8* coding sequence (Cheung et al. 2008). Therefore, cryptic intragenic transcription can be measured by the growth of *his3Δ* cells containing this reporter construct on medium lacking histidine. For this assay, strains expressing plasmid-borne *SPT16* or the indicated *spt16* mutant alleles were monitored for growth on medium lacking histidine. When grown in galactose-containing medium, all but two of the *spt16* mutants allowed cells to grow in the absence of histidine, indicative of robust transcription initiation from the cryptic promoter within the *FLO8* coding sequence (Figure 5B). For the most part, these data correlate well with the Spt- phenotypic data, suggesting that the molecular defects...
resulting in these two phenotypes are likely related. Interestingly, only those mutations within the N-terminal domain of Spt16 allowed cells to grow in glucose-containing medium lacking histidine, suggesting that these mutants permit cryptic transcription initiation even in the absence of significant levels of transcription across this region.

Finally, we tested the spt16 mutants for their ability to suppress a cold-sensitive (cs) phenotype of a histone mutant, H3 L61W, a phenotype that has been previously described for a distinct class of mutations located within the Spt16 M-domain (Myers et al. 2011). For this assay, spt16Δ cells containing the H3 L61W mutant as the sole source

Figure 4 Effect of spt16 mutants on chromatin structure at SER3. (A) Diagram of the SER3 locus. The gray ovals mark the position of nucleosomes when wild-type cells are grown in SER3-repressing conditions (YPD). The block arrow indicates SRG1 transcription. (B–H) Nucleosome scanning assays were performed on spt16Δ cells (YJ1091 and YJ1092) carrying plasmids expressing either wild-type SPT16 or mutant spt16 alleles as indicated. Mononucleosome-sized DNA fragments were generated by micrococcal nuclease (MNase) digestion of formaldehyde-treated chromatin that was isolated from cells grown to \(\sim 2 \times 10^7\) cells/mL in YPD media at 30°C. MNase protection across the SER3 locus relative to a positioned nucleosome within the GAL1 promoter was determined by qPCR. For each PCR amplicon, the mean MNase protection \(\pm\) SEM from three independent experiments is plotted at its midpoint. Shown below each graph is a diagram of the SER3 locus indicating the positions of nucleosomes (gray ovals) extrapolated from the MNase protection data for each spt16 mutant. The light-gray ovals are indicative of less dramatic reductions in MNase protections compared with the wild-type control shown in panel A. (I) Histone H3 ChIP was performed on chromatin isolated from the same strains used in panels B–H. The amount of immunoprecipitated DNA was determined by qPCR as a fraction of the input that was then normalized to a control region in chromosome V and made relative to strains expressing wild-type SPT16 (arbitrarily set to 1). Each bar represents the mean \(\pm\) SEM of three independent experiments. Below the graph is a schematic of SER3 with black bars corresponding to the regions amplified by qPCR.
of histone H3 (YADP50) and plasmid-borne copies of wild-type or mutant versions of *SPT16* were monitored for growth on YPD at 15°C (Figure 5C). When compared with the *spt16-E735G* control (kindly provided by A. Duina), none of our identified *spt16* mutants suppressed the cold sensitivity of H3 L61W. Therefore, the *spt16* mutants we isolated as being defective for *SER3* repression represent a distinct class of mutants from those that suppress the cs phenotype of the H3 L61W mutant and may define functionally distinct regions of the *Spt16*-D and *Spt16*-M domains.

