Gene promoters dictate histone occupancy within genes

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Spt6 is a transcriptional elongation factor and histone chaperone that reassembles transcribed chromatin. Genome-wide H3 mapping showed that Spt6 preferentially maintains nucleosomes within the first 500 bases of genes and helps define nucleosome-depleted regions in 5' and 3' flanking sequences. In Spt6-depleted cells, H3 loss at 5' ends correlates with reduced pol II density suggesting enhanced transcription elongation. Consistent with its ‘Suppressor of Ty’ (Spt) phenotype, Spt6 inactivation caused localized H3 eviction over 1–2 nucleosomes at 5' ends of Ty elements. H3 displacement differed between genes driven by promoters with 'open'/DPN and 'closed'/OPN chromatin conformations with similar pol II densities. More eviction occurred on genes with ‘closed’ promoters, associated with ‘noisy’ transcription. Moreover, swapping of ‘open’ and ‘closed’ promoters showed that they can specify distinct downstream patterns of histone eviction/deposition. These observations suggest a novel function for promoters in dictating histone dynamics within genes possibly through effects on transcriptional bursting or elongation rate.

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Introduction

Nucleosome occupancy can affect transcription by governing the access of transcription factors to promoters and imposing an obstacle to elongating pol II within genes. Along each gene, chromatin structure is determined by the opposing forces of histone eviction and replacement that are coupled to transcription elongation (Kristjuhan and Svejstrup, 2004; Schwabisch and Struhl, 2004; Dion et al, 2007; Jamai et al, 2007; Rufiange et al, 2007; reviewed in Owen-Hughes and Gikopoulos, 2012). Histone chaperones facilitate this replication-independent histone exchange and thereby help determine nucleosome occupancy (Schwabisch and Struhl, 2004, 2006; Adkins and Tyler, 2006; De Koning et al, 2007; Zaugg and Luscombe, 2012). Spt6 is a histone H3, H4 chaperone (Bortvin and Winston, 1996) that binds nucleosomes (McDonald et al, 2010) and is conserved between yeast and metazoans. Spt6 is localized throughout actively transcribed genes (Kim et al, 2004; Mayer et al, 2010) and binds directly to the phosphorylated C-terminal domain of pol II through its SH2 domains (Mayer et al, 2012). In seminal experiments, Spt6 inactivation was found to uncover transcription-dependent loss of nucleosomes and activation of cryptic promoters (Kaplan et al, 2003; Cheung et al, 2008).

SPT6 was identified as a suppressor of the His− phenotype caused by insertion of a Ty 6 element at the 5′ end of HIS4 (Winston et al, 1984). The basis for this suppression is not completely understood.

A study of 180 protein-coding genes on yeast ChrIII showed that Spt6 is most important for replacement of nucleosomes evicted from highly transcribed genes and that altered chromatin structure in the mutant does not always correlate with changes in mRNA abundance (Ivanovska et al, 2011). Spt6 inactivation also causes transcriptional derepression of some genes due to histone depletion at promoters (Adkins and Tyler, 2006; Jensen et al, 2008; Hainer et al, 2011; Ivanovska et al, 2011), but it is not known how generally Spt6 is required to maintain transcriptional repression. The fate of histones evicted from chromatin when Spt6 function is impaired is also not well understood; however, the pool of free histones increases (Morillo-Huesca et al, 2010).

Spt6 was first implicated as a transcription elongation factor by genetic interaction with TFIIIS (Hartzog et al, 1998) and it can accelerate transcription of a naked DNA template (Endoh et al, 2004). On Drosophila heat-shock genes, Spt6 knock-down elevated pol II density consistent with slower elongation (Ardehali et al, 2009) but its effects on pol II occupancy have yet to be investigated genome-wide.

Replication-independent histone exchange is greatest at highly transcribed genes (Dion et al, 2007; Rufiange et al, 2007; Jamai et al, 2009) but in addition to pol II density within a gene, it has been suggested that there are additional unknown factors that govern rates of histone exchange (Gat-Viks and Vingron, 2009). One such factor may be elongation rate, since histone eviction in vitro is enhanced by faster transcription (Bitlu et al, 2011). How Spt6 affects histone dynamics at fast and slow exchanging genes has not been determined. Moreover, it is unclear whether Spt6-mediated chromatin reassembly operates uniformly throughout transcribed genes or whether it is more active at specific locations.

In budding yeast, two major promoter classes are distinguished by their patterns of nucleosome occupancy (Tirosh and Barkai, 2008; Cairns, 2009; Zaugg and Luscombe, 2012).
‘Open’ promoters have a greater distance between the −1 and +1 nucleosomes (Zaugg and Luscombe, 2012) and they overlap extensively with the ‘depleted proximal nucleosome’ (DPN) class (Tirosh and Barkai, 2008). ‘Closed’ promoters have −1 and +1 nucleosomes closer together and they coincide extensively with the ‘occupied proximal nucleosome’ (OPN) class. ‘Open’/DPN promoters have fewer TATA boxes, more Htz1 in promoter nucleosomes, more constitutive transcription, and slower rates of histone turnover than ‘closed’/OPN promoters (Cairns, 2009). ‘Closed’/OPN promoters are thought to drive ‘noisy’ expression associated with bursts of transcription initiation (Raser and O’Shea, 2004; Zenklusen et al, 2008; Chubb and Liverpool, 2010). It is not known whether transcribed chromatin downstream of ‘open’ and ‘closed’ promoters differs in its histone dynamics. In this report, we employed a new full-length ts degron mutant to investigate how Spt6 affects histone and pol II occupancy throughout the yeast genome. We found that while net histone eviction often occurs on highly expressed genes when Spt6 is inactivated, there is not always a correlation between histone loss and pol II density on a gene. Surprisingly, histone occupancy is affected differently within genes driven by ‘open’/DPN and ‘closed’/OPN promoters, suggesting a novel effect of promoters on histone eviction.

