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Isw1 Chromatin Remodeling ATPase Coordinates Transcription Elongation and Termination by RNA Polymerase II

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Summary

We demonstrate that distinct forms of the yeast chromatin-remodeling enzyme Isw1p sequentially regulate each stage of the transcription cycle. The Isw1a complex (Iswlp/Ioc3p) represses gene expression at initiation through specific positioning of a promoter proximal dinucleosome, whereas the Isw1b complex (Iswlp/Ioc2p/Ioc4p) acts within coding regions to control the amount of RNA polymerase (RNAPII) released into productive elongation and to coordinate elongation with termination and pre-mRNA processing. These effects of Isw1b are controlled via phosphorylation of the heptad repeat carboxy-terminal domain (CTD) of RNAPII and methylation of the chromatin template. The transcription elongation factor Spt4p antagonizes Isw1p and overcomes the Isw1p dependent pausing of RNAPII at the onset of the elongation cycle. Overall these studies establish the central role played by Isw1p in the coordination of transcription.

Introduction

Chromatin plays an important role in transcriptional regulation and is generally considered to impede transcription initiation and elongation by RNA polymerase II (RNAPII). Chromatin structures over promoters are regulated by enzymes that covalently modify histones (Strahl and Allis, 2000) or alter chromatin by ATP-dependent disruption of DNA-histone interactions (Becker and Horz, 2002). These proteins are generally recruited to promoters by sequence-specific DNA binding proteins (Cosma et al., 1999). During the elongation phase of transcription, RNAPII also uses a wide range of accessory factors to facilitate its movement through chromatin (Hartzog, 2003) and several RNAPII-associated complexes have been identified (Shilatifard et al., 2003). For example, in yeast, some complexes, such as PAF (Krogan et al., 2002), Spt4/5, and TFIIS (Dst1p) (Pokholok et al., 2002) are all associated with RNAPII throughout the elongation phase. However, other factors are localized to the 5′ region of genes such as capping enzymes (Cho et al., 1997, 1998; McCracken et al., 1997; Rodriguez et al., 2000) or to the 3′ region of genes such as the cleavage/polyadenylation complex CF1A (Licatalosi et al., 2002). Differential association between complexes and RNAPII appears to be a function of the heptapeptide repeat (Tyr-Ser-Pro-Thr-Ser-Pro-Ser) (Allison et al., 1985) of the carboxy-terminal domain (CTD) of the largest subunit, Rpb1p, which is subject to differential phosphorylation. Hyperphosphorylation at Ser5 of the CTD by the Kin28p kinase subunit of TFIIH promotes disengagement of the enzyme from the promoter into the elongation phase of transcription (Rodriguez et al., 2000) coupled with the exchange of initiation factors for elongation factors (Pokholok et al., 2002). Ser5 phosphorylation then diminishes in the coding region while levels of Ser2 phosphorylation increase as RNAPII moves toward the 3′ region (Komarnitsky et al., 2000). Significantly capping factors (Cho et al., 1998; McCracken et al., 1997; Rodriguez et al., 2000; Schroeder et al., 2000) and the Set1p complex (Ng et al., 2003) interact with phospho-Ser5 CTD while CF1A (Barilla et al., 2001; Licatalosi et al. 2002), and the Set2p histone methylase (Krogan et al., 2003; Li et al., 2003; Xiao et al., 2003), interact with phospho-Ser2 CTD. Thus, the differential association of complexes to the CTD links the various phases of pre-mRNA synthesis, processing, and export. Furthermore, differential patterns of histone H3 methylation by Set1p (H3-K4) and Set2p (H3-K36) may respectively mark out the early and later stages of elongation and promote association of additional factors to the chromatin template to regulate these events (Krogan et al., 2003; Ng et al., 2003; Xiao et al., 2003). Candidates for this function are the FACT complex that facilitates transcription through chromatin (Orphanides et al., 1999), and the chromodomain containing chromatin-remodeling ATPase, Chd1p (Woodage et al., 1997), recently proposed to function as an elongation factor (Simic et al., 2003). Chd1p has also been identified as a termination factor at some yeast genes but at other genes Chd1p functions redundantly with the imitation switch (ISWI) chromatin-remodeling ATPases, Isw1p and Isw2p (Alen et al., 2002). Furthermore, we have previously observed a highly localized chromatin organization within the coding regions of a number of yeast genes that is dependent on the catalytic activity of Isw1p chromatin remodeling activity (Kent et al., 2001). We therefore address in this study the potential role played by Isw1p in the elongation and termination of transcription.

Chromatin remodeling enzymes of the ISWI type (Clapier et al., 2001) are widely found in eukaryotes and are implicated in events leading to repression of expression. Thus, ISWI is associated with nontranscribed regions of polytene chromosomes in *Drosophila* (Deuring et al., 2000) and may displace TBP from promoters in *Xenopus* (Kikyo et al., 2000) and yeast (Moreau et al., 2003). However, a positive role for ISWI cannot be excluded as it is required for the expression of some genes in *Drosophila* (Badenhorst et al., 2002). In addition, microarray data in yeast show that Isw1p and Isw2p, the two ISWI homo-

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logs (Tsukiyama et al., 1999), have both positive and negative effects on patterns of gene expression (Fazzio et al., 2001; Vary et al., 2003). Isw2p represses meiotic genes by configuring nucleosomes on promoters (Goldmark et al., 2000; Kent et al., 2001) and may function in collaboration with the Sin3/Rpd3 histone deacetylase (Fazzio et al., 2001). Although less is known about the function of Isw1p, it is present in two distinct complexes, Isw1a and Isw1b, which confer distinct properties to the ATPase (Vary et al., 2003). Isw1a contains Ioc (*i*sw *o*ne *c*omplex) 3p while Isw1b contains Ioc2p and Ioc4p. Here, we show that while Isw1a functions at promoters to repress genes, Isw1b possesses a bipartite function that coordinates both transcription elongation and termination.