**Occupancy of mutant versions of Spt16 is reduced across *SRG1* and the *SER3* promoter region**

We next considered the possibility that these mutant versions of Spt16 fail to be recruited normally to transcribed regions, which may account for their multiple phenotypes related to defects in transcription-coupled nucleosome occupancy. Therefore, we performed ChIP experiments to assess the binding of selected *Spt16* mutant proteins across the *SRG1* transcription unit (Figure 6A). In general, we detected reduced binding of most of the mutant versions of *Spt16* that parallels the loss of histone H3 occupancy across this region that we observed in these mutant versions (compare Figure 6A with Figure 4I). The lone exception is the *spt16-K579E* mutant where we detected a stronger decrease in the occupancy of the mutant protein expressed from this allele than expected based on a relatively modest decrease in histone H3 occupancy. Because Spt16 strongly colocalizes with RNA Pol II across transcribed genes, we tested whether the decrease in the occupancy of the mutant versions of Spt16 might be indirect due to a decrease in RNA Pol II occupancy at *SER3*. To this end, we performed ChIP analysis of Rpb3, a subunit of RNA Pol II, over *SRG1* (Figure 6B). Consistent with our Northern analysis (Figure 3), we found that all but one of these *spt16* mutants did not cause a decrease in RNA Pol II occupancy compared with cells expressing wild-type *SPT16*. Interestingly, the *spt16-L669S* mutant did cause a slight but significant decrease (*P < 0.05*) in Rpb3 binding across *SRG1*. However, by normalizing the binding of this mutant version of Spt16 to Rpb3 binding, it is clear that this minor decrease in Rpb3 binding alone cannot account for the reduced binding of this mutant version of *Spt16* across *SRG1* (Figure 6C). Taken together, these data indicate that the amino acids defined by these mutants are required to maintain Spt16 colocalization with RNA Pol II across *SRG1*.

**Effect of *spt16* mutants on histone H3, Spt16, and RNA Pol II occupancy at other genes**

To investigate whether the *spt16* mutants that reduce nucleosome occupancy across *SRG1* have a general effect in transcription-coupled nucleosome occupancy, we measured histone H3 occupancy across the coding sequences of a subset of yeast genes by ChIP (Figure 7A). At three highly transcribed genes, *PMA1* (100 mRNA/hr), *PTK1* (95 mRNA/hr), and *ADH1* (125 mRNA/hr) (Holstege et al. 1998), histone H3 levels were reduced in all of the mutants to a similar extent as we observed across *SRG1*. Conversely, histone H3 occupancy at three lowly transcribed genes, *GALI* (repressed), *TUB2* (12 mRNA/hr), and *CYC1* (10 mRNA/hr) (Holstege et al. 1998), was unaffected in the mutants.

We next examined the occupancy of these mutant derivatives of Spt16 across the coding sequence of this subset of yeast genes (Figure 7B). Consistent with our results at *SRG1*, we found that at the highly transcribed genes, *PMA1*, *PTK1*, and *ADH1*, the binding of the mutant *Spt16* proteins were generally reduced in accordance with the decrease in histone H3 levels across these regions. Interestingly, the decrease in the occupancy of these mutant *Spt16* proteins was greater at the 5’ end of these genes compared with regions toward the 3’ end. For the most part, these changes in Spt16 binding occurred in the absence of any change in RNA Pol II binding to these regions (Figure 7C). Interestingly, a small but significant decrease in Rpb3 levels in the *spt16-L669S* mutant (*P < 0.05*) was detected at these highly transcribed genes, comparable to what we observed at *SRG1*. In contrast to what we observed at highly transcribed genes, occupancy of the mutant *Spt16* proteins and Rpb3 at three lowly transcribed genes, *GALI*, *TUB2*, and *CYC1*, were largely unaffected. Importantly, we
show that when these mutants are grown in galactose-containing medium to induce high levels of GAL1 expression, we detected reduced occupancy of both H3 and the mutant Spt16 proteins to the GAL1 coding sequence similar to what we observed for SRG1 and other highly transcribed genes (Figure S1). Thus, we have identified mutant spt16 alleles that cause reduced occupancy of both the mutant version of Spt16 encoded by these alleles and histones specifically over highly transcribed regions of the genome. Taken together, our studies suggest that the integrity of the Spt16-D and Spt16-M domains is generally required to maintain nucleosome occupancy at highly transcribed genes, possibly by facilitating Spt16 recruitment to those genes.

