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Evidence that dissociation of Spt16 from transcribed genes is partially dependent on RNA Polymerase II termination

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ABSTRACT

FACT (FAcilitates Chromatin Transactions) is a highly conserved histone chaperone complex in eukaryotic cells that can interact and manipulate nucleosomes in order to promote a variety of DNA-based processes and to maintain the integrity of chromatin throughout the genome. Whereas key features of the physical interactions that occur between FACT and nucleosomes *in vitro* have been elucidated in recent years, less is known regarding FACT functional dynamics *in vivo*. Using the *Saccharomyces cerevisiae* system, we now provide evidence that at least at some genes dissociation of the FACT subunit Spt16 from their 3′ ends is partially dependent on RNA Polymerase II (Pol II) termination. Combined with other studies, our results are consistent with a two-phase mechanism for FACT dissociation from genes, one that occurs upstream from Pol II dissociation and is Pol II termination-independent and the other that occurs further downstream and is dependent on Pol II termination.

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Spt16; FACT complex; nucleosomes; Pol II; transcription termination

Introduction

In eukaryotic cells, the process of gene transcription takes place in the context of chromatin, a DNA-protein complex that includes the nucleosome as its fundamental unit. Typical nucleosomes are composed of ~147 base pairs of DNA wrapped around a histone octamer containing a histone H3-H4 tetramer and two histone H2A-H2B dimers and generally have repressive roles during transcription [1]. Several mechanisms have evolved that enable cells to manipulate nucleosomes in various ways to achieve fine-tuned levels of gene expression throughout the genome. These include ATP-driven changes in nucleosome occupancy and composition, alterations in the types and location of histone post-translational modifications, and ATP-independent mechanisms that promote nucleosome assembly and disassembly (for some recent reviews, see [2–8]). These latter processes are carried out by a group of factors known as histone chaperones, with the highly conserved FACT (FAcilitates Chromatin Transactions) complex being among the more thoroughly investigated ones to date [9–11].

Mammalian FACT, a heterodimeric complex composed of hSpt16 and SSRP1, was originally identified as a factor required for efficient transcription elongation through chromatinized templates *in vitro* [12,13]. Additional studies showed that FACT can also regulate the initiation phase of transcription and is also involved in other chromatin-based processes including DNA replication and repair [10,14]. During transcription elongation, FACT is thought to travel across genes in conjunction with RNA polymerase II (Pol II) and to promote both the disassembly of nucleosomes ahead of Pol II passage and the reassembly of nucleosomes behind the polymerase [10,11,15–18]. Whereas both the nucleosome disassembly and reassembly functions of FACT have been well-documented *in vitro*, it has recently been proposed that FACT's function in maintaining genome integrity through its nucleosome-reassembly activity may be its most critical role in the *in vivo* setting [9].

A variety of elegant biochemical studies in recent years have provided a wealth of information on the nature of FACT-nucleosomes interactions *in vitro*. These studies have led to a model in which various regions of FACT interact with both DNA and histones in coordinated and synergistic ways to facilitate both nucleosome disassembly to allow for DNA access to the relevant enzyme (Pol II in the case of

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transcription) and nucleosome reassembly once the task at hand is completed [19–27]. This model posits that FACT reassembles nucleosomes using the same histones that had been present at that location prior to FACT's arrival, thus maintaining the epigenetic state in that genomic region intact – a notion also consistent with *in vivo* experiments in yeast [28].

The model for FACT-nucleosome interactions derived from *in vitro* studies is likely descriptive of FACT's action on nucleosomes during transcription *in vivo*, but does not provide immediate insight into the mechanisms that govern FACT recruitment to genes nor its dissociation from genes once the transcription process is complete. Experiments carried out in various model systems have provided evidence that a number of protein factors and histone modifications are directly or indirectly involved in FACT recruitment to transcribed genes – these include Pol II itself, the chromatin remodelers Chd1 and Fun30, the mRNA capping enzyme Cet1, heterochromatin protein 1 (HP1), the protein complexes Paf1C and NuA3, acetylated histone H3 tails, and the histone modifications H3-K36me3, H3-K4me3 and H2B-K123Ub ([29–32] and reviewed in [11]). The nucleosome acidic patch has also been shown to play roles in promoting FACT association with genes [33–35] and other recent studies have provided compelling evidence that nucleosomes that have been disrupted through interactions with transcribing Pol II can also promote FACT localization over transcribed genes [36].

The mechanisms that regulate FACT dissociation from genes upon transcription termination, on the other hand, are much less well understood. Our laboratory has been using the budding yeast *Saccharomyces cerevisiae* model system to gain insights into this question. Similarly to mammalian FACT, yeast FACT (yFACT) is also a heterodimeric complex, with Spt16 and Pob3 being homologous to hSpt16 and SSRP1, respectively [37–39]. However, Pob3 lacks the HMGB-like domain that is part of SSRP1, and, as a result, yFACT relies on the assistance of the HMGB protein Nhp6 for interactions with nucleosomes [40]. Our past work has provided evidence that the integrity of a specific region of the nucleosome is important for ensuring proper yFACT departure from genes at the end of the transcription process. This region, which we refer to as the ISGI (Influences Spt16-Gene Interactions) region, is located on the side of the nucleosome and our results point to the charge landscape across this region as being an important determinant in promoting normal Spt16-gene dissociation [41,42]. In this work, we now present evidence that Spt16 dissociation from some genes is also in part dependent on Pol II termination. These results expand our understanding of the mechanisms at play during yFACT dissociation from genes following transcription and provide a framework with which the dissociation of other transcription elongation factors from transcribed genes may be assessed in future studies.