Results

Isw1p Configures the MNase Cleavage Site between Nucleosomes −1 and +1 at *MET16*

A systematic analysis in vivo of Isw1p-dependent chromatin structures (Kent et al., 2001) identified several promoter proximal regions including *MET16* (Figure 1A). This gene is induced when methionine is absent from the growth medium (−Met) but repressed when it is present (+Met). The position of nucleosomes over *MET16* under repressed conditions is inferred from their ability to protect DNA from MNase cleavage at sites present in naked DNA (Figure 1A, lanes 2 and 3). In strains containing a deletion of *ISW1* (*isw1*Δ) or encoding an ATPase deficient version of Isw1p (*isw1*^{K227R}), MNase cleavage (thick arrow) at the region between Nuc −1 (light bar) and +1 (dark bar) is significantly reduced (Figures 1A and Supplemental Figure S1 available at http://www.cell.com/cgi/content/full/115/4/425/DC1). Thus, Isw1p configures two nucleosomes at the repressed *MET16* promoter. We examined whether this chromatin configuration changes on gene activation (−Met) compared to the repressed state (+Met). This analysis, at high resolution (Figure 1B) and low resolution (Figure 3C), revealed multiple changes to the chromatin structure centered around Nuc −1 and Nuc +1. First, the MNase cleavage at −110 becomes less distinct and the TATA region (−40) becomes hypersensitive to MNase cleavage. This would be consistent with remodeling of Nuc −1 associated with rearrangement of the Cbf1/Met4/Met28 activator complex at −110 to −130 (Kuras et al., 2002) and binding of TBP to the TATA box. Furthermore, both the cluster of MNase cleavage sites around +193 and the hypersensitive sites around +20 are substantially reduced on activation and the regions upstream and downstream of the +20 site cleave like naked DNA. This pattern of cleavage supports remodeling of Nuc +1 on activation of *MET16*. Finally, no further changes in MNase cleavage sites in the coding region are observed (data not shown). We conclude that under repressed conditions the *MET16* promoter is blocked by the Isw1p-dependent positioning of two nucleosomes −1 and +1. On activation, these nucleosomes are remodeled allowing exposure of the promoter to the transcription machinery.

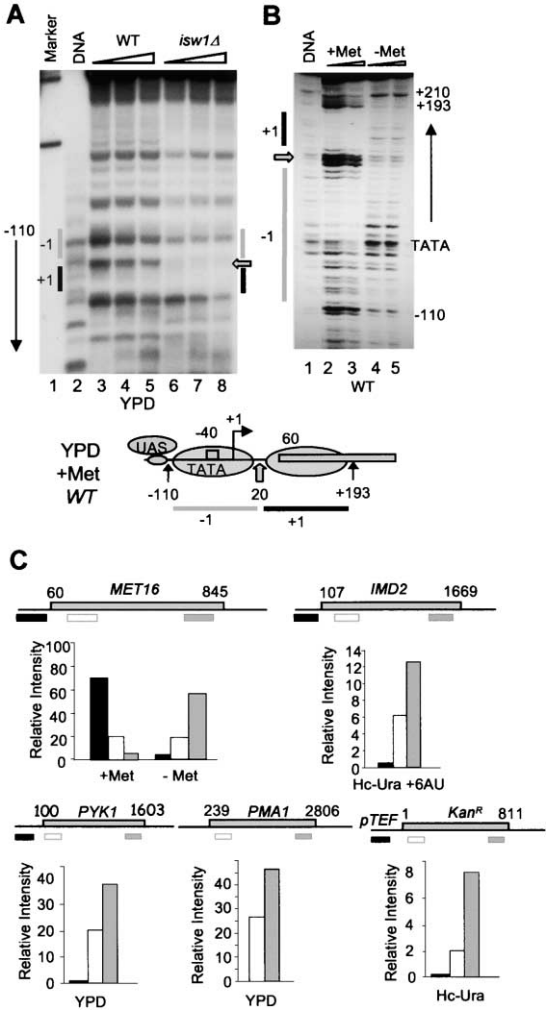


Figure 1. Isw1p Configures Nucleosomes at *MET16*

Isw1p-dependent change in micrococcal nuclease (MNase) accessibility in yeast chromatin at the *MET16* locus mapped by indirect end-label analysis. The position of the Isw1p dependent structure is marked with a thick arrow.

(A) Lane 1, marker digest (BglII −1.76 kb); lane 2, Naked DNA control; lanes 3–8, chromatin in permeabilized cells digested with 75 (lanes 3 and 6), 150 (lanes 4 and 7) and 300 (lanes 5 and 8) units/ml MNase. DNA was subject to 2° digestion with EcoRI (−7.2 kb to +0.798 kb) and a Southern blot of DNA hybridized to an EcoRI (+0.796 kb)/*Msc*1 (+0.473 kb) fragment in the coding region allowing analysis toward the promoter region. The arrow represents the direction of the *MET16* gene on the fragment starting at +1 (RNA initiation site).

(B) Lane 1, Naked DNA control; lanes 2–5 permeabilized cells digested with 5 and 10 U/ml MNase after growth ± 1 mM Met (+Met) (lanes 2–3); (−Met) (lanes 4–5) and cleavage sites analyzed by high resolution on the transcribed strand from the HpaII site at −0.21 kb to the HpaII site at +386. The arrow represents the direction of the *MET16* gene on the fragment starting at +1. A schematic showing the position of nucleosomes in +Met is shown. The position of major MNase cleavage sites around Nuc −1 (light line) and Nuc +1 (dark line) is show relative to the ATG at +60, TATA region (black box −40), UAS (upstream of −110).

(C) Distinct distribution of the Isw1 ATPase on active or repressed genes. Schematics of the genes showing the position of the PCR products, relative to the transcription initiation site (+1; except *pTEF:Kan*^{R}), used in real-time PCR to detect DNA after immunoprecipitation of chromatin with antibodies to the myc epitope (9E10; Sigma) from W303-1a based strains expressing Isw1-myc cultured in YPD (+Met), methionine free medium (Hc-Met), Hc minus uracil +6AU (induced for 60 min) or Hc minus uracil.

Isw1p Is Associated with the Transcribed Regions of Active Genes

As Isw1p is required to configure the MNase cleavage site between Nuc −1 and +1, we asked whether Isw1p is found at this region of chromatin. We immunoprecipitated fixed chromatin (ChIP) from a strain expressing myc tagged Isw1p and used real-time PCR to amplify DNA with primers specific to the region occupied by nucleosome (Nuc) −1 (black box), +1 (white box), and +5 (gray box) (Figure 1C). In the repressed state (+Met), Isw1-myc is strongly associated with chromatin at Nuc-1, more weakly at Nuc +1 and at low levels with Nuc +5. When expression is derepressed (−Met), the ChIP signals at −1 are more than 10-fold lower than the +Met signal while at +1 they are similar. By contrast, high levels of Isw1p could be detected at +5. We conclude that the distribution of Isw1p across *MET16* depends on whether the gene is repressed or active. To see if the association of Isw1p with the coding regions of active genes is more general, we tested four more genes. *IMD2* is induced strongly by the drug 6 azauracil (6AU) that mimics nucleotide depletion (Shaw and Reines, 2000). *PMA1* and *PYK1* are examples of highly expressed genes that are widely used to study gene expression (Komarnitsky et al., 2000; Ng et al., 2003). *pTEF-Kan*^{R} is a hybrid gene comprising the *A. gossypii TEF* promoter linked to the bacterial *Kan*^{R} gene, widely used as a marker gene in yeast (Wach, 1996). Isw1-myc could be detected associated with the coding region of all four genes when actively transcribing (Figure 1C).