**DISCUSSION**

Spt16 is an essential, highly conserved component of the FACT elongation complex with a dual role in transcription elongation—the disassembly of nucleosomes to allow the passage of RNA Pol II and their reassembly in the wake of RNA Pol II [reviewed in Duina (2011), Formosa (2011), Reinberg and Sims (2006), and Winkler and Luger (2011)]. In this work, we provide evidence indicating that the integrity of both the Spt16-D and Spt16-M domains are required to support the histone chaperone activities of Spt16 during transcription elongation. We utilized a previously characterized system in which this activity of Spt16 is required for SRG1 transcription—dependent repression of the S. cerevisiae SER3 gene (Hainer et al. 2011) to identify a largely novel class of mutations in SPT16 that derepress SER3. Six mutations that most strongly derepress SER3 contain single amino acid substitutions in either the Spt16-D or Spt16-M domain. For this subset of mutants, SRG1 transcription—coupled nucleosome occupancy over the SER3 promoter is reduced to degrees that generally correlate with SER3 derepression. Moreover, we provide evidence that these mutations broadly disrupt transcription-coupled nucleosome occupancy at highly transcribed regions of the yeast genome. Finally, we show that while these mutant versions of Spt16 are expressed at wild-type levels, their association with highly transcribed genes is significantly reduced. These data suggest that the integrity of the Spt16-D and Spt16-M domains are required for transcription-coupled nucleosome occupancy, possibly by promoting or maintaining FACT association with transcribed regions of the genome.

With one exception (spt16-E857K), the spt16 mutants that we identified in this work are distinct from those that have been previously identified by other genetic approaches (Formosa et al. 2002; Malone et al. 1991; Myers et al. 2011; O’Donnell et al. 2009; Stevens et al. 2011). Although most of this new class of spt16 mutants confers an Spt phenotype similar to many previously characterized spt16 mutants, additional phenotypic studies indicate that there are important functional differences between these mutants. First, these mutants do not confer lethality at elevated temperature as is common for many previously characterized spt16 mutant alleles (Formosa et al. 2002; Myers et al. 2011; O’Donnell et al. 2009). This result suggests that the amino acid substitutions caused by these mutations are not likely to affect the general stability of the Spt16 protein. Furthermore, these results indicate that the ability of Spt16 to promote nucleosome assembly during transcription is not essential for viability. Second, these mutants do not confer a growth defect in the presence of hydroxyurea, a phenotype conferred by other spt16 mutants (Formosa et al. 2002; Myers et al. 2011; O’Donnell et al. 2009) that is indicative of a defect in DNA replication and/or DNA repair (Hampey 1997). Therefore, this new group of spt16 mutants may define an activity for Spt16 that is specific to its role in transcription elongation rather than a histone chaperone activity that may be generally required for all of Spt16 functions. Third, these spt16 mutants do not suppress a cold-sensitive growth defect conferred by a histone H3 L61W as has been recently described for a distinct set of spt16 mutant alleles (Myers et al. 2011). This is somewhat surprising given that both groups of spt16 mutants have amino acid substitutions within the Spt16-M domain. Moreover,
Figure 7 Relative occupancy of histone H3, Spt16, and Rpb3 across the coding regions of a subset of yeast genes. Histone H3 (A), Spt16 (B), and Rpb3 (C) was measured by ChIP within the coding region of three highly transcribed genes: PMA1, PYK1, and ADH1 (top panels in A, B, and C) and over three lowly transcribed genes: GAL1, TUB2, and CYC1 (bottom panels in A, B, and C) as described in Figure 6. The regions assayed by qPCR are marked with the black bars in the diagram provided for each gene. All values represent the mean ± SEM of three independent experiments.
one of the spt16 mutants isolated as a suppressor of the cold sensitivity of the histone H3 L61W mutant contains a glutamine substitution of glutamic acid residue at position 847, the same residue that, when substituted for a lysine, confers strong SER3 derepression and transcription-coupled nucleosome assembly defects. However, the lysine substitution did not suppress the cold sensitivity of the H3 L61W mutation. Taken together, these data show that we have identified a new class of spt16 mutants that interferes with Spt16 activity specific to its role in transcription-coupled nucleosome assembly rather than its generally required functions in transcription, cell viability, and/or DNA replication/DNA repair.