Materials and methods

Yeast strains and growth media. All yeast strains used in this study and their corresponding genotypes are listed in Table 1. The yADP strains are *GAL2*$^{+}$ derivatives of the *S288C* background [43] and strains FD4D and FD4A, generously provided by Nick Proudfoot, have been described previously [44]. Strains containing *HHT2*(WT)-*URA3* or *hht2*(H3-L61T)-*URA3* harbor an integration of the *URA3* gene downstream from the corresponding *HHT2* allele to allow for selection of the histone H3-expressing gene. Standard yeast media and genetic

Table 1. *Saccharomyces cerevisiae* strains.

Strain	Genotype
yADP121	*MATa his3* [a] *leu2* [b] *ura3* [c] *trp1Δ63 lys2-128δ can1Δ::MFA1pr-HIS3 (hht1-hhf1)Δ::LEU2 hht2*(H3-L61T)-*URA3*
yADP122	*MATa his3* [a] *leu2* [b] *ura3* [c] *trp1Δ63 lys2-128δ can1Δ::MFA1pr-HIS3 (hht1-hhf1)Δ::LEU2 HHT2*(WT)-*URA3*
yADP123	*MAT***a** *his3Δ200 leu2Δ1 ura3-52 lys2-128δ (hht1-hhf1)Δ::LEU2*
yADP124	*MAT***a** *his3* [a] *leu2* [b] *ura3* [c] *lys2-128δ (hht1-hhf1)Δ::LEU2 rtt103Δ::KanMX4*
yADP125	*MAT***a** *his3* [a] *leu2* [b] *ura3* [c] *lys2-128δ (hht1-hhf1)Δ::LEU2 hht2(H3-L61T)-URA3*
yADP126	*MAT***a** *his3* [a] *leu2* [b] *ura3* [c] *lys2-128δ (hht1-hhf1)Δ::LEU2 rtt103Δ::KanMX4 hht2(H3-L61T)-URA3*
FD4D	*MATa leu2* [d] *ura3* [e] *trp1Δ63*
FD4A	*MAT***a** *leu2* [d] *ura3* [e] *rat1-1 sen1-1*

[a]The allele at this locus is either *his3Δ200* or *his3Δ1*
[b]The allele at this locus is either *leu2Δ1* or *leu2Δ0*
[c]The allele at this locus is either *ura3-52* or *ura3Δ0*
[d]The exact nature of this *leu2* mutant allele is not known
[e]The exact nature of this *ura3* mutant allele is not known

techniques used in these studies have been described previously [45]. Canavanine (50mg/L, Sigma C1625) and G418 (200mg/L, Sigma G5013) were added to some of the plates for the SGA screen.

Synthetic Gene Array (SGA) screen and related tests. yADP121 was used as the query strain in the SGA screen. yADP121 cells were mated with the ~4800 strains from the non-essential yeast deletion library [46] and the resulting diploids were selected on SC-URA-TRP medium. Following induction of meiosis, a subset of meiotic products were selected on SC-HIS-ARG+Canavanine medium, and *(hht1-hhf1)Δ::LEU2 hht2*(H3-L61T)-*URA3 yfgΔ::KanMX4* cells were subsequently selected on SC-LEU-URA-HIS-ARG+Canavanine+G418 medium (*yfgΔ* indicates a representative deletion from the deletion set) and growth was assessed at 30°C and 14°C. Candidates displaying growth defects were then retested for genetic interactions in a secondary screen in which *yfgΔ::KanMX4* cells were taken through the same steps as in the original screen, but each candidate was crossed with yADP121 as well as yADP122 in order to identify *yfgΔ::KanMX4* mutations displaying genetic interactions specifically with the H3-L61T mutant. Candidates that appeared to show H3-L61T-specific genetic interactions were then reanalyzed through standard crosses and tetrad analysis. For these tests, rich medium (YPD) was used to assess growth patterns.

Chromatin Immunoprecipitation (ChIP) assays. ChIP assays were carried in a manner similar to that described previously [47]. Briefly, cells were grown to logarithmic phase in rich medium (YPD) and cross-linked with formaldehyde (final concentration of 1%). Chromatin was collected and sheared to average size of ~200–500 base-pairs using a Bioruptor 300 (Diagenode). Immunoprecipitations were carried out using 1µl rabbit polyclonal antibody specific for Spt16 (a generous gift of Tim Formosa) or 1µl mouse monoclonal antibody specific for Rpb3 (BioLegend, 665003). Antibody-chromatin complexes were affinity purified using Protein G-coupled Dynabeads (Thermo Fisher Scientific, 10004D) and wash extensively. Following steps to elute the beads from the chromatin, reverse the crosslinks, and degrade the proteins, the immunoprecipitated DNA as well as input DNA for each sample was quantified by qPCR using a StepOnePlus Real-Time PCR system (Applied BioSystems) to obtain the % immunoprecipitation value for each region tested. For the 37°C experiments, prior to the cross-linking step, cells growing logarithmically at 30°C were collected by centrifugation, resuspended in 37°C pre-warmed YPD medium, and incubated in a shaker incubator set at 37°C for 2 hours. Specificity of the antibodies was ensured by carrying out mock-immunoprecipitations done in the absence of antibodies. The gene-specific primers used in these experiments and the corresponding regions they amplify are shown in Table 2. Amplification of the NO ORF region (a gene-free location on chromosome V) was carried out using primers OAD377 and OAD378 described previously [47].