Results

Spt6 depletion in a degron mutant

We investigated histone and pol II occupancy in a ts Spt6-degron mutant (spt6-td) under the control of a doxycycline (Dox) repressible promoter (Seward et al, 2007). This mutant expresses the full-length Spt6 fused to an N-terminal degron and grows normally at 25°C. Under non-permissive conditions (37°C + Dox), there is a severe growth defect and the protein is substantially depleted within 30 min (Figure 1A). Furthermore, Spt6 binding to genes as measured by ChIP is strongly diminished under non-permissive conditions (Supplementary Figure S1a and b, lanes 11 and 12). The Spt6-degron mutant has potential advantages over the spt6-td allele used previously (Kaplan et al, 2003; Adkins and Tyler, 2006; Jensen et al, 2008; Ivanovska et al, 2011) because the latter has an in-frame deletion of the helix-hairpin helix domain (residues 931–994). This deletion destabilizes the protein and causes transcriptional defects at the permissive temperature (Kaplan et al, 2005). Genome-wide ChIP-Chip of the HA-tagged Spt6-degron fusion protein showed that it was enriched throughout highly transcribed genes and is highest at 3′ ends (Figure 1B) in agreement with previous mapping of TAP-tagged Spt6 (Mayer et al, 2010). Spt6 was also enriched on non-coding genes for cryptic unstable transcripts (CUTs), stable unannotated transcripts (SUTs) and small nucleolar RNAs (snoRNAs) genes, but not on tRNA or rRNA genes (Supplementary Figure S2a–e).

Spt6 maintains 5′ and 3′ nucleosome-depleted regions

To examine how Spt6 affects chromatin, we assayed H3 occupancy in independent ChIP-seq and ChIP-Chip experiments in isogenic WT and spt6-td strains shifted to 37°C + Dox for 1 h. Spt6 is required for nucleosome maintenance at several inducible promoters (Adkins and Tyler, 2006; Jensen et al, 2008; Ivanovska et al, 2011) and we examined how it affects histone occupancy at promoters generally (>6500 genes). Spt6 depletion caused the average dip in histone occupancy at promoter-associated nucleosome-depleted regions (NDRs) to flatten out and become less well defined (Figure 1C). A relative decrease in histone occupancy at the boundaries together with a small increase within the NDR are responsible for this effect. A similar flattening of the histone occupancy profile across the NDR was observed at ‘OPN’, ‘DPN’, ‘open’ and ‘closed’ promoters (Supplementary Figure S3a–d) (Tirosh and Barkai, 2008; Zaugg and Luscombe, 2012).

We also asked whether Spt6 affected 3′ NDRs near poly(A) sites (Mavrich et al, 2008; Fan et al, 2010; Yadon et al, 2010). Comparison of average H3 ChIP profiles around 2900 3′ ends with NDRs (Yadon et al, 2010) showed that removal of Spt6 strongly diminished the extent of nucleosome depletion at 3′ ends so that 3′ NDRs like 5′ NDRs became less well defined. Similar results were obtained in independent ChIP-Chip and ChIP-seq experiments (Figure 1D; Supplementary Figure S3e). Together, these observations show that Spt6 functions widely throughout the genome to maintain NDRs at 5′ and 3′ ends of genes.

Transcription and histone eviction in Spt6-depleted cells

As expected, degron-mediated depletion of Spt6 diminished histone occupancy within many protein-coding genes. Our results agreed well with those for the spt6-1004 ts mutant at 86 genes on chromosome III where histone loss occurred (Ivanovska et al, 2011) (Supplementary Figure S2f). Comparison of highly transcribed coding regions showed extensive H3 loss throughout the length of short genes (<800 bp), whereas on longer genes there is greater loss at 5′ ends than at 3′ ends in both ChIP-seq and ChIP-Chip experiments (Figure 1E and F). Most H3 displacement in Spt6-depleted cells occurred within the first 500 bases of the transcription unit (Figure 1C). Unexpectedly relative histone occupancy in the 3′ flanking regions was enhanced when Spt6 was depleted, especially for longer genes (Figure 1E and F). These results suggest that although Spt6 is most abundant at 3′ ends, the majority of Spt6-dependent nucleosome re-placement occurs within the first 500 bases of most genes. They furthermore suggest the possibility that histones evicted from within genes when Spt6 is compromised may be deposited in intergenic regions downstream of genes.