Opposite Effects of Isw1p and Spt4p on the Density of Transcriptionally Engaged RNAPII

Factors that localize to internal regions of transcription units are likely to function in the control of transcript elongation (Pokholok et al., 2002). To directly monitor the production of nascent transcripts, we have employed transcription run on (TRO) analysis across *pTEF:Kan*^{R} (Figures 2A and 2B; Supplemental Figure S2 available on *Cell* website), since this measures the relative density of engaged RNAPII molecules along a gene. As reference points we compared the profile of *isw1*^{K227R} to a wild-type (*WT*) strain as well as a strain (*spt4*Δ) lacking the positive transcript elongation factor, Spt4p (Hartzog et al., 1998). In *WT*, robust TRO signals were observed on the transcription unit that dropped to background levels (M13) just past the poly(A) site in the *GAL10-7* intergenic region indicating efficient termination of transcription. In *spt4*Δ, RNAPII at the 5′ region is actively transcribing as a strong signal is observed at probe 1. However, much lower signals are observed at the 3′ end of the gene, suggesting that much of the engaged RNAPII at the 5′ region is not released into the productive phase of transcript elongation. Thus, RNAPII may be in an abortive transcription cycle at the 5′ part of the transcription unit. This suggests that Spt4p promotes productive elongation by RNAPII. By contrast, in *isw1*^{K227R} the TRO profile was significantly different with strong signals at both the 5′ and 3′ regions of the transcription unit. Moreover, this signal extended past the poly(A) site into the *GAL10-7* intergenic region. This indicates the presence of engaged RNAPII past the poly(A) site and strongly supports a role for the ATPase of Isw1p

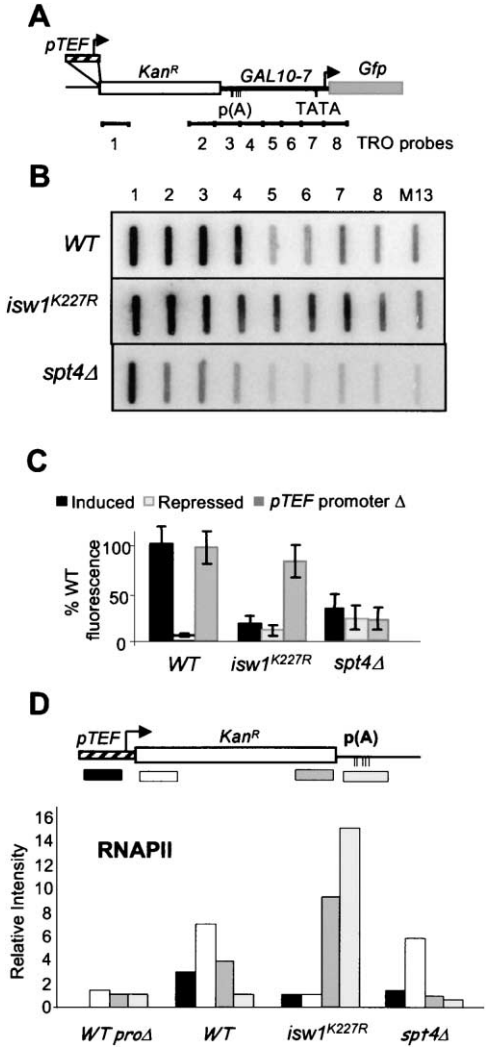


Figure 2. Isw1p and Spt4p Have Opposite Effects on RNAPII

(A) Schematic of the transcription run on (TRO) and transcriptional interference (TI) assay constructs. A p*TEF:Kan*^{R} fusion is positioned upstream of the *GAL10-7* intergenic region driving expression of the G*fp* gene. A second version lacks the *pTEF* promoter (Δpro). Positions of DNA transcription run on (TRO) probes (1–8) are under the schematic.

(B) Autoradiograph of a representative TRO experiment showing the hybridization signal to each probe (1–8). M13 represents the background hybridization. Quantitation of three independent experiments is shown in Supplemental Figure S2 available on *Cell* website.

(C) The effect of transcription interference at the *GAL7* promoter monitored by GFP production (expressed as a % of wild-type for four independent experiments) in synthetic medium containing galactose (*GAL7* induced; black box) or glucose (*GAL7* repressed; pale box) or a promoter less version induced with galactose (gray box).

(D) Chromatin immunoprecipitation experiment to detect RNAPII (H-244 Santa Cruz) across *pTEF:Kan*^{R}. WT *pro*Δ indicates a strain transformed with the promoter-less version of the construct. The PCR products generated by real-time PCR are indicated on the schematic.

in pre-mRNA 3′ end formation (Alen et al., 2002). To confirm these results, we used a previously described transcriptional interference (TI) assay (Figure 2C; Morillon et al., 2003). TI occurs between the tandem *GAL10* and *GAL7* genes when 3′ end formation (termination) at

GAL10 is disrupted by deletion of the poly(A) signal (Greger and Proudfoot, 1998). In this assay, a *Gfp* reporter gene is fused to the *GAL7* promoter and the degree of interference at the *GAL7* promoter measured by the reduction in GFP produced (Figure 2A). When induced by galactose, *WT* was able to express Gfp from the *GAL7* promoter regardless of whether the promoter at the interfering upstream gene was present or not, since efficient termination occurs at the *GAL10* poly (A) site (Figure 2C). As expected, *spt4*Δ expressed Gfp poorly under all conditions. By contrast, we observe a dramatic interference effect in *isw1*^{K227R}. There is a 4-fold reduction in the GFP signal that is dependent on expression of the upstream gene. These data confirm that functional Isw1p ATPase is required for *GAL10* termination.

Opposite Effects of Isw1p and Spt4p on the Distribution of RNAPII across the Transcription Unit

Next we compared the density of total RNAPII across the *pTEF:Kan*^{R} gene detected by chromatin immunoprecipitation (Figure 2D) with the TRO profiles (Figure 2B). The ChIP profiles of RNAPII in *WT* and *spt4*Δ broadly recapitulate the TRO signals. In *WT*, a gradient of RNAPII is observed from the beginning to the end of the *pTEF:Kan*^{R} gene. In contrast, for *spt4*Δ very little RNAPII is detected beyond the genes 5′ end. This may reflect control over the amount of RNAPII released into productive elongation, which is influenced by the action of Spt4p.