Results

A genetic screen uncovers a potential connection between Rtt103 function and Spt16 gene dissociation

Our previous work in yeast identified the nucleosomal ISGI region as an important contributor to proper Spt16 dissociation from genes at the end of the transcription process [42]. To identify additional factors that promote Spt16 dissociation from genes, we carried out a synthetic gene array (SGA) screen to probe for genetic interactions between an ISGI mutant and deletions in each of ~4800 genes available in a yeast haploid non-essential gene deletion library [46]. For this screen we used the H3-L61T ISGI mutant as the query strain – the H3-L61T mutant causes a strong defect in Spt16 dissociation from the 3′ ends of genes but does not confer a growth defect at 30°C and only moderate growth defects at 14°C and 37°C ([48] and Figure 1a). We reasoned that the combined effects of H3-L61T and deletion of a gene encoding a protein involved in Spt16 gene dissociation would lead to a defect in Spt16 gene dissociation severe enough to result in a synthetic sick or lethal phenotype. Following mating between the query and the deletion strains and sporulation of the resulting diploids, double mutant meiotic products were screened for synthetic growth defects at 30°C and 14°C (see Materials and methods). The latter temperature was chosen because in previous studies we found that the three ISGI mutants that cause the most severe Spt16 gene dissociation defects – H3-L61K, H3-L61W, and H4-R36A – also confer strong growth defects at 14°C

Table 2. Regions analyzed in ChIP-qPCR experiments.

Region	Primers used for amplification of region	Coordinates of amplified region of corresponding gene[a]
PMA1 R1	OAD394-OAD395	+198 through +260
PMA1 R2	OAD645-OAD646	+2904 through +3018
PMA1 R3	OAD647-OAD648	+3081 through +3170
PMA1 R4	OAD383-OAD384	+3373 through +3442
FBA1 R1	OAD419-OAD420	+135 through +197
FBA1 R2	OAD423-OAD424	+1174 through +1295
FBA1 R3	OAD713-OAD714	+1323 through +1390
FBA1 R4	OAD707-OAD708	+1584 through +1661
ADH1 R1	OAD701-OAD702	+81 through +150
ADH1 R2	OAD715-OAD716	+1175 through +1236
ADH1 R3	OAD703-OAD704	+1317 through +1383
ADH1 R4	OAD717-OAD718	+1682 through +1763
RPL9b R1	OAD657-OAD658	+165 through +226
RPL9b R2	OAD659-OAD660	+682 through +724
RPL9b R3	OAD661-OAD662	+974 through +1059
RPL9b R4	OAD663-OAD664	+1144 through +1223
RPS11b R1	OAD665-OAD666	+167 through +239
RPS11b R2	OAD667-OAD668	+1029 through +1116
RPS11b R3	OAD669-OAD670	+1202 through +1260
RPS11b R4	OAD719-OAD720	+1401 through +1471

[a]Coordinates are in relation to the +1 position, which corresponds to the first base pair of the coding region of the corresponding gene.

(Cs^- phenotype [41,48,49]), thus pointing to a possible correlation between strong Spt16 gene dissociation defects and cold sensitivity.

Following this initial screen, 390 gene deletions were identified as potentially displaying moderate to strong genetic interactions with the H3-L61T mutant. To remove candidates displaying growth defects in a manner independent of the H3-L61T mutant, these candidates were subjected to a secondary screen using a strain isogenic to the query strain but expressing wild-type histone H3 (H3-WT) instead of the H3-L61T mutant. This resulted in the removal of over 50% of the samples, leaving 171 gene deletions as candidates for displaying genetic interactions specifically with H3-L61T. Within this group, 47 encode proteins previously implicated in transcription and chromatin functions. Thirty-two of these proteins represent components of ten well-characterized proteins complexes – we thus continued our analysis with one representative from each of these complexes as well as the remaining 15 candidates with roles in transcription and chromatin processes. Strains harboring deletions in each of these 25 gene candidates were then once again crossed with an H3-L61T strain, but this time they were analyzed individually through standard tetrad dissection and analysis to confirm the authenticity of the genetic interactions we identified in the screens. To our surprise, the majority of these crosses (21 out of 25) did not clearly recapitulate the genetic interactions we thought we had uncovered in the screens. One possible reason for this discrepancy could be related to the difference in media used for the last step of the screens versus that used for tetrad analysis (the former being synthetic medium lacking certain amino acids and containing drugs and the latter being rich medium, see Materials and methods). Regardless of the reason, however, these results point to the importance of confirming genetic interactions uncovered in SGA screens through the use of standard genetic crosses and tetrad analysis.