To investigate the relationship between Spt6-dependent histone deposition/eviction and transcription, we plotted H3 occupancy at 37°C in spt6-td relative to WT in three groups of genes (1–3) (Venters and Pugh, 2009) with low, intermediate and high expression, respectively. As expected, the greatest histone loss occurred among the most highly transcribed genes (group 3, Figure 2A; Supplementary Figure S3f). To examine this relationship in greater detail, we plotted the change in average H3 occupancy within genes caused by Spt6 depletion, against average pol II occupancy in WT cells at 25°C as determined by ChIP-seq, or transcriptional activity as determined by global nuclear runon sequencing (NRO-seq) (Mckinlay et al, 2011) (Figure 2B and C). The results show significantly greater histone loss in more highly transcribed genes with the strongest correlation between histone loss and nuclear runon signal. Average histone loss across genes is less on long genes (>2 kb, red dots in Figure 2B) probably because it occurs mostly near their 5′ ends (Figure 1E and
We noted that among cytoplasmic ribosomal protein genes there is more heterogeneity of NRO-seq signals than for pol II ChIP signals (Figure 2B and C, green dots) consistent with the suggestion that not all polymerases have equivalent transcriptional activity (Pelechano et al., 2009). The discordance that can occur between pol II density and H3 loss in the spt6 mutant is exemplified by the PRB1 and EXG2 genes. PRB1 is strongly depleted of H3 in the absence of Spt6.

**Figure 1** Spt6 degradation and histone eviction in the spt6-td ts degron mutant. (A) Upper panel: Growth defect of the spt6-td degron mutant (DBY875) relative to WT (DBY311) at 37°C + Doxycycline (Dox, 2 μg/ml). Lower panel: Western blot of HA-Spt6 degron fusion at time points after shifting to 37°C + Dox. Pgk1 is a loading control. (B) Average ChIP-Chip (ChCh) profiles of pol II and HA-Spt6 degron at 25°C normalized to the values at the start of the transcription unit on 462 highly transcribed genes 800–2000 bases long as described (Kim et al., 2010). Transcription units are divided into 10 equal intervals (dotted line) with 1 kb of 5’ and 3’ flanking sequences (smooth line). Note enrichment at the 3’ end (arrow). Dashed lines mark the central 80% of genes. (C, D) Average distributions of histone H3 ChIP-seq (Ch-seq) signals in the spt6-td and WT strains (37°C + Dox) at 5’ NDRs of all protein-coding genes and 3’ NDRs of 2900 genes as defined previously (Yadon et al., 2010). Note that within genes most of the H3 loss occurs within the first 500 bases. (E, F) Average distributions of H3 ChIP-seq and ChIP-Chip signals in the spt6-td degron normalized to WT (37°C + Dox) in highly transcribed short (<800 bp), medium (800–2000 bp) and long (>2000 bp) genes described previously (Kim et al., 2010). ORFs are divided into 10 equal bins. Note greater histone loss near 5’ ends and increased occupancy in 3’ flanking regions.
whereas **EXG2** is relatively unaffected, yet they have similar levels of pol II occupancy (Figure 2D and E).

Spt6 inactivation can either downregulate or upregulate transcription. We investigated the effects on transcription by comparing pol II ChIP-Chip in WT and *spt6-td* strains. We identified 553 genes where average pol II ChIP signals were reduced by >2.0-fold in *spt6-td* relative to two replicates of WT (DBY311 37°C, this paper and previously published Kim et al., 2010) (Supplementary Table S1) including the Dox-repressed SPT6 gene itself and **CLN3**, which is also downregulated in *spt6-1004* (Morillo-Huesca et al., 2010) (Supplementary Figure S4a). We also found 109 genes whose average pol II ChIP signal was elevated by >2.0-fold in the *spt6-td* mutant relative to WT at 37°C (Supplementary Table S1) including **CHA1** and **HSP104** (Figure 2F and G) that are also derepressed in *spt6-1004* (Jensen et al., 2008; Ivanovska et al., 2011) and the sporulation-specific genes **SGA1**, **AMA1** and **SPO20** (Supplementary Figure S4b; Supplementary Table S1). Among these upregulated genes are 18 that produce cryptic transcripts in *spt6-1004* (Cheung et al., 2008) including **STE11** (Figure 2I). The amount of histone H3 loss within the ORF varied widely among upregulated genes (Supplementary Table S1; Figure 2F–H) and we did not find that histone depletion within ORFs correlated with transcriptional derepression (Supplementary Figure S4c). In summary, though transcription is necessary for histone eviction when Spt6 function is impaired (Kaplan et al., 2003), these results show that the extent of histone loss is not always related in a simple way to average pol II density.

**Spt6 and transcription elongation**

We examined how Spt6 affects transcription elongation by comparing profiles of pol II density along genes in the WT and
Spt6-degron strains under non-permissive conditions. On exceptionally long genes (>4 kb) such as MDN1, Spt6 depletion reduced pol II density at the 3' end relative to the 5' end suggesting impaired transcriptional elongation, without much effect on histone H3 density (Figure 3A and B). This observation is consistent with a chromatin-independent stimulation of elongation by Spt6 (Endoh et al., 2004). The most common effect of Spt6 depletion on pol II localization, however, was to reduce its density at 5' ends relative to 3' ends. This shift in pol II distribution occurred on both poorly and highly transcribed genes including many ribosomal protein genes (Figure 3C–F) and is consistent with faster transcription elongation at 5' ends where histones are preferentially lost.