Surprisingly, the profile of RNAPII across *pTEF:Kan*^{R} is markedly different from the TRO profile in *isw1*^{K227R}. First, the amount of RNAPII detectable in the 5′ part of the transcription unit is severely reduced even though TRO profiles obtained from cells grown in the same cultures indicate robust transcription. Thus, the ATPase of Isw1p may be required for the accumulation of RNAPII at the 5′ promoter proximal region. Second, we observe a 15-fold increase in the amount of RNAPII at the genes 3′ end as compared to *WT*. Since TRO analysis shows an even profile over both gene and 3′ flanking regions in *isw1*^{K227R}, the large accumulation of 3′ flanking RNAPII is likely to reflect RNAPII that is not actively engaged in transcription. This accumulation of disengaged RNAPII may represent a failure to dissociate RNAPII from the 3′ region of the template. Taken together, these data suggest that the Isw1p ATPase influences the level and activity of RNAPII at both the 5′ and 3′ regions of the transcription unit. To explore further how the Isw1p ATPase regulates RNAPII, we next addressed how the positive elongation factors such as Spt4p or Dst1p (Morillon et al., 2003; Simic et al., 2003) influence Isw1p.

A Mutation in *ISWI* Suppresses the 6AU Sensitivity of *dst1*Δ and *spt4*Δ

Strains lacking Spt4p and Dst1p show a marked 6AU^{S} phenotype (Figure 3A) (Archambault et al., 1992; Hartzog et al., 1998; Kulish and Struhl, 2001). 6AU depletes the cellular pools of UTP and GTP and is believed to render RNAPII unable to elongate efficiently without positive transcription elongation factors (Exinger and Lacroute, 1992). In contrast, *isw1*Δ or *isw1*^{K227R} show the unusual

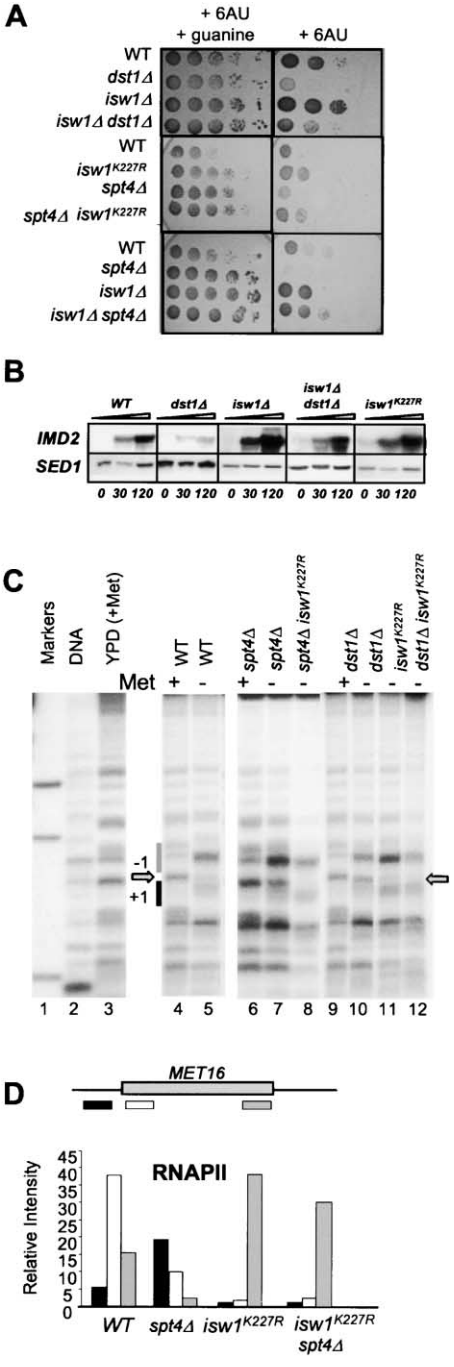


Figure 3. Genetic Interactions between *ISW1*, *DST1*, and *SPT4*

(A) Sensitivity to 6-azauracil (6AU). 10-fold serial dilutions were plated onto Hc-uracil +6AU ± guanine (top 75 ug/ml, middle 100 ug/ml, bottom 150 ug/ml 6AU and guanine).

(B) Northern blot analysis of total RNA induced with 100 μg/ml 6AU for 0, 30, or 120 min. Radioactive probes were used to detect *IMD* (top) or *SED1* transcripts (bottom).

(C) Micrococcal nuclease digestion of chromatin. See Figure 1 for details. The Isw1p dependent cleavage is marked with a thick arrow between Nuc −1 (light line) and Nuc +1 (dark line). Lane 1, marker digest; lane 2, naked DNA; lanes 3–12, chromatin in permeabilized cells digested with 300 units/ml MNase after growth in YPD (lane 1); Hc –uracil ± 1 mM Met (+ lanes 4, 6, and 9; − lanes 5, 7, 8, 10–12).

(D) Cells were grown overnight in Hc-met, crosslinked chromatin precipitated with antibodies to RNAPII (H-244 Santa Cruz) and DNA detected using real-time PCR to amplify regions shown at *MET16*.

characteristic of resistance to 6AU ($6AU^R$) (Figure 3A). This suggests that *isw1*Δ does not generate the $6AU^S$ phenotype associated with *dst1*Δ or *spt4*Δ, consistent with opposing modes of action. Moreover, ablation of Isw1p is sufficient to partially suppress the sensitivity of *dst1*Δ and to completely suppress the $6AU^S$ of *spt4*Δ (Figure 3A). This suggests that $6AU^R$ associated with the *isw1* mutations is dominant over the $6AU^S$ associated with *dst1*Δ or *spt4*Δ.

Resistance to 6AU treatment is connected with the ability to rapidly induce expression of the *IMD* genes, demonstrated targets of Isw1p ATPase regulation (Supplemental Figure S6 available on *Cell* website). In *WT*, induction occurs within 30 min of 6AU treatment and RNA continues to accumulate for 2 hr (Shaw and Reines, 2000) (Figure 3B). Consistent with the $6AU^R$ phenotype, the *IMD* genes are strongly induced over a two hour induction period in *isw1*Δ and importantly, this mutation suppresses the induction defect in *dst1*Δ (Figure 3B) or *spt4*Δ (data not shown). We conclude that in $isw1^{K227R}$, RNAPII is no longer dependent on transcription elongation factors even under conditions of nucleotide depletion.