One of the four deletions that display confirmed and clear genetic interactions with H3-L61T is within the gene encoding the transcription termination factor Rtt103. As shown in Figure 1a, the H3-L61T *rtt103Δ* double mutation confers a moderate synthetic growth defect at the standard growth temperature of 30°C and more marked synthetic growth defects at 14°C and 37°C. Previous work has shown that Rtt103 associates with the carboxy-terminal domain (CTD) of Pol II at 3′ ends of genes and is thought to aid in the recruitment of the RNA exonuclease Rat1 to sites of transcription termination [50–56]. According to the torpedo model for transcription termination, Rat1 then engages with the free 5′ end of the RNA molecule still tethered to Pol II following processing of the RNA by the cleavage/polyadenylation factors and progressively digests the RNA until it eventually disengages Pol II from the DNA substrate [57,58]. The genetic interactions we have uncovered establish a functional relationship between the H3-L61T mutant and Rtt103, and, by extension, provide a possible link between Spt16 dissociation and Pol II termination.

Deletion of RTT103 *causes marginal defects in Spt16 dissociation from the 3′ ends of two transcribed genes*

To determine if Rtt103 plays a role in promoting Spt16 dissociation from 3′ ends of transcribed genes, we assessed occupancy levels of Spt16 across three highly transcribed and constitutively expressed genes commonly used in transcription elongation and termination studies in various genetic backgrounds using chromatin immunoprecipitation (ChIP) assays. As shown in Figure 1

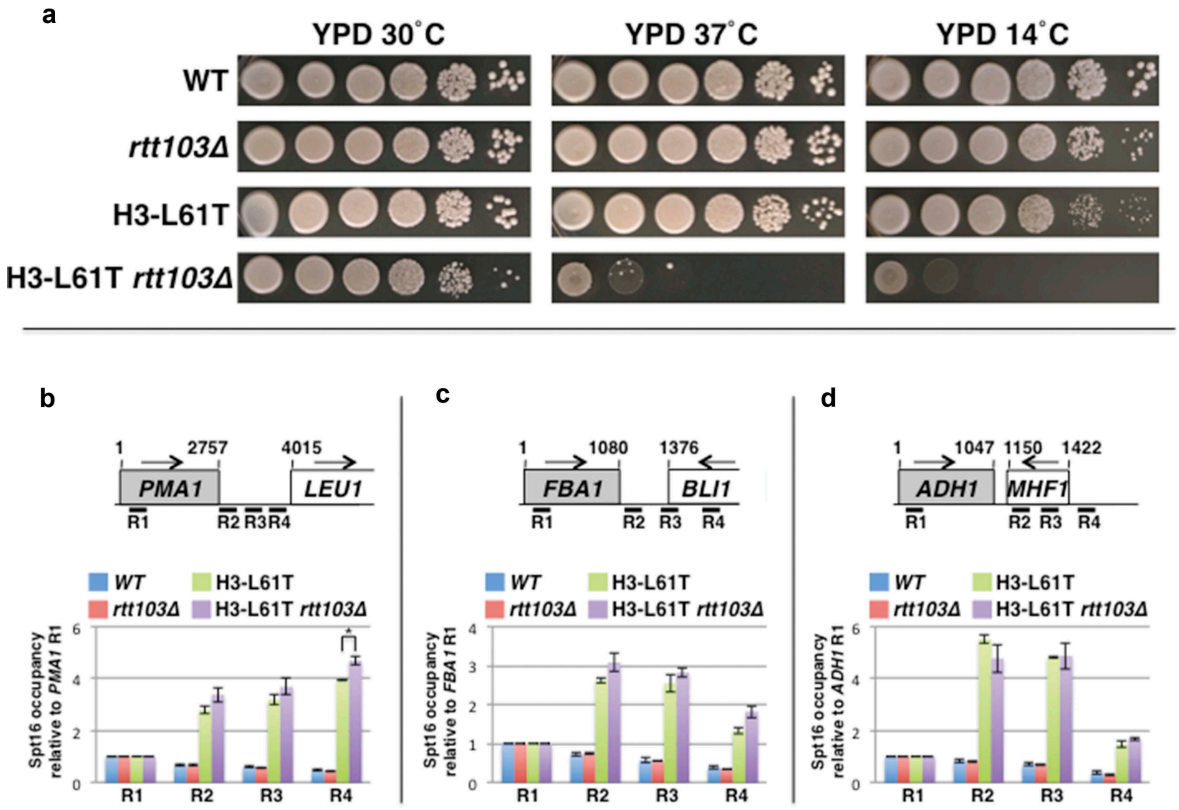

Figure 1. Genetic interactions between H3-L61T and *rtt103Δ* and effects of *rtt103Δ* on Spt16 occupancy across *PMA1*, *FBA1*, and *ADH1*. (a) Cells of the indicated genotypes were spotted in 10X-dilution series on rich medium (YPD) such that the most concentrated spot (left-most spot on each row) contained ~2X10^6 cells. Plates were photographed following incubations at 30°C for 2 days, 37°C for 2 days, or 14°C for 8 days. The strains used for these experiments were yADP123-yADP126. (b–d) Results from ChIP assays measuring Spt16 occupancy levels across *PMA1*, *FBA1*, and *ADH1* in the indicated four genetic backgrounds. For each gene, four regions were assayed for Spt16 binding (R1-R4, see diagram on top of each panel). The coordinates of R1-R4 for each of the three genes assayed in these studies are provided in Table 2. Arrows indicate the direction of transcription. In all cases, Spt16 occupancy levels are shown relative to Spt16 occupancy at the 5′ region (R1) of the corresponding gene. For each sample, data are expressed as mean ± S.E.M. from three independent experiments. The asterisk denotes a statistically significant difference as assessed by the Student's *t*-test ($P < 0.05$). The strains used in these experiments are the same as those used in the experiments shown in panel a.