During our phenotypic analyses, we found that most of the spt16 mutants that were isolated based on their ability to derepress SER3 also confer sensitivity to cyclohexamide, a phenotype that has not been previously described for spt16 mutant alleles. Cyclohexamide is a potent inhibitor of eukaryotic protein synthesis that is normally toxic to yeast cells (Mccusker and Haber 1988). However, at low doses, a sensitivity to this drug has been shown to reveal mutations that reduce protein synthesis or impair cell-cycle progression (Hampey 1997). Therefore, although the identification of this phenotype may be interesting, the interpretation of the data are unclear. We hypothesize that the subset of spt16 mutants causing cyclohexamide sensitivity do so as a result of the misregulation of one or more genes encoding proteins that are essential for viability, regulate protein synthesis, or regulate intracellular levels of cyclohexamide.

Interestingly, the spt16-E857K allele, which we found to confer a dominant negative effect on SER3 repression, was previously isolated as a dominant suppressor of the transcription defects of δ element insertions just 5′ of both the LYS2 and HIS4 genes (O’Donnell et al. 2009; Stevens et al. 2011). This is not surprising given the striking similarities between SER3 repression by SRG1 transcription and LYS2 and HIS4 repression by the δ element insertions (Clark-Adams and Winston 1987; Martens et al. 2004; Winston et al. 1984). Both SRG1 and the δ element insertion promote transcription across the promoters of their adjacent genes, SER3 and either LYS2 or HIS4, respectively. Our finding that SER3 derepression in the spt16-E857K mutant is the result of reduced SRG1 transcription–dependent nucleosome assembly at the SER3 promoter suggests that a similar transcription defect in nucleosome occupancy may play a role in alleviating repression of LYS2 and HIS4 caused by these δ element insertions. Interestingly, we found that whereas three of the five double mutants containing the E857K substitution also act in a dominant manner, two of these combinations, spt16-I626T/E857K and spt16-T651A/H741Y/E857K, do not. Moreover, we found that the level of SER3 derepression in these two mutant alleles to be significantly lower to that caused by the E857K substitution alone (B. A. Charsar, unpublished data). Therefore, I626T and one or both of T651A and H741Y substitutions appear to suppress the negative effects of the E857K substitution.

Our analysis of the single amino acid substitutions in the Spt16-D and Spt16-M domains revealed a strong correlation between defective transcription-dependent nucleosome assembly and reduced association of these mutant versions of Spt16 at highly transcribed regions of the yeast genome. Several possible models could account for these observations. First, these mutant versions of Spt16 may interfere with the normal recruitment of FACT to transcribed DNA. In this model, the reduced recruitment of FACT would be the cause of the defect in transcription-coupled nucleosome assembly. Although several studies have determined that FACT physically associates with DNA that is being transcribed (Duina et al. 2007; Kim et al. 2004; Mason and Struhl 2003; Mayer et al. 2010), the molecular mechanism of this association is not known. Previous studies have implicated a number of factors that may facilitate Spt16 association with transcribed DNA, including the Chd1 chromatin-remodeling factor, the Paf1 elongation complex, RNA Pol II, and histone proteins (Adelman et al. 2006; Biswas et al. 2007; Formosa et al. 2001; Mason and Struhl 2003; Prunessi et al. 2011; Simic et al. 2003; Winkler et al. 2011). It is conceivable that the amino acid substitutions within the Spt16-D and Spt16-M domains that interfere with transcription-coupled nucleosome assembly do so by altering FACT interactions with one or more of these factors. Second, the reduction in Spt16 association with transcribed regions may be a consequence of the reduced nucleosome occupancy due to a defect in transcription-coupled nucleosome assembly. In this model, the amino acid substitutions in the Spt16-D and Spt16-M domains would not alter initial Spt16 recruitment to transcribed DNA or its ability to associate with nucleosomal DNA but, rather, interfere with its nucleosome remodeling activity that leads to disassembly and/or reassembly of nucleosomes during transcription. Additional molecular and biochemical experiments to investigate the effect of these mutants on FACT interactions with other proteins and the nucleosome remodeling activity of Spt16 will be necessary to distinguish between these models.