Spt4p and Dst1p Are Required to Remodel Nucleosome +1 at *MET16*

Isw1p possesses ATPase activity that functions to slide nucleosomes on DNA while both Dst1p and Spt4p are proposed to help RNAPII overcome the nucleosomal barrier to elongation (Fish and Kane, 2002; Hartzog et al., 2002). We reasoned that Isw1p might provide a block to elongation, possibly nucleosomal, while Dst1p and Spt4p are required to overcome this block. If so, in the absence of the Isw1p-dependent block, Dst1p and Spt4p should become redundant. We therefore examined the local chromatin organization at *MET16* in *spt4*Δ, d*st1*Δ, and $isw1^{K227R}$ mutant strains (Figure 3C; see Figure 1). The chromatin organization in the repressed state (+Met) in *spt4*Δ resembles *WT* (Figure 3C compare lanes 4 and 6). However, on activation (−Met) the TATA region becomes hypersensitive, as observed in *WT*, but the hypersensitivity at the +20 region (thick arrow) is not lost in *spt4*Δ (compare lanes 5 and 7). This suggests that in the majority of cells Nuc +1 is not fully remodeled. In the double $isw1^{K227R}$ *spt4*Δ mutant, we observed a structure similar to that in the single $isw1^{K227R}$ ATPase mutation, consistent with full remodeling of Nuc +1 (compare lanes 8 and 11). Thus, in $isw1^{K227R}$, Spt4p is no longer required for remodeling of Nuc +1. A similar observation was made for *dst1*Δ (lanes 9–12). We conclude that both Spt4p and Dst1p are required for remodeling of Nuc +1 at *MET16*. These data suggest that general elongation factors play in major role in the release of RNAPII into the elongation phase of transcription from a block imposed by the Isw1p ATPase. Indeed, based on ChIP analysis, RNAPII accumulates at the *MET16* promoter in *spt4*Δ in an $isw1^{K227R}$ dependent fashion (Figure 3D).

Normal CTD Phosphorylation Profiles on RNAPII Require the Isw1p ATPase

We have recently demonstrated that RNAPII in *dst1*Δ or *spt4*Δ shows aberrant patterns of CTD phosphorylation (Morillon et al., 2003). This prompted us to ask whether the function of the Isw1p ATPase might also be reflected in CTD phosphorylation profiles.

We employed antibodies that recognize RNAPII phosphorylated at Ser5 or Ser2 of the CTD to perform ChIP analysis with real-time PCR over *MET16* (Figure 4). In $isw1^{K227R}$ the levels of Ser5 and Ser2 phosphorylation are drastically reduced throughout *MET16* compared to *WT* (Figures 4A and 4B). The low levels of Ser5 phosphorylation are likely to result from mislocalized recruitment of the Kin28p Ser5 kinase in $isw1^{K227R}$ (Figure 4C). Strains lacking the Isw1p ATPase also show defects in the association of the pre-mRNA processing enzymes, Ceg1p and Rna15p, at the 5′ and 3′ regions (Figures 4D and 4E). To confirm that *MET16* is still expressed in $isw1^{K227R}$, total RNA was hybridized to a *MET16* probe followed by a loading control (Figures 4F). The levels of transcript are similar in $isw1^{K227R}$ and *WT*. This suggests that although there are marked defects in the early and late elongation phases of transcription and associated cotranscriptional processing of the pre-mRNA, these do not prevent the accumulation of transcript.

The *spt4*Δ strain shows a marked defect in the Ser2 phosphorylation profile of the CTD and associated Rna15p recruitment (Figures 4B and 4E). Remarkably, high levels of Ser2 phosphorylation and Rna15p are associated with the promoter and the 5′ part of the transcription unit. This suggests the normal dynamic of CTD phosphorylation is disrupted. It is also evident that introduction of $isw1^{K227R}$ into *spt4*Δ suppresses the CTD phosphorylation defects as the double mutants all show the same characteristics as $isw1^{K227R}$. We conclude that the opposing actions of Isw1p ATPase and Spt4p toward RNAPII are reflected in CTD phosphorylation profiles.

Distinct Isw1p-Associated Proteins on the Active or Repressed *MET16* Chromatin

To determine whether the Isw1p associated with the *MET16* gene (Figure 5A) is associated with different partners, we epitope-tagged Ioc4p and Ioc2p, components of the Isw1b complex and Ioc3p, a component of the Isw1a complex and followed their distribution across *MET16*. The results obtained show a clear difference depending on whether *MET16* is expressed or not. When *MET16* is repressed by growth in YPD (+Met), the Ioc3p ChIP is centered at the promoter (Figure 5B). By contrast, there was no Ioc2p or Ioc4p signal detectable at the promoter or coding region. This suggests that Ioc3p (Isw1a) is preferentially associated with the inactive promoter. On activation of expression (−Met), the amount of Ioc3p-myc detectable at *MET16* was drastically reduced. By contrast, significant levels of Ioc2p and Ioc4p are detectable within the *MET16* coding region, especially at the end of the gene (Figures 5C and 5D), matching the Isw1p pattern following activation (Figure 5A). Thus, Isw1a is at the promoter of the repressed gene while Isw1b is associated with the coding region of the active gene.

Isw1a Represses Gene Expression

To see whether Isw1p or Ioc3p are required for *MET16* repression in the presence of methionine, we used ChIP and real-time PCR to measure the occupancy of RNAPII at the promoter and within the coding region in *WT*,