panels b-d, an *rtt103Δ* mutation in an otherwise wild-type background does not cause measurable defects in Spt16 dissociation from the 3′ ends of the *PMA1*, *FBA1*, and *ADH1* genes. However, at *PMA1* and *FBA1*, the *rtt103Δ* mutation slightly exacerbates the Spt16 dissociation defect conferred by the H3-L61T mutant (compare results for H3-L61T and H3-L61T *rtt103Δ* in Figure 1, panels b and c). We note that with the exception of one region (region 4 [R4] of *PMA1*, see Figure 1a), these effects do not cross the threshold for statistical significance. However, if the subtle increase in Spt16 retention at 3′ ends of genes is a widespread phenomenon across the genome, the combined effects might be detrimental enough to cause the growth defects seen in H3-L61T *rtt103Δ* cells.

Mutations that interfere with Pol II termination cause Spt16 dissociation defects at the PMA1 *gene*

Since Rtt103 has been implicated in promotion of Pol II termination through the torpedo mechanism, our genetic and ChIP results point to a possible connection between Pol II termination and Spt16 gene dissociation. Despite its proposed role, however, deletion of *RTT103* does not cause a measurable retention of Pol II at the 3′ end of *PMA1* [50] – thus, if Spt16 dissociation from genes is indeed dependent on Pol II termination, it may not be surprising that an *rtt103Δ* does not cause marked defects in Spt16 dissociation from the 3′ ends of the genes we tested in our studies.

As an alternative way to test for a possible connection between Pol II termination and Spt16 gene dissociation, we assessed Spt16 occupancy across genes in a genetic background that is more severely impaired for the torpedo mechanism. Previous studies have shown that a mutation in the Rat1 exonuclease causes abnormally high levels of Pol II at the 3′ end of the *PMA1* gene, and that this defect is exacerbated in conjunction with a mutation in *SEN1*, a gene encoding a protein with RNA helicase activity thought to assist Rat1 function in transcription termination [44,50]. To probe more directly for a requirement for Pol II termination in Spt16 gene dissociation, we carried out ChIP experiments to assess the occupancy levels of Spt16, as well as those of Pol II itself, across the *PMA1* gene in wild-type and *rat1-1 sen1-1* mutant strains. These strains were kindly provided by the Proudfoot laboratory and are those used in their studies to show a role for Rat1 and Sen1 in Pol II dissociation from *PMA1* [44]. Since *rat1-1* and *sen1-1* are temperature sensitive mutations, we performed our experiments at 30°C as well as following a shift to 37°C (see Materials and methods).

Consistent with previous studies [44], we found that the *rat1-1 sen1-1* double mutation causes Pol II dissociation defects at *PMA1* (Figure 2). These defects are manifested by a reduction in relative Pol II occupancy at the 5′ end of *PMA1* (region 1 [R1]) presumably as a result of reduced Pol II availability for gene recruitment due to Pol II termination defects genome-wide and, more importantly, an increase in Pol II occupancy at the 3′ end of *PMA1* (R4) relative to the amount of Pol II recruited to the 5′ region (R1) of the gene (see Figure 2, panels b and d). These defects were seen at both 30°C and 37°C, but were somewhat more pronounced at the latter temperature. Thus, while the *rat1-1 sen1-1* mutations are somewhat temperature sensitive for this defect, they do confer marked defects in Pol II termination at 30°C as well.

Interestingly, Spt16 occupancy across *PMA1* is altered in the *rat1-1 sen1-1* strain in a manner similar to that seen for Pol II (Figure 2, panels c and e). More specifically, the *rat1-1 sen1-1* mutations cause increased occupancy of Spt16 at R4 relative to R1 at both 30°C and 37°C. These experiments also point to a two-phase mechanism for Spt16 dissociation at *PMA1*. The first phase occurs over regions 2 and 3 (R2 and R3) of the gene and appears to be independent of Pol II termination, as indicated by the facts that (i) Pol II occupancy over these regions in wild-type cells is not markedly reduced compared to the levels seen over the 5′ region, and (ii) the drop in Spt16 occupancy over these regions is not appreciably affected by the *rat1-1 sen1-1* mutations. On the other hand, the second phase, which occurs over R4, appears to be dependent on Pol II termination since not only does Pol II occupancy decrease over this region in wild-type cells, but, more importantly, Spt16 occupancy is increased in the context of the *rat1-1 sen1-1* double mutation. These results thus suggest that Spt16 dissociates from *PMA1* through two separate mechanisms, one of which is dependent on Pol II termination.