Although the possibility that the Spt16-D and Spt16-M domains may directly mediate protein-protein interactions or FACT nucleosome remodeling activity is intriguing, we cannot rule out a more indirect role for these domains. For example, it is possible that the three mutations in the Spt16-D domain may simply disrupt the Spt16-Pob3 interface (VanDemark et al. 2006, 2008). However, if this were the case, we would expect any changes in the Spt16-Pob3 dimer interface to be subtle, specifically affecting the activity of FACT in transcription-dependent nucleosome assembly rather than in a more general histone chaperone role. Large perturbations in the Spt6-Pob3 interaction would most likely lead to more broad defects in cell growth and DNA replication/repair, which were not detected in these mutants by our phenotypic assays.

In summary, we have identified a novel class of spt16 mutants that specifically impairs transcription-coupled nucleosome occupancy across highly transcribed regions of the S. cerevisiae genome and results in reduced association of the mutant Spt16 proteins to these regions. These mutants are likely to be useful molecular tools to further elucidate the dynamic function of Spt16 in maintaining chromatin architecture during transcription.

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LITERATURE CITED
Adelman, K., W. Wei, M. B. Ardehali, J. Werner, R. Zhu et al., 2006 Drosophila PaF1 modulates chromatin structure at actively transcribed genes. Mol. Cell. Biol. 26: 250–260.
Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman et al. (Editors), 1991 Current Protocols in Molecular Biology. John Wiley and Sons, New York.
Bai, L., and A. V. Morozov, 2010 Gene regulation by nucleosome positioning. Trends Genet. 26: 476–483.
Belotserkovskaya, R., S. Oh, V. A. Bondarenko, G. Orphanides, V. M. Studitsky et al., 2003 FACT facilitates transcription-dependent nucleosome alteration. Science 301: 1090–1093.
Biswas, D., R. Dutta-Biswas, and D. J. Stillman, 2007 Chd1 and yFACT act in opposition in regulating transcription. Mol. Cell. Biol. 27: 6279–6287.
Brickner, D. G., I. Cajigas, Y. Fondufe-Mittendorf, S. Ahmed, P. C. Lee et al., 2007 H2A.Z-mediated localization of genes at the nuclear periphery confers epigenetic memory of previous transcriptional state. PLoS Biol. 5: e81.
Cairns, B. R., 2009 The logic of chromatin architecture and remodelling at promoters. Nature 461: 193–198.
Carrozza, M. J., B. Li, L. Florens, T. Suganuma, S. K. Swanson et al., 2005 Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. Cell 123: 581–592.
Cheung, V., G. Chua, N. N. Batada, C. R. Landry, S. W. Michnick et al., 2008 Chromatin- and transcription-related factors repress transcription from within coding regions throughout the Saccharomyces cerevisiae genome. PLoS Biol. 6: e277.
Clark-Adams, C. D., and F. Winston, 1987 The SPT6 gene is essential for growth and is required for delta-mediated transcription in Saccharomyces cerevisiae. Mol. Cell. Biol. 7: 679–686.