Highly localized histone maintenance by Spt6 at Ty elements

SPT6 mutants were discovered as suppressors of a δ insertion at the 5' end of HIS4 (Winston et al., 1984) and we therefore examined H3 occupancy on Ty and δ elements. Although ChIP signals are not specific to individual Ty elements because of their repetitive nature, the results of independent ChIP-Chip and ChIP-seq experiments nevertheless established that on average there is localized displacement of H3 in a discrete region corresponding to 1–2 nucleosomes ~200–500 bases downstream of the TSS when Spt6 is depleted (Figure 4A–C; Supplementary Figure S4d). These results suggest that Spt6 can act in a highly localized way to maintain histone occupancy within a transcription
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Distinct effects of Spt6 at genes with fast and slow histone exchange rates

Replication-independent histone exchange in yeast is pervasive at promoters and within highly transcribed genes (Dion et al., 2007; Rufiange et al., 2007). We compared H3 occupancy within groups of ~1000 genes with the fastest and slowest rates of replication-independent histone exchange (Supplementary Table S1; Rufiange et al., 2007) in WT and Spt6-depleted cells and found a marked difference between them. At ‘fast’ exchanging genes relative H3 occupancy declined as expected, whereas at ‘slow’ exchanging genes, it was elevated (Figure 4D). Much the difference between how Spt6 inactivation affects chromatin on fast and slow exchanging genes is probably explained by the higher pol II occupancy on ‘fast’ exchanging genes (Dion et al., 2007; Rufiange et al., 2007) (data not shown). In summary, these results suggest that Spt6-mediated histone replacement is a major determinant of nucleosome occupancy within genes with high histone exchange rates where active displacement correlates with high pol II occupancy.

Promoter-specific effects on histone occupancy in spt6

We asked whether promoter structure might influence histone eviction by comparing subsets of genes with open/DPN and closed/OPN promoters (Tirosh and Barkai, 2008; Zaugg and Luscombe, 2012) that have approximately equivalent average pol II occupancy. At DPN genes, there was modest histone loss in the ORF when Spt6 was depleted, whereas at OPN genes there was greater loss, particularly near their 5’ ends. Independent anti-H3 ChIP-Chip and ChIP-seq experiments yielded equivalent results (Figure 5A and B). Similarly, genes driven by ‘open’ promoters had less histone eviction that those driven by ‘closed’ promoters (Figure 5D and E). The distinct patterns of histone eviction on genes driven by different promoter classes are not explained by differences in their pol II density. In fact, there was slightly higher pol II density on the DPN/open genes that have less histone eviction (Figure 5C and F). Nor are the different histone eviction patterns correlated with differences in histone occupancy in WT cells (Supplementary Figure S5a and b) or gene length. (Median gene lengths for ‘open’, ‘closed’, ‘DPN’ and ‘OPN’ genes are 1552, 1480, 1378 and 1465 bases.) The OPN/Closed genes analysed had somewhat lower Spt6 occupancy that the DPN/Open genes (Supplementary Figure S5c and d), in accordance with their slightly lower pol II densities (Figure 5C and F). Together, these results suggest that histone eviction within coding regions is influenced by promoters in a way that is independent of average pol II density.

Promoters influence histone occupancy within genes

To test whether promoters can influence downstream histone occupancy, we constructed spt6-td strains in which the promoter of the chromosomal YLR454W gene was swapped...
for that of GCD1 or ADH1. GCD1 has an ‘open’ (DPN) promoter whereas ADH1 has a ‘closed’ (OPN) promoter. The YLR454W promoter that was replaced is of the ‘closed’ class but its response to Spt6 inactivation (Figure 6A bottom panel) is atypical. The effects of Spt6 depletion on H3 occupancy at the endogenous GCD1 and ADH1 genes (Figure 6A) are typical of their respective gene classes (Figure 5). Both GCD1 and ADH1 promoters drove higher levels of expression than the endogenous YLR454W promoter as shown by RT–PCR (Supplementary Figure S5e) and pol II ChIP signals were somewhat higher on the ADH1-YLR454W gene (Figure 6B and D) fragment. Transcription from both promoters was only modestly affected when Spt6 was inactivated (Figures 6B and D). We assayed H3 occupancy on the GCD1- and ADH1-YLR454W genes in isogenic WT and spt6-td strains at 37°C + doxycycline. ChIP results were quantified by Q-PCR with a panel of 11 amplicons relative to a telomere control (Figure 6C and E). Remarkably, Spt6 depletion had distinct effects on chromatin within the common YLR454W sequence in the two chimaeras. In the GCD1-YLR454W gene, H3 occupancy was little affected at the 5’0 end, but increased at the 3’0 end (amplicons 1766–8900) when Spt6 was depleted (Figure 6C). In contrast, at ADH1-YLR454W, H3 occupancy decreased markedly at the 5’0 end (amplicons ADH1-YLR–2400) and increased at the 3’0 end (amplicons 5903–8900, Figure 6E). These changes in histone occupancy resembled the changes within the ADH1 and GCD1 genes themselves in spt6-td. Thus, at GCD1, the major effect of Spt6 inactivation was an enrichment of H3 over the 3’0 portion of the gene (Figure 6A) similar to the GCD1-YLR454W chimaera (Figure 6C). At ADH1, H3 was strongly depleted throughout the gene (Figure 6A) and at ADH1-YLR454W it was also depleted, but the effect was restricted to the 5’0 end (amplicons ADH1-YLR—2400, Figure 6E). Similar results were obtained for chimaeras of YLR454W driven by the APA1 (YCL050C, DPN) and PDC1 (OPN) promoters.
YLR454W
Note the distinct effects of Spt6 inactivation on H3 occupancy within 37° (Figure 1A). Our results show that Spt6 functions widely
occupancy in budding yeast using a new ts degron mutant
ronal elongation factor, Spt6, affects histone H3 and pol II
We investigated how the histone chaperone and transcrip-
Discussion
APA1-YLR454W
small effect at 30 (Supplementary Figure S6). Spt6 inactivation had only a
depletion at the 5° end. In contrast, both fusions driven by OPN promoters,
PDC1 and ADH1, responded to Spt6 inactivation with H3 depletion at the 5′ end and enrichment at the 3′ end. While we cannot eliminate the possibility that differences in pol II occupancy within YLR454W fusion genes play some role, the promoter-swapping experiments are fully consistent with the idea that the differences in histone dynamics between closed/OPN and open/DPN genes (Figure 5) correlate with their promoters rather than their coding sequences.