Partial dependence for Spt16 gene dissociation on Pol II termination is not limited to the PMA1 *gene*

To test whether the dependency for Spt16 dissociation on Pol II termination is restricted to *PMA1* or is a more generalized phenomenon, we assessed Pol II and Spt16 occupancy across *FBA1* and *ADH1* in both wild-type and *rat1-1 sen1-1* cells. As anticipated, the *rat1-1 sen1-1* double mutation causes defects in Pol II dissociation from the 3′ ends of both of these genes (Figure 3, panels b and e). Similarly to the results seen at *PMA1*, the *rat1-1 sen1-1* double mutation also impairs Spt16 dissociation from these genes (Figure 3, panels c and f), thus pointing to a more generalized requirement for Pol II termination on proper Spt16 dissociation from genes. The existence of a two-phase Spt16 dissociation process like that seen at *PMA1* is hinted at but is not as clearly observable at these genes – for example, whereas in wild-type cells Spt16 (but not Pol II) seems to subtly dissociate from R2 of *ADH1*, this dissociation appears to partly depend on Rat1/Sen1. We note that due to the short intergenic space between *FBA1* and *BLI1* and between *ADH1* and *MHF1*, some of the regions assayed in these experiments lie within the genes directly downstream from *FBA1* or *ADH1*. However, at *FBA1* dependency of Spt16 dissociation on Rat1/Sen1 is seen at Region 2, which is located very near the end of the *FBA1* coding region, thus indicating that the effects observed are related to *FBA1* transcription. Whereas at *ADH1* all

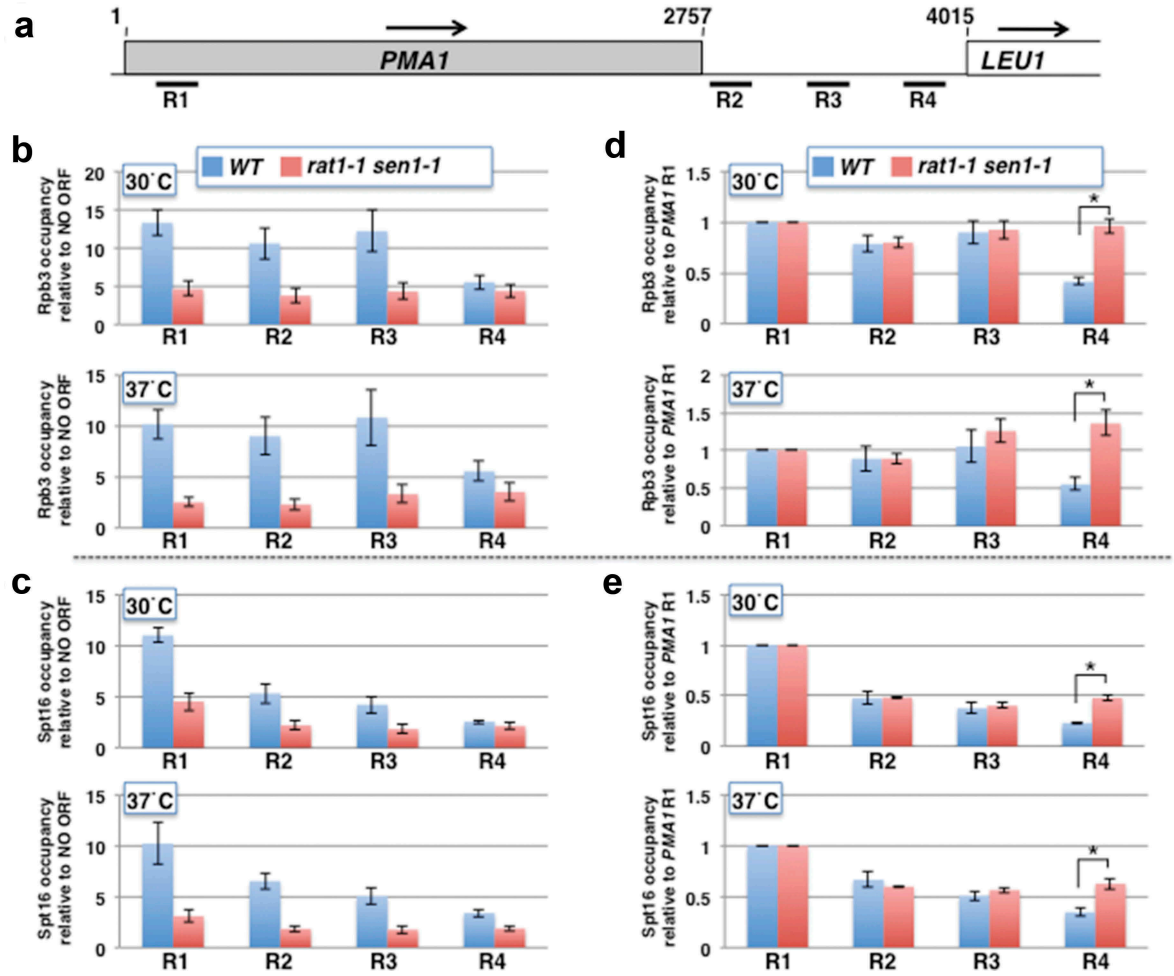

Figure 2. Effects of the *rat1-1 sen1-1* double mutation on Rpb3 and Spt16 occupancy across the *PMA1* gene. (a) Diagram of the *PMA1* locus with the four regions assayed for Rpb3 and Spt16 binding indicated (R1-R4). Arrows indicate the direction of transcription. (b) Results from ChIP assays measuring occupancy levels of Rpb3 (a subunit of Pol II) across *PMA1* relative to a region of the genome devoid of genes (NO ORF region, see Materials and methods) in wild-type and *rat1-1 sen1-1* cells. Data are shown for experiments carried out at 30°C and after a shift to 37°C (see Materials and methods). (c) Results from ChIP assays as described in panel b but in which Spt16 occupancy was measured instead of Rpb3. (d,e) Results from the experiments described in panels b and c, respectively, but expressed relative to Rpb3 or Spt16 binding to the 5′ region (R1) of the gene instead of the NO ORF region. For each sample, data are expressed as mean ± S.E.M. from three independent experiments. Asterisks denote statistically significant differences for the data shown in panels d and e as assessed by the Student's *t*-test ($P < 0.05$). The strains used in these experiments are FD4D and FD4A.