Clark-Adams, C. D., D. Norris, M. A. Osley, J. S. Fassler, and F. Winston, 1988 Changes in histone gene dosage alter transcription in yeast. Genes Dev. 2: 150–159.
Collart, M. A., and S. Oliviero, 2001 Preparation of yeast RNA.Curr. Protoc. Mol. Biol. Chapter 13: Unit 13.12.
Cox, J. S., R. E. Chapman, and P. Walter, 1997 The unfolded protein response coordinates the production of endoplasmic reticulum protein and endoplasmic reticulum membrane. Mol. Biol. Cell 8: 1805–1814.
Duina, A. A., 2011 Histone chaperones Spt16 and FACT: similarities and differences in modes of action at transcribed genes. Genet. Res. Int. 2011: Article ID 625201.
Duina, A. A., A. Rufiange, J. Bracey, J. Hall, A. Nourani et al., 2007 Evidence that the localization of the elongation factor Spt16 across transcribed genes is dependent upon histone H3 integrity in Saccharomyces cerevisiae. Genetics 177: 101–112.
Evans, D. R., N. K. Brewster, Q. Xu, A. Rowley, B. A. Altheim et al., 1998 The yeast protein complex containing cdc68 and p0b3 mediates core-promoter repression through the cdc68 N-terminal domain. Genetics 150: 1393–1405.
Floer, M., X. Wang, V. Prabhu, G. Berrozo, S. Narayan et al., 2010 A RSC/nucleosome complex determines chromatin architecture and facilitates activator binding. Cell 141: 407–418.
Fornos, T., 2008 FACT and the reorganized nucleosome. Mol. Biosyst. 4: 1085–1093.
Fornos, T., 2011 The role of FACT in making and breaking nucleosomes. Biochem. Biophys. Acta. 1819: 247–255.
Fornos, T., P. Eriksson, J. Wittmeyer, J. Ginn, Y. Yu et al., 2001 Spt16-P0b3 and the HMG protein Nhp6 combine to form the nucleosome-binding factor SPN. EMBO J. 20: 3506–3517.
Fornos, T., S. Ruone, M. D. Adams, A. E. Olsen, P. Eriksson et al., 2002 Defects in Spt16 or P0b3 (γFACT) in Saccharomyces cerevisiae cause dependence on the Hir/Hpc pathway: polymerase passage may degrade chromatin structure. Genetics 162: 1557–1571.
Hainer, S. J., and J. A. Martens, 2011 Identification of histone mutants that are defective for transcription-coupled nucleosome occupancy. Mol. Cell. Biol. 31: 3557–3568.
Hainer, S. J., J. A. Prunescu, R. D. Mitchell, R. M. Monteverde, and J. A. Martens, 2011 Intergenic transcription causes repression by directing nucleosome assembly. Genes Dev. 25: 29–40.
Hampsey, M., 1997 A review of phenotypes in Saccharomyces cerevisiae. Yeast 13: 1099–1133.
Holsteg, F. C., E. G. Jennings, J. J. Wyrick, T. I. Lee, C. J. Hengartner et al., 1998 Dissecting the regulatory circuitry of a eukaryotic genome. Cell 95: 717–728.
Jami, A., A. Puglisi, and M. Strubin, 2009 Histone chaperone Spt16 promotes repositioning of the original h3-h4 histones evicted by elongating RNA polymerase. Mol. Cell 35: 377–383.
Kaplan, C. D., L. Laprade, and F. Winston, 2003 Transcription factors repress transcription initiation from cryptic sites. Science 301: 1096–1099.
Kim, M., S. H. Ahn, N. J. Krogan, J. F. Greenblatt, and S. Buratowski, 2004 Transitions in RNA polymerase II elongation complexes at the 3’ ends of genes. EMBO J. 23: 354–364.