Discussion
We investigated how the histone chaperone and transcriptional elongation factor, Spt6, affects histone H3 and pol II occupancy in budding yeast using a new ts degron mutant (Figure 1A). Our results show that Spt6 functions widely throughout the genome to maintain NDRs both at promoters and at 3′ ends. In Spt6-depleted cells, NDRs became less well defined as manifested by flatter H3 occupancy profiles with loss from their upstream and downstream boundary regions and some deposition within the depleted regions (Figure 1C and D). Together, the results suggest that Spt6 helps to deposit or stabilize nucleosomes that flank NDRs and to prevent nucleosome encroachment within NDRs. This function of Spt6 complements other chaperones and remodellers including Asf1, Isw2, RSC and SWI/SNF that prevent histone accumulation within NDRs (Whitehouse et al, 2007; Badis et al, 2008; Parnell et al, 2008; Hartley and Madhani, 2009; Tolkunov et al, 2011).

In agreement with previous work on selected genes (Kaplan et al, 2003; Jensen et al, 2008; Ivanovska et al, 2011), our genome-wide study revealed net histone eviction within highly transcribed genes in Spt6-depleted cells. In addition, we found that under these conditions, eviction occurs preferentially at genes with fast rates of histone exchange. Our results revealed a marked 5′-3′ bias with most histone eviction occurring within the first 500 bases of the transcription unit when Spt6 was depleted (Figures 1E, F and 4D). This 5′-3′ bias is consistent with the idea that transcription complexes that have elongated a short distance are more effective at displacing nucleosomes than those that

Figure 6 Promoters can influence histone eviction/deposition within genes. (A) Histone H3 occupancy at GCD1, ADH1 and YLR454W in the spt6-td degron and WT strains (B, D) Pol II ChIP for the integrated GCD1- and ADH1-YLR454W genes in isogenic spt6-td strains DBY1327, 1330 and WT strains DBY1369, 1370 at 37° C + Dox quantified by Q-PCR. Signals were normalized to the YLR454W – 215 flanking region. Means of PCRs from two biological replicates (two PCRs of each) and standard deviations (s.d.) are shown. Values refer to positions of the centre of the amplicons relative to the ATG of YLR454W. GCD1-YLR and ADH1-YLR are amplicons that span the boundary of these sequences at the 5′ end. (C, E) Histone H3 ChIP Q-PCR normalized to telomere VIR (TEL) for the GCD1- and ADH1-YLR454W genes in WT and spt6-td strains at 37° C + Dox. Maps of the amplicons are not to scale. Means of PCRs from two biological replicates (three PCRs of each) with s.d. are shown. Note the distinct effects of Spt6 inactivation on H3 occupancy within YLR454W driven by different promoters.

(Supplementary Figure S6). Spt6 inactivation had only a small effect at APA1-YLR454W with notably little loss of H3 occupancy, similar to the GCD1 fusion and DPN genes as a whole. In contrast, both fusions driven by OPN promoters, PDC1 and ADH1, responded to Spt6 inactivation with H3 depletion at the 5′ end and enrichment at the 3′ end. While we cannot eliminate the possibility that differences in pol II occupancy within YLR454W fusion genes play some role, the promoter-swapping experiments are fully consistent with the idea that the differences in histone dynamics between closed/OPN and open/DPN genes (Figure 5) correlate with their promoters rather than their coding sequences.
have elongated a long distance. It will be of interest in future
to examine whether the dynamics of elongation factor re-
cruitment or CTD phosphorylation influence co-transcrip-
tional nucleosome displacement.

We noted that relative histone occupancy was elevated
at specific loci in Spt6-depleted cells particularly within
genes with slow rates of histone exchange, and in 3' flanking
intergenic regions (Figure 1E, F, 4D and 6C; Supplementary
Figures S2g, S4e and f). One possible explanation for this
observation is that when Spt6 activity is low, histones evicted
from actively transcribed regions could be re-deposited
elsewhere by other chaperones such as Asf1 and FACT
(De Koning et al., 2007).