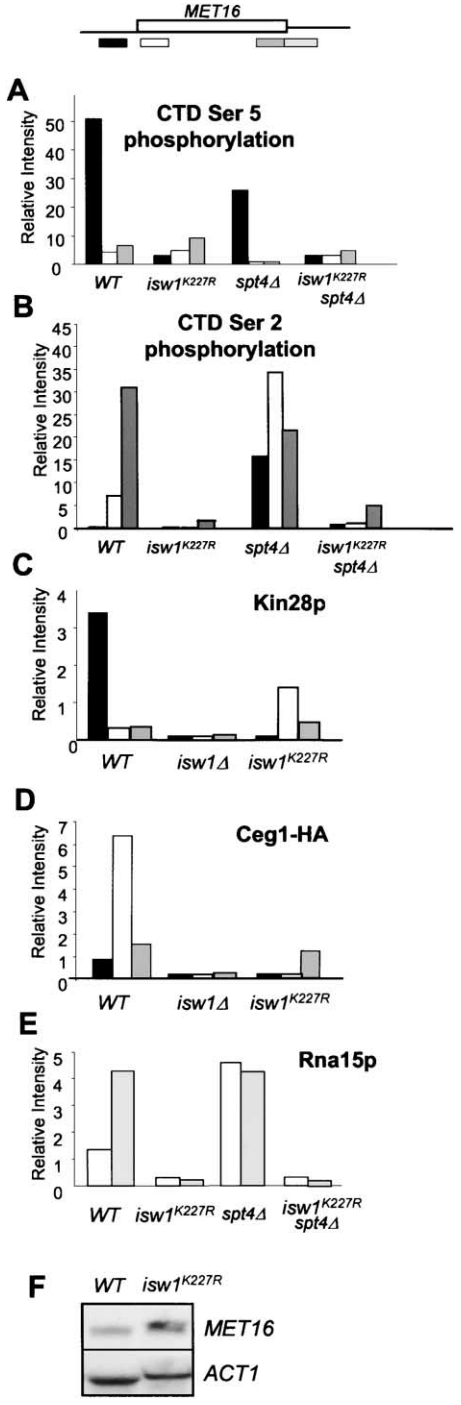


Figure 4. Isw1p and Spt4p Have Opposite Effects on RNAPII CTD Phosphorylation

Crosslinked chromatin from cells grown in Hc-met was precipitated with antibodies to (A) phosphorylated Ser5 (H14; Covance); (B) phosphorylated Ser2 (H5; Covance) (C) Kin28p; (D) the HA epitope on Ceg1p (12CA5 [Roche]); (E) Rna15p and real-time PCR used to amplify regions on *MET16*. (F) Northern blot analysis of total RNA prepared from strains grown in Hc-met. Radioactive probes were used to detect *MET16* (top image) or *ACT1* transcripts (bottom image).

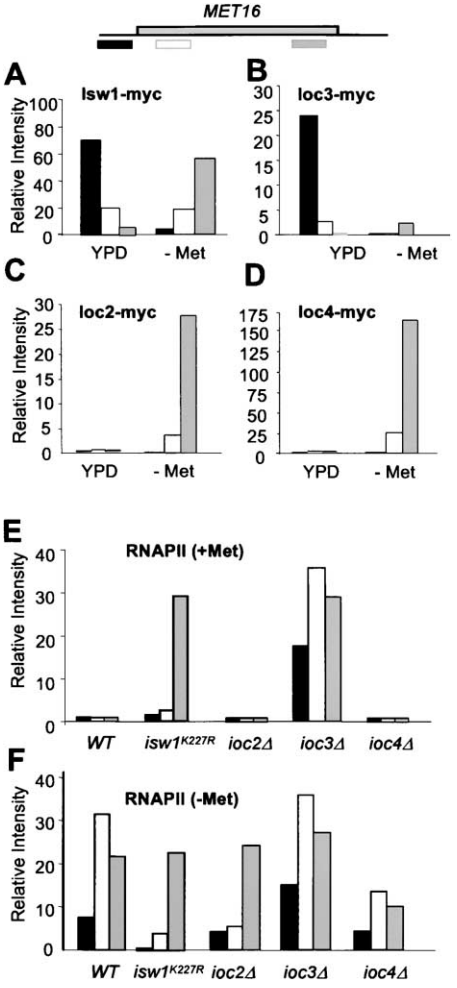


Figure 5. Distinct Forms of the Isw1 ATPase Influence RNAPII

Strains expressing Isw1-myc (A), Ioc3-myc (B), Ioc3-myc (C), Ioc4-myc (D), or with genotype indicated (E and F) were cultured in YPD (+Met; repressed) or Hc-Met (active), crosslinked chromatin precipitated with antibodies to the myc epitope (9E10; Sigma; A–D) or RNAPII (H-224; Santa Cruz; E–F) and detected with real-time PCR using primers indicated.

isw1^{K227R}, and *ioc3*Δ and compared these results to those obtained in strains lacking Ioc2p (*ioc2*Δ) and Ioc4p (*ioc4*Δ) (Figure 5E). No signal for RNAPII above background could be detected over the coding region of the repressed *MET16* gene in WT, *ioc2*Δ, or *ioc4*Δ. By contrast, RNAPII showed a skewed distribution in *isw1*^{K227R}, supporting a role for the ATPase in both repression and control of the distribution of RNAPII over the active gene. These two functions can be separated in *ioc3*Δ as the distribution of RNAPII resembles that in the induced *WT*, although reproducibly more enzyme is found throughout the gene (Figures 5E and 5F). In *ioc3*Δ, the residual Isw1p activity, probably in the Isw1b complex, ensures the normal distribution of the enzyme over the gene. This suggests that the Isw1a complex, but not the Isw1b complex, represses *MET16*. Furthermore, the ATPase of Isw1p is required for repression.

The Isw1 ATPase Activity and Ioc2p Control the Distribution of RNAPII across Activated Genes

The next question we addressed was the role of the Isw1 complex proteins in transcription of *MET16* when expression is activated (−Met). In *ioc3*Δ and *ioc4*Δ, the

distribution of RNAPII resembles *WT* (Figure 5F). The overall levels of RNAPII are significantly reduced in *ioc4*Δ, suggesting that Ioc4p plays a positive role in transcription. In *ioc2*Δ, RNAPII distribution was skewed to the 3′ regions, in a similar profile to *isw1*^{K227R}. Thus, the Ioc2p component of the Isw1b complex is likely to function to control the distribution of RNAPII across genes. Moreover, this data suggests that Ioc2p and Ioc4p have distinct functions.

Ioc2p Is Required for Ser5 Phosphorylation of the CTD

We examined the effect of mutations in *IOC2, IOC3*, and *IOC4* on the profile of Ser5 phosphorylation at *MET16*. In *ioc3*Δ and *ioc4*Δ, the pattern of Ser5 phosphorylation resembled the profile in *WT* (Figure 6A). However, in *ioc2*Δ and *isw1*^{K227R}, Ser5 phosphorylation at the CTD throughout *MET16* was barely detectible. These data suggest that Ser5 phosphorylation is a function of the Ioc2p component of the Isw1b complex. Moreover, this data links a normal RNAPII distribution with Ser5 phosphorylation of the CTD, as both require the Ioc2p component of Isw1b (Figure 5F).

Ioc4p Is Required for Ser2 Phosphorylation of the CTD

The *ioc4*Δ and the *ioc2*Δ strains show distinct profiles for Ser2 phosphorylation of the CTD and associated Rna15p recruitment (Figures 6B and 6C). The signals are greatly reduced at the promoter and throughout the gene in both the *ioc4*Δ and *isw1*^{K227R}. In contrast for *ioc2*Δ, the levels of Ser2 phosphorylation and Rna15p are elevated throughout the gene. Thus, Ioc4p is required for Ser2 phosphorylation and the associated events, while Ioc2p appears to regulate the activity and/or timing of these events. In this respect Ioc2p functions like Spt4p (Figure 4), as both influence the timing or activity of the Ser2 CTD kinase.