Komaritsky, P., E. J. Cho, and S. Buratowski, 2000 Different phosphorylated forms of RNA polymerase II and associated mRNA processing factors during transcription. Genes Dev. 14: 2452–2460.
Krogan, N. J., M. Kim, S. H. Ahn, G. Zhong, M. S. Kohor et al., 2002 RNA polymerase II elongation factors of Saccharomyces cerevisiae: a targeted proteomics approach. Mol. Cell. Biol. 22: 6979–6992.
Li, B., M. Carey, and J. L. Workman, 2007 The role of chromatin during transcription. Cell 128: 707–719.
Liu, Y., H. Huang, B. O. Zhou, S. S. Wang, Y. Hu et al., 2010 Structural analysis of Rtt106p reveals a DNA binding role required for heterochromatin silencing. J. Biol. Chem. 285: 4251–4262.
Luger, K., 2006 Dynamic nucleosomes. Chromosome Res. 14: 5–16.
Luger, K., A. W. Mader, R. K. Richmond, D. F. Sargent, and T. J. Richmond, 1997 Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature 389: 251–260.
Malone, E. A., C. D. Clark, A. Chiang, and F. Winston, 1991 Mutations in Spt16/Cdc68 suppress cis- and trans-acting mutations that affect promoter function in Saccharomyces cerevisiae. Mol. Cell. Biol. 11: 5710–5717.
Martens, J. A., L. Laprade, and F. Winston, 2004 Intergenic transcription is required to repress the Saccharomyces cerevisiae SER3 gene. Nature 429: 571–574.
Martens, J. A., P. Y. Wu, and F. Winston, 2005 Regulation of an intergenic transcript controls adjacent gene transcription in Saccharomyces cerevisiae. Genes Dev. 19: 2695–2704.
Mason, P. B., and K. Struhl, 2003 The FACT complex travels with elongating RNA polymerase II and is important for the fidelity of transcriptional initiation in vivo. Mol. Cell. Biol. 23: 8323–8333.
Mayer, A., M. Lidschreiber, M. Siebert, K. Leike, J. Sodding et al., 2010 Uniform transitions of the general RNA polymerase II transcription complex. Nat. Struct. Mol. Biol. 17: 1272–1278.
McCullough, L., R. Rawlins, A. Olsen, H. Xin, D. J. Stillman et al., 2011 Insight into the mechanism of nucleosome reorganization from histone mutants that suppress defects in the FACT histone chaperone. Genetics 188: 835–846.
McCutcher, J. H., and E. J. Haber, 1988 Cycloheximide-resistant temperature-sensitive lethal mutations of Saccharomyces cerevisiae. Genetics 119: 303–315.
Myers, C. N., G. B. Berner, J. H. Hlothhoff, K. Martinez-Fonts, J. A. Harper et al., 2011 Mutant versions of the S. cerevisiae transcription elongation factor Spt16 define regions of Spt16 that functionally interact with histone H3. PLoS ONE 6: e20847.
Narlikar, G. J., H. Y. Fan, and R. E. Kingston, 2002 Cooperation between complexes that regulate chromatin structure and transcription. Cell 108: 475–487.
O’Donnell, A. F., N. K. Brewster, J. Kurniawan, L. V. Minard, G. C. Johnston et al., 2004 Domain organization of the yeast histone chaperone FACT: the conserved N-terminal domain of FACT subunit Spt16 mediates recovery from replication stress. Nucleic Acids Res. 32: 5984–5906.
O’Donnell, A. F., J. R. Stevens, R. Keplay, C. A. Barnes, G. C. Johnston et al., 2009 New mutant versions of yeast FACT subunit Spt16 affect cell integrity. Mol. Gen. Genomics 282: 487–510.