Spt6 inactivation caused remarkably highly localized his-
tone depletion over a region corresponding to 1–2 nucleo-
somes near the 5' ends of Ty and δ elements about 200–500
bases from the TSS (Figure 4A–C; Supplementary Figure S4d).
This phenomenon may explain why spt6 mutants were first
isolated as suppressors of the transcriptional defect caused by
insertion of a δ element at the 5' end of H1S4 (Winston et al.,
1984). We speculate that induction of such a discrete NDR at a
δ element could rescue HIS4 transcription from a cryptic
promoter by providing access to the pol II transcriptional
apparatus. The DNA sequence of the H3-depleted region in
Ty elements does not have a low predicted nucleosome
occupancy score (Field et al., 2008). Why this region is so
exquisitely dependent on Spt6 for nucleosome maintenance is
an interesting open question.

We observed two effects of Spt6 depletion on pol II
distribution that likely reflect changes in transcription elon-
gation. On a few exceptionally long genes, pol II density
decreased 5'-3' in the absence of Spt6 suggesting that it is
required to maintain processivity over long distances (Figure
3A and B). More generally, Spt6 depletion depressed pol II
density at 5' ends relative to 3' ends, a bias that coincides
with greater histone loss at 5' ends. Together, these results
suggest that removal of 5' nucleosomes permits faster tran-
scriptional elongation and hence lower steady-state pol II
occupancy. Co-transcriptional pre-mRNA splicing in yeast
is thought to be coordinated with pol II pausing (Oesterreich
et al., 2011). It is possible that disrupted pausing near 5' ends
contributes to the spt6-associated splicing defect (Burckin
et al., 2005).

Histone loss in spt6 cells requires ongoing transcription
(Kaplan et al., 2003); however, our results show that there is
not a straightforward relationship between net histone
eviction and the density of pol II on a gene. Histone loss
 correlates with pol II density and more strongly with nuclear
runon signals (Figure 2B and C). However, there is consider-
able variation in histone eviction among genes with similar
transcriptional activities (Figure 2), suggesting that additional
factors influence this phenomenon (Gat-Viks and Vingron,
2009).

We investigated whether promoters help determine histone
occupancy within genes when Spt6 is depleted. There are two
major classes of promoters in yeast with distinct chromatin
conformations and transcription characteristics (Tirosh and
Barkai, 2008; Cairns, 2009; Zaugg and Luscombe, 2012).
‘Open’ promoters with ‘depleted proximal nucleosomes’
(DPN) are predominantly found at constitutively expressed
genes lacking TATA boxes. ‘Closed’ promoters with ‘occupied
proximal nucleosomes’ (OPN) often have TATA boxes, are
more sensitive to regulation by chromatin remodelers, and
drive ‘noisy’ expression associated with bursts of
transcription initiation (Raser and O’Shea, 2004; Zenklusen
et al., 2008; Chubb and Liverpool, 2010). A major conclusion
of this report is that chromatin within genes driven by these
two classes of promoter is affected differently by Spt6
depletion. Within genes driven by DPN/open promoters,
there was relatively modest histone eviction when Spt6 was
inactivated whereas at genes driven by OPN/closed
promoters with equivalent or lower pol II density, eviction
was greater and extended further towards 3' ends (Figure 5).
This connection between promoter class and downstream
histone occupancy suggests that promoters in some way
influence chromatin dynamics within their cognate transcrip-
tion units. A second possibility is that sequence elements
within the genes driven by DPN/open and OPN/closed
promoters specify different levels of histone occupancy in the
absence of the Spt6 chaperone. To distinguish between these
possibilities, we performed a promoter swapping experiment.
We compared two chromosomal reporter genes with a com-
mon transcribed sequence, the YLR454W ORF, driven by
open/DPN (GCD1, APA1) or closed/OPN (ADH1, PDC1)
promoters. This experiment revealed distinct outcomes for
how histone occupancy was affected by Spt6 inactivation, strongly suggesting that promoters can influence chromatin
dynamics within adjacent transcribed sequences (Figure 6;
Supplementary Figure S6). Moreover, the changes in histone
occupancy within YLR454W sequences in the chimaeric
genes, approximated those in the cognate natural genes
when Spt6 was depleted (Figure 6; Supplementary Figure S6
compare a, c and e). The latter result suggests that the nature of
a promoter might exert a qualitative effect on histone dynamics
within the adjacent transcription unit. This promoter effect
on histone occupancy is not easily accounted for by differences
in average pol II density within the downstream gene.