Ioc4p Is Required for Histone H3 Tail Methylation

A significant peak in Set1p-dependent H3 K4 trimethylation is observed in the early coding region of active genes including *MET16* gene (Figure 6D) (Ng et al., 2003; Santos-Rosa et al., 2002). Furthermore, the Set2p-dependent histone H3 K36 dimethylation accumulates toward the 3′ region of many genes including *MET16* (Figure 6E). Both the Isw1p ATPase activity and Ioc4p are required for normal levels of H3K4 trimethylation (Figure 6D) and H3K36 dimethylation (Figure 6E). Ioc2p appears to play a role in regulating these events. As expected, the profile of H3K36 dimethylation follows that of Ser2 phosphorylation in *ioc2*Δ, with a high signal across the whole gene (Figure 6E). Thus, both Ioc2p and Ioc4p contribute different functions to control the profiles of H3 K4 trimethylation and H3 K36 dimethylation.

The Generality of Isw1p Function at Yeast Genes

To address the generality of Isw1p function in regulating transcription elongation, we demonstrate a role for Isw1p in controlling the distribution of RNAPII and other associated parameters at the four genes, *IMD2*, *PMA1*, *PYK1*, and *pTEF:Kan*^{R}, known to have the Isw1 protein associated with the transcribed region (Figure 1C and

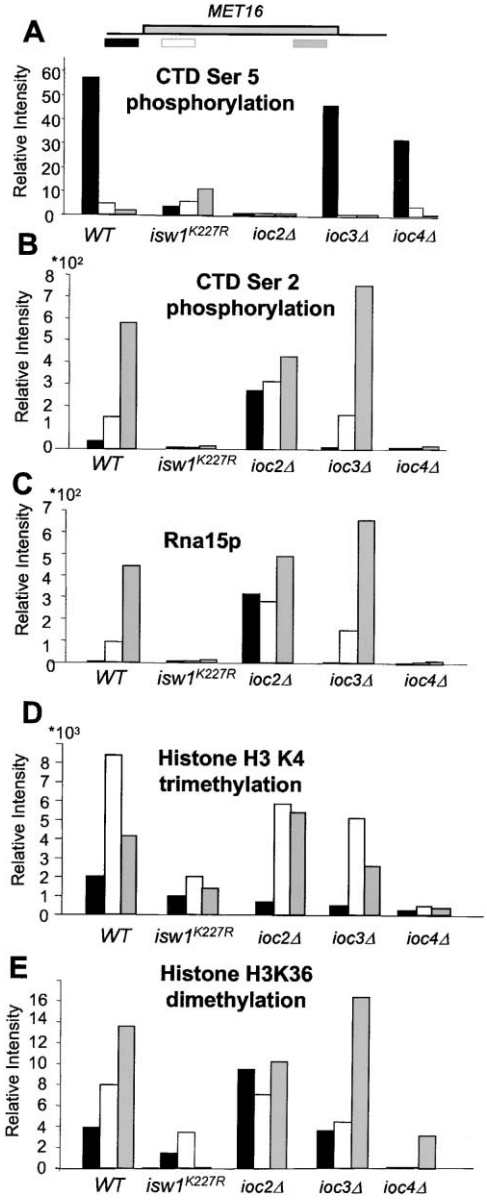


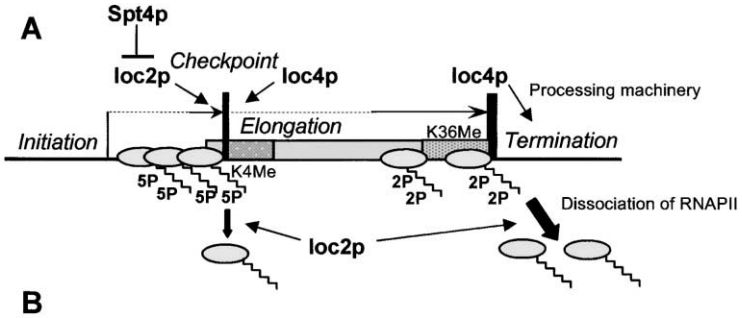
Figure 6. Distinct Forms of Isw1p ATPase Influence RNAPII CTD Phosphorylation and Chromatin Methylation

After overnight growth in Hc-methionine, crosslinked chromatin was precipitated with antibodies to (A) phosphorylated Ser5 (H14; Covance); (B) phosphorylated Ser2 (H5; Covance) (C) Rna15p; (D) Histone H3 trimethylated K4 (Abcam); (E) Histone H3 dimethylated K36 (Abcam) and DNA detected using real-time PCR to amplify regions shown at *MET16*.

Supplemental Figures S3–S6 available on *Cell* website). Thus, many different genes in *S. cerevisiae* are likely to be subject to regulation by Isw1p.

Discussion

Previous studies implicated Isw1p in the repression of RNAPII transcribed genes (Moreau et al., 2003). We show here that Isw1p displays additional key functions in modulating the passage of RNAPII through all stages of gene transcription. This is achieved by separate Isw1p



B

	Form	Ser5P	Ser2P	H3K4Me	H3K36Me	Rna15
Isw1p		+	+	+	+	+
Ioc2p	Isw1b	+	m	m	m	m
Ioc3p	Isw1a	-	-	-	-	-
Ioc4p	Isw1b	-	+	+	+	+
Spt4p		-	m	ND	ND	m

Figure 7. Model Outlining Roles for Spt4p, Ioc2p, and Ioc4p in the Regulation of RNA Polymerase II during the Transcription Cycle

(A) The Isw1p/Ioc2p component of Isw1b influences Kin28p phosphorylation of Ser5 (5P) on the CTD (zigzag) that is required for accumulation of RNAPII (oval) at the transcription elongation checkpoint (solid line). In *ioc2*Δ, failure to phosphorylate Ser5 (and subsequent events) leads to a reckless RNAPII that proceeds into elongation without pausing and therefore initiates the subsequent Ioc4p-mediated events prematurely; K4Me, K36Me (shaded boxes), Ser2 (2P) phosphorylation of the CTD and recruitment of the 3′ end processing machinery. Ioc2p is also proposed to promote the dissociation of RNAPII at the checkpoint and so controls the amount of RNAPII in productive elongation. Spt4p (and possibly Ioc4p) may antagonize (blocked line) the actions of Ioc2p to promote efficient elongation.

(B) Summary of the roles played by each individual factor in facilitating modifications to RNAPII and chromatin. (+) indicates factor is required for the modification; (−) indicates factor is not required and (m) indicates a modification occurs prematurely. ND, not determined

complexes: Isw1a (containing Ioc3p) and two functionally different forms of Isw1b containing either Ioc2p or Ioc4p. Mutation of *ISW1*, encoding the common subunit of these three different complexes, has consequences at all stages of transcription. Instead, separate mutation of *IOC3*, *IOC2*, and *IOC4* allows a functional dissection of the different roles that these Isw1p complexes play at each stage of the transcription cycle. Our data demonstrate that the Isw1a complex (Isw1p/Ioc3p) is required for promoter inactivation while the Isw1b (Isw1p/Ioc4p/Ioc2p) correlates with the active state. Moreover, distinct positive (Ioc4p) and negative (Ioc2p) functions for the components of Isw1b are evident (Figure 7).