Orphanides, G., G. LeRoy, C. H. Chang, D. S. Luse, and D. Reinberg, 1998 FACT, a factor that facilitates transcript elongation through nucleosomes. Cell 92: 105–116.

Orphanides, G., W. H. Wu, W. S. Lane, M. Hampsey, and D. Reinberg, 1999 The chromatin-specific transcription elongation factor FACT comprises human SPT16 and SSRP1 proteins. Nature 400: 284–288.

Paff, M. W., 2001 A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29: e45.

Prunesi, J. A., S. J. Hainer, K. O. Petrov, and J. A. Martens, 2011 The Paf1 complex represses SER3 transcription in Saccharomyces cerevisiae by facilitating intergenic transcription-dependent nucleosome occupancy of the SER3 promoter. Eukaryot. Cell 10: 1283–1294.

Reinberg, D., and R. J. Sims 3rd, 2006 de FACTo nucleosome dynamics. Proc. Natl. Acad. Sci. USA 105: 8884–8889.

Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast laboratory strains useful for studies on the budding yeast Saccharomyces cerevisiae. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schwabish, M. A., and K. Struhl, 2004 Evidence for eviction and rapid deposition of histones upon transcriptional elongation by RNA polymerase II. Mol. Cell. Biol. 24: 10111–10117.

Shira, M. K., S. E. Rogers, D. E. Alexander, and K. M. Arndt, 2005 The Snf1 protein kinase and Sit4 protein phosphatase have opposing functions in regulating TATA-binding protein association with the Saccharomyces cerevisiae INO1 promoter. Genetics 169: 1957–1972.

Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics 122: 19–27.

Simic, R., D. L. Lindstrom, H. G. Tran, K. L. Roinick, P. J. Costa et al., 2003 Chromatin remodeling protein Chd1 interacts with transcription elongation factors and localizes to transcribed genes. EMBO J. 22: 1846–1856.

Smith, E., and A. Shilatifard, 2010 The chromatin signaling pathway: diverse mechanisms of recruitment of histone-modifying enzymes and varied biological outcomes. Mol. Cell 40: 689–701.

Squazzo, S. L., P. J. Costa, D. L. Lindstrom, K. E. Kumer, R. Simic et al., 2002 The Paf1 complex physically and functionally associates with transcription elongation factors in vivo. EMBO J. 21: 1764–1774.

Stevens, J. R., A. F. O’Donnell, T. E. Perry, J. J. Benjamin, C. A. Barnes et al., 2011 FACT, the Bur kinase pathway, and the histone co-repressor HirC have overlapping nucleosome-related roles in yeast transcription elongation. PLoS ONE 6: e25644.

VanDemark, A. P., M. Blanksma, E. Ferris, A. Heroux, C. P. Hill et al., 2006 The structure of the yFACT Pob3-M domain, its interaction with the DNA replication factor RPA, and a potential role in nucleosome deposition. Mol. Cell 22: 363–374.

Van Demark, A. P., H. Xin, L. McCullough, R. Rawlins, S. Bentley et al., 2008 Structural and functional analysis of the Spt16p N-terminal domain reveals overlapping roles of yFACT subunits. J. Biol. Chem. 283: 5058–5068.

Winkler, D. D., and K. Luger, 2011 The histone chaperone FACT: structural insights and mechanisms for nucleosome reorganization. J. Biol. Chem. 286: 18369–18374.

Winkler, D. D., U. M. Muthurajan, A. R. Hieb, and K. Luger, 2011 Histone chaperone FACT coordinates nucleosome interaction through multiple synergistic binding events. J. Biol. Chem. 286: 41883–41892.

Winston, F., D. T. Chaleff, B. Valent, and G. R. Fink, 1984 Mutations in regulating TATA-binding protein association with the Saccharomyces cerevisiae HIS4 gene. Genetics 107: 179–197.

Winston, F., C. Dollard, and S. L. Ricupero-Hovasse, 1995 Construction of a set of convenient Saccharomyces cerevisiae strains that are isogenic to S288C. Yeast 11: 53–55.

Wittmeyer, J., and T. Formosa, 1997 The Saccharomyces cerevisiae DNA polymerase alpha catalytic subunit interacts with Cdc68/Spt16 and with Pob3, a protein similar to an HMG1-like protein. Mol. Cell. Biol. 17: 4178–4189.

Zheng, S., J. J. Wyrick, and J. C. Reese, 2010 Novel trans-tail regulation of H2B ubiquitylation and H3K4 methylation by the N terminus of histone H2A. Mol. Cell. Biol. 30: 3635–3645.

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