Promoters have been suggested to control aspects of
gene expression beyond the amount of pol II that loads
onto a gene including CTD phosphorylation (Kim et al.,
2010), alternative splicing (Cramer et al., 1997; Kornblihtt,
2005) and mRNA degradation (Bregman et al., 2011; Trycek
et al., 2011). Our results raise the question ‘How could
promoters specify different patterns of chromatin dynamics
within a common downstream sequence?’ One possibility is
that they affect Spt6 recruitment or activity, however, we did
not observe a marked difference between Spt6 levels relative
to pol II on ‘closed’/OPN versus ‘open’/DPN genes
(Supplementary Figure S5c and d). Promoters could also
control recruitment of elongation factors that might affect
histone eviction. Pol II transcription elongation in metazoans
is influenced by transcription factors, promoters and enhan-
cers (Bentley, 1995) and histone eviction in vitro is enhanced
by faster elongation (Bintu et al., 2011). Alternatively,
promoters could affect histone eviction by controlling the
timing of initiation events which can occur as uncorrelated
events or as bursts (Chubb and Liverpool, 2010). Transcription factors and promoter elements influence
bursting activity in mammalian cells (Stavreva et al., 2009;
Suter et al., 2011). In yeast, transcriptional bursting and
consequent noisy expression is associated with TATA box-
containing promoters (Raser and O’Shea, 2004; Zenklusen
et al., 2008) with ‘closed’/OPN chromatin conformations
where we observed the most extensive histone eviction.
We suggest that clusters of pol II molecules at 5' ends of genes resulting from bursts of initiation are more effective at evicting nucleosomes than single isolated polymerases. This idea is consistent with the strong 5'-3' bias in histone depletion evident on long genes (Figure 1E and F) and with greater depletion on genes driven by 'noisy' promoters in sp6-td cells (Figure 5). Precedent for this model comes from the fact that pairs of E. coli RNA polymerase molecules cooperate to elongate through a nucleosome barrier better than single polymerases (Jin et al, 2010). It will be of interest in future to investigate how transcriptional bursting and elongation rate affect histone exchange within genes.

Materials and methods

Yeast strains

Yeast strains used in this study are described in Table I. The Sp6-td-3HA strain (DBY875) was constructed in two steps: first, the C-terminal HA3 C-terminal tag was added by integration of a PCR fragment amplified from pAF3-3HAkanMX6 into DBY311 to make DBY871. Second, the N-terminal ts degron Sp6 fusion plasmid (pRS306 Sp6-td) was integrated into DBY871 as described (Seward et al, 2007) at the endogenous SP6 locus. We cannot exclude the possibility that substitution of the tet repressible promoter alters Sp6 expression under permissive conditions (25°C); however, we did not detect a large effect on HA-Sp6 ChIP signals relative to the globin recovery control (Supplementary Figure S1a and b compare lanes 9 and 11). YLR454W promoter swapping plasmids were constructed by insertion of PCR-amplified promoter regions of GDC1 (−154 to +150 relative to the ATG), ADH1 (−840 to +240), APA1 (YCL050C, −517 to +150) and PDC1 (−889 to +141) into the BamHI-SacI sites of pFL445 YLR454W-hygro which contains two fragments of YLR454W (−590 to −149 and +1 to +522) with flanking Norl sites situated on either side of the hygromycin resistance gene. Norl fragments excised from the plasmids were integrated into DBY875 and DBY311 (Table I).

ChIP

Cells were grown in YPD at 25°C to OD ~0.8 and crosslinked for 15 min at room temperature in 1% formaldehyde for ChIP as described (Perales et al, 2011). Because we used total sheared chromatin rather than micrococcal nuclease-resistant fragments, our analysis does not permit localization of individual positioned nucleosomes. For inactivation of the Sp6 td degron, cells were pelleted and resuspended in YPD at 37°C with 2–10 μg/ml doxycycline (Dox) and incubated at 37°C for 1 h. Q-PCR and primers have been described (Zhang et al, 2005; Perales et al, 2011) and are available on request.

ChIP-Chip and ChIP-seq

For anti-pol II and anti-H3 ChIP-Chip of DBY311 and DBY875, immunoprecipitated DNA was amplified by ligation-mediated PCR (23 cycles) and hybridized to Nimblegen whole genome arrays (cat. no. C4214-00-01 with 378 664 50mers tilled every 32 bases) and analysis with the ChIP-Viewer program using the Tk/Tcl platform implemented in the R statistics package as described (Kim et al, 2010). Log2 ratios of ChIP signal/input were scaled to be centred around zero as described (Nimblegen Epigenetics Userguide) and were averaged over 20 base intervals and smoothed using the locopoly Gaussian kernel algorithm in the R package. Note that in the figures Y axes for overlaid log2 ChIP signals may differ between genotypes. The H3 ChIP-Chip signals from overlapping sets of 50mers within the arrays were highly reproducible (Supplementary Figure S1e and f). For metagene analysis, data points within each gene were scaled and averaged over 10 equal intervals with fixed lengths of 5' and 3' flanking sequences. Normalization of ChIP-Chip results according to Peng et al (2007) did not alter any of the results reported here.