three 3′ regions assayed lie within the *MHF1* gene, it is unlikely that the ChIP signals seen in these experiments are significantly affected by transcription originating from the *MHF1* promoter since binding of Pol II at the *MHF1* promoter region (R4) is very low and near background levels in wild-type cells (Figure 3 and data not shown), which in turn argues against high levels of transcription initiation activity at *MHF1*.

Whereas previous studies conducted at the single-gene level showed that Spt16 dissociates from the genes tested (which included *PMA1* and *ADH1*) at roughly the same location as that from which Pol II dissociates [59], genome-wide studies have provided evidence that at most genes Spt16 dissociates at regions upstream from Pol II dissociation sites [60,61]. Given the difference in Spt16 and Pol II dissociation patterns, we anticipated that dissociation of Spt16 from these genes would be independent of Pol II. To test this, we assessed Pol II and Spt16 occupancies across *RPL9b* and *RPS11b*, two genes for which the genome-wide studies showed clear Spt16 departure upstream from Pol II dissociation [60]. Consistent with the results by Mayer *et al.* [60] we found that at *RPL9b* Spt16 occupancy decreases upstream from where Pol II dissociates (Figure 3h,i, compare Spt16 and

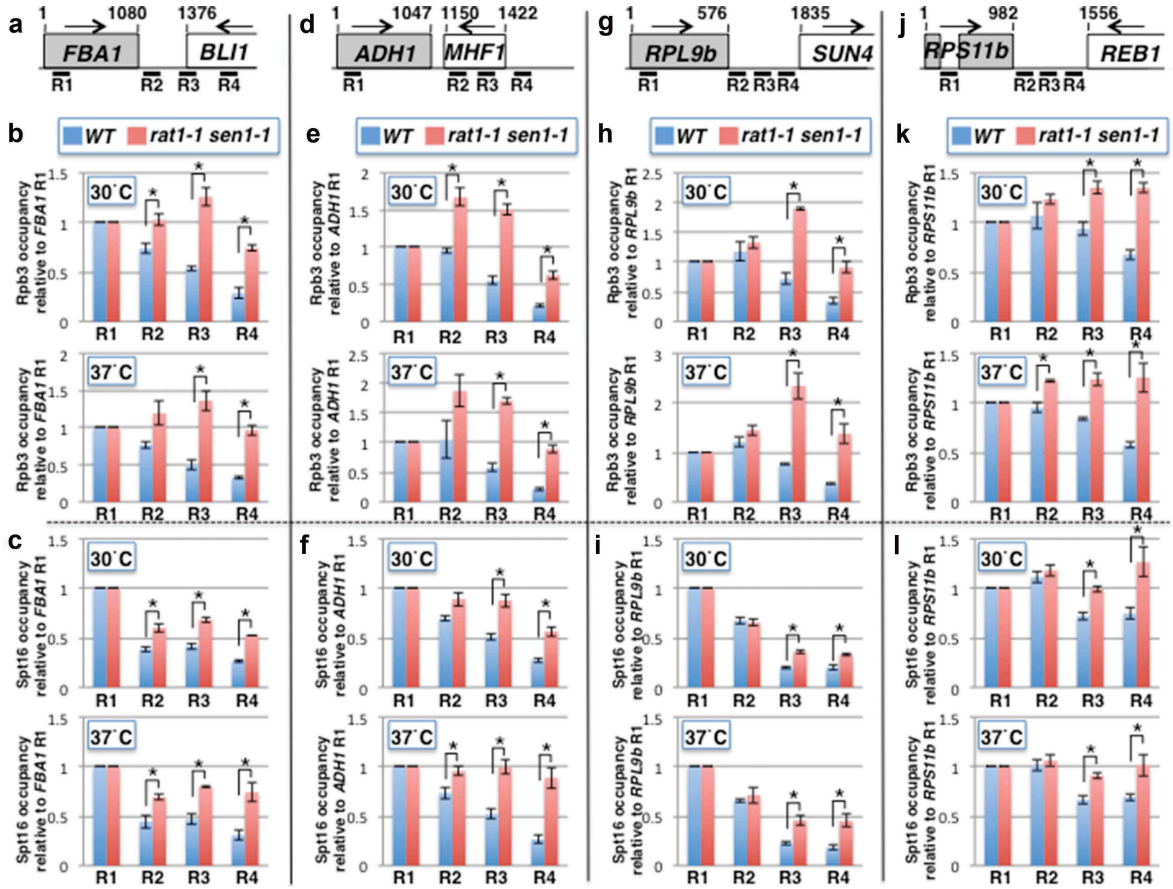

Figure 3. Effects of the *rat1-1 sen1-1* double mutation on Rpb3 and Spt16 occupancy across *FBA1, ADH1, RPL9b*, and *RPS11b*. (a, d, g, j) Diagrams of four additional genes used to assess effects of *rat1-1 sen1-1* on Rpb3- and Spt16-gene interactions and the corresponding four regions used in each case (R1-R4). The coordinates corresponding to R1-R4 for *RPL9b* and *RPS11b* are included in Table 2 along with all the other regions assayed in this study. Arrows indicate the direction of transcription. (b, c, e, f, h, i, k, l) Results from Rpb3 and Spt16 ChIP assays performed in wild-type and *rat1-1 sen1-1* cells across the indicated genes at 30°C and 37°C presented in the same manner as those shown in Figure 2, panels d and e. For each sample, data are expressed as mean ± S.E.M. from three independent experiments. Asterisks denote statistically significant differences as assessed by the Student's *t*-test ($P < 0.05$). The strains used in these experiments are FD4D and FD4A.

Pol II occupancy at R2). Spt16 dissociation from this location is unaffected by the *rat1-1 sen1-1* mutations, supporting the notion that it is indeed independent of Pol II termination. However, Spt16 occupancy is further reduced downstream from this location (*i.e.*, at R3 and R4), and in this case Spt16 dissociation appears to be dependent on Pol II termination since it is impaired by the *rat1-1 sen1-1* mutations. This two-phase mechanism for Spt16 dissociation is similar to that seen at *PMA1*, thus suggesting that it may be a widespread phenomenon. Unlike the results from the Mayer *et al.* studies, our ChIP assays at *RPS11b* do not show marked Spt16 departure upstream from Pol II dissociation sites (Figure 3, panels k and l) – this discrepancy may be due to the inability of the primers used in the qPCR step of our experiments to convincingly capture Spt16's early departure. However, these experiments do show dependency for Spt16 dissociation from *RPS11b* on Rat1/Sen1 (especially clear at R3 and R4). Taken together, our studies provide evidence that Spt16 dissociation from the genes tested is partially dependent on Pol II termination, and that at some of them, Spt16 dissociates in two phases, with the upstream phase being independent on Pol II termination.

Discussion

In this work, we have provided evidence the dissociation of Spt16, and by extension the FACT complex, from the 3′ ends of some transcribed genes in

yeast is in part dependent on Pol II termination. Our results also provide evidence that at least at some genes Spt16 dissociation occurs in two distinct phases. The first phase takes place upstream from where Pol II dissociates and does not require the functions of Rat1/Sen1, whereas the second phase occurs further downstream and is dependent on Pol II dissociation.

Results from early experiments done at the individual gene level pointed to the dissociation of Spt16 occurring at the same sites where Pol II dissociates [59], whereas more recent genome-wide studies have indicated that at an average gene Spt16 dissociates upstream from the sites from which Pol II dissociates [60,61]. Our data may in part reconcile these apparently conflicting results as they suggest that both events – upstream departure (Pol II independent) and further downstream departure (Pol II dependent) – may in fact be occurring at at least some genes across the genome. Additional evidence in support of the presence of a Pol II-independent mechanism for Spt16 dissociation (upstream from the Pol II-dependent sites) comes from previous studies showing that specific mutations within the ISGI region, while causing strong retention of Spt16 at 3′ ends of genes, confer only minimal perturbations in Pol II occupancy [41,49].

We envision two possible non-mutually exclusive mechanisms – one direct and the other indirect – that could explain the partial dependence for Spt16 gene dissociation on Pol II termination. For the direct mechanism, Pol II dissociation from the DNA template through the torpedo mechanism simply "drags" Spt16 off of the DNA as a result of Spt16 being physically associated with Pol II during transcription – abnormal retention of Pol II at 3′ ends of genes through impairment of Rat1/Sen1 would therefore also cause Spt16 to be abnormally retained. The indirect mechanism builds upon recent work from the Howe laboratory that has provided strong evidence indicating that nucleosomes that are structurally altered through their engagement with transcribing Pol II serve to recruit the FACT complex to chromatin [36]. In this scenario, retention of Pol II at the 3′ ends of genes through impairment of the torpedo mechanism would result in abnormally higher levels of Pol II-altered nucleosomes at those locations, which would then in turn cause abnormal retention of Spt16 as well. An implication of this mechanism is that the encounter of Spt16 with a canonical nucleosome (*i.e.*, not engaged with Pol II) might be part of the normal process that leads to Spt16 dissociation from chromatin following gene transcription.

Genome-wide studies have shown that different transcription elongation factors display different patterns of dissociation from transcribed genes, with some factors departing genes upstream from the sites of Pol II termination and some departing from sites further downstream [60]. It will be of interest to determine if any of these factors also depend on Pol II dissociation for their own departure from genes upon transcription termination. Additional topics for future studies include identifying the mechanisms that promote the first phase of Spt16 dissociation from genes (*i.e.*, the phase that operates upstream of Pol II termination) and elucidating which phase of the Spt16 dissociation process depends on the integrity of the nucleosomal ISGI region.

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Author contributions

JBC performed the experiments associated with the results shown in Figures 2 and 3, MJE performed the experiments associated with the results shown in Figure 1, and SAO performed the experiments associated with the results shown in Figures 1–3. AAD designed and supervised all experiments and wrote the paper. All authors have read this paper and agree with the content.

Disclosure statement

No potential conflict of interest was reported by the authors.

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