ChIP-seq libraries were made as described (Kim et al, 2011) in experiments independent of the ChIP-Chip. True-Seq indices were added in the final PCR amplification (18 cycles) and sequencing was done on the Illumina HiSeq platform at UCDenver. Single-end reads (after removing barcodes) were mapped to the SacCer3 genome (April 2011, GeneBank Assembly GCA_00140454.5) with Bowtie version 0.12.5 (Langmead et al, 2009). Using option -m 2 in Bowtie, we obtained 1.33 × 10^7 (92% mapping) reads for anti-pol II in W303 (25°C), and 2.11 × 10^7 (90% mapping) and 1.01 × 10^7 (90% mapping) reads for anti-H3 ChIP-seq in DBY871 (WT, 37°C) and DBY1330 (sp6-td 37°C), respectively. Signals are expressed as reads per bin per million mapped reads (RPBM). For mapping to repetitive elements, we used the following bowtie options: '-m 2 -best -strata'. The numbers of read counts were weighted by the inverse of the number of multiple hits. Bedgraph profiles were made using 50 bp bins assuming a 200-bp fragment size shifting effect. There was good agreement between ChIP-Q-PCR, ChIP-Chip and ChIP-seq results (Supplementary Figure S1a–d). For meta-analysis, genes were divided into bins as defined in the figures and for each bin the mean number of aligned reads per bp normalized to the total number

Table I  Yeast strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Parent</th>
<th>Genotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBY311</td>
<td>W303-1a</td>
<td>MAT a ura3-1 his3-11 leu2-3,112 ade2-1 trp1-1 LEU2::pCM245 TetR-SSN6 His3::pRS403His3::pRS403-188 CMV I TA [pFL45SUBR1]</td>
<td>Seward et al (2007)</td>
</tr>
<tr>
<td>DBY871</td>
<td>DBY311</td>
<td>MAT a ura3-1 his3-11 leu2-3,112 ade2-1 trp1-1 LEU2::pCM245 TetR-SSN6 His3::pRS403His3::pRS403-188 CMV I TA [pFL45SUBR1] SPT6::HA3Kan</td>
<td>This study</td>
</tr>
<tr>
<td>DBY875</td>
<td>DBY871</td>
<td>MAT a ura3-1 his3-11 leu2-3,112 ade2-1 trp1-1 LEU2::pCM245 TetR-SSN6 His3::pRS403His3::pRS403-188 CMV I TA [pFL45SUBR1]</td>
<td>This study</td>
</tr>
<tr>
<td>DBY1327</td>
<td>DBY875</td>
<td>MAT a ura3-1 his3-11 leu2-3,112 ade2-1 trp1-1 LEU2::pCM245 TetR-SSN6 His3::pRS403His3::pRS403-188 CMV I TA [pFL45SUBR1]</td>
<td>This study</td>
</tr>
<tr>
<td>DBY1330</td>
<td>DBY875</td>
<td>MAT a ura3-1 his3-11 leu2-3,112 ade2-1 trp1-1 LEU2::pCM245 TetR-SSN6 His3::pRS403His3::pRS403-188 CMV I TA [pFL45SUBR1]</td>
<td>This study</td>
</tr>
<tr>
<td>DBY1369</td>
<td>DBY1330</td>
<td>MAT a ura3-1 his3-11 leu2-3,112 ade2-1 trp1-1 LEU2::pCM245 TetR-SSN6 His3::pRS403His3::pRS403-188 CMV I TA [pFL45SUBR1]</td>
<td>This study</td>
</tr>
<tr>
<td>DBY1370</td>
<td>DBY1369</td>
<td>MAT a ura3-1 his3-11 leu2-3,112 ade2-1 trp1-1 LEU2::pCM245 TetR-SSN6 His3::pRS403His3::pRS403-188 CMV I TA [pFL45SUBR1]</td>
<td>This study</td>
</tr>
<tr>
<td>DBY1392</td>
<td>DBY1370</td>
<td>MAT a ura3-1 his3-11 leu2-3,112 ade2-1 trp1-1 LEU2::pCM245 TetR-SSN6 His3::pRS403His3::pRS403-188 CMV I TA [pFL45SUBR1]</td>
<td>This study</td>
</tr>
<tr>
<td>DBY1396</td>
<td>DBY1392</td>
<td>MAT a ura3-1 his3-11 leu2-3,112 ade2-1 trp1-1 LEU2::pCM245 TetR-SSN6 His3::pRS403His3::pRS403-188 CMV I TA [pFL45SUBR1]</td>
<td>This study</td>
</tr>
<tr>
<td>DBY1398</td>
<td>DBY875</td>
<td>MAT a ura3-1 his3-11 leu2-3,112 ade2-1 trp1-1 LEU2::pCM245 TetR-SSN6 His3::pRS403His3::pRS403-188 CMV I TA [pFL45SUBR1] APA1::YLR454W-Hygro</td>
<td>This study</td>
</tr>
<tr>
<td>DBY1402</td>
<td>DBY875</td>
<td>MAT a ura3-1 his3-11 leu2-3,112 ade2-1 trp1-1 LEU2::pCM245 TetR-SSN6 His3::pRS403His3::pRS403-188 CMV I TA [pFL45SUBR1] APA1::YLR454W-Hygro</td>
<td>This study</td>
</tr>
</tbody>
</table>
of mapped reads for the genes analysed was calculated. In some cases, the number of genes included for meta-analysis of H3 ChIP-seq was slightly less than that for ChIP-Chip because those with extensive regions lacking reads were excluded. Data sets have been deposited at GEO accession GSE49928.

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**Author contributions:** RP, LZ and DB designed the experiments; RP, LZ, BE and EV performed ChIP and strain construction; RP, BE and HK performed data analysis; RP and DB wrote the manuscript.

**Conflict of interest**

The authors declare that they have no conflict of interest.

**References**


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