Repression by the Isw1a complex is likely to be mediated by a local chromatin structure that prevents transcription factors, TBP or RNAPII associating with the promoter. A number of protein complexes containing Isw1p, Ioc3p, Mot1p, and Spt15 (TBP) have been identified (Gavin et al., 2002) and a role for Isw1p in mediating repression by aiding TBP displacement at the *PHO8* promoter has recently been demonstrated (Moreau et al., 2003).

The presence of Isw1p on inactive promoters ideally positions it to play a major role in regulating the switch to the activated state. During this switch, there may be an exchange of Ioc3p (in Isw1a) for Ioc2 and Ioc4p (in Isw1b) and a major change in the distribution of the Isw1p complex from the promoter to within the coding region of the gene. At this stage, there will also be an exchange of general initiation factors for the transcription elongation factors, marked by TFIIH (Kin28p)-dependent phosphorylation of Ser5 on the CTD of RNAPII. Support for the function of Isw1b early in transcription elongation comes from its requirement for the normal association of the Kin28p Ser5 CTD kinase with the promoter. In *Drosophila*, phosphorylation of Ser5 of the CTD is required for promoter proximal pausing of engaged RNAPII at heat shock promoters (Schwartz et al., 2003). Here, we demonstrate that in *ioc2*Δ and *isw1*^{K227R} strains, where Ser5 CTD phosphorylation is defective, RNAPII is uncontrolled and accumulates at the 3′ end of the gene. This links Ioc2p to Kin28 recruitment and the phosphorylation of Ser5 of the CTD and supports a role for Ioc2p in the accumulation of yeast RNAPII at promoter proximal positions (Figure 7). By contrast, Ioc4p controls Ser2 phosphorylation, H3K36 methylation, and recruitment of factors such as Rna15p for 3′ end formation, events that coordinate cleavage and polyadenylation of the pre-mRNA. Moreover, Ioc2p and Spt4p are required to prevent premature Ser2 phosphorylation by Ioc4p. Thus, our data demonstrate that the Isw1b complex links Ser5 and Ser2 phosphorylation of the CTD on elongating RNAPII and coordinates the timing of these events. In addition, Ioc2p may promote release of RNAPII from the template. In *ioc2*Δ and *isw1*^{K227R} mutants, disengaged RNAPII accumulates at the 3′ end of the gene and there is the strong correlation between RNAPII with Ser5 CTD phosphorylation and the effective dissociation of RNAPII from the template. Thus, together Ioc2p and Ioc4p impose control on RNAPII allowing the coordination of transcription elongation and termination and effective cotranscriptional pre-mRNA processing including capping, 3′ end cleavage, and polyadenylation. Even though capping is likely to be defective in *isw1* mutants, since they fail to recruit Ceg1p, this is not expected to prevent transcript accumulation (Dower and Rosbash, 2002). Moreover, the exosome, associated with the degradation of improperly processed transcripts (Hilleren et al., 2001), is linked to elongating RNAPII in *Drosophila* (Andrulis et al., 2002), and in yeast this may also be dependent on Isw1p function.

Chromatin structure and chromatin remodeling activities are implicated in the control of transcription elongation (Belotserkovskaya et al., 2003; Saunders et al., 2003) and localized recruitment of the SWI/SNF ATPase drives a remodeling reaction necessary for efficient transcript elongation in mammals (Corey et al., 2003). Here, we propose that the Isw1p-positioned nucleosomes are central to control of transcript elongation in yeast. By

this model, nucleosome +1 at *MET16*, positioned by Isw1p, impedes progress of RNAPII while the elongation factors, Spt4p and Dst1p, facilitate RNAPII movement through this nucleosome. Moreover, in *isw1* mutants, RNAPII is no longer dependent on positive transcription elongation factors, such as Spt4p, and insensitive to drug induced arrest.

It is striking that the local effect of Set1p trimethylation corresponds to two nucleosomes at the beginning of coding regions (Ng et al., 2003), very similar to the effect of Isw1p on promoter proximal chromatin structures observed here. Highly localized Isw1p-dependent chromatin structures and peaks of trimethylation are also associated within the 5′ region of other yeast genes including *CLB2*, *SWI5*, *FIG1*, *INO1, IMD2*, and *DRS2* (data not shown). As the increase in H3 K4 trimethylation at 5′ regions is dependent on Isw1p/Ioc4p, modifications to the histone proteins themselves may define this chromatin configuration. Moreover, Isw1p links events at the beginning and end of the transcription unit. It is well established that early elongation is marked by a combination of CTD Ser5 phosphorylation and H3K4 trimethylation by Set1p. Our results now demonstrate that Isw1b coordinates these events with subsequent CTD Ser2 phosphorylation and H3K36 dimethylation by Set2p marking the termination phase of the transcription cycle. Thus, we propose that the Isw1p-dependent chromatin configuration acts as part of a promoter proximal transcription elongation checkpoint (TEC), involving components of the Isw1b complex and Spt4p, to coordinate the early and late events in the transcription cycle (Figure 7). The interplay of Ioc4p, Ioc2p, and Spt4p would control the amount of RNAPII prematurely aborted or released into productive elongation. Ioc4p and Spt4p may limit the propensity of Ioc2p to promote dissociation of RNAPII from the template at the checkpoint, allowing more RNAII into productive elongation. This would be entirely consistent with the positive roles proposed for Spt4p (Rondon et al., 2003) and Ioc4p in transcript elongation. In summary, we propose that Isw1p sequentially regulates each stage of the transcription cycle, linking events at the 5′ and 3′ end of the transcription unit and controlling the amount of RNAPII entering productive elongation.

Experimental Procedures

Strains

Strains were constructed using PCR-mediated deletion and modification of genomic OFRs, using KanMX, *TRP1*, or *HIS3MX* as selectable markers and myc or HA epitope tags, exactly as described (Wach, 1996). Details of strains are given in Supplemental Data available on *Cell* website.

Growth Conditions

Minimal medium for liquid culture and plates was based on Hartwell's synthetic complete (Hc) (Adams et al., 1997) minus uracil supplemented with 150 ug/ml 6 azauracil (Sigma) (40 mg/ml stock in 0.1 M NaOH) (+6AU) or 100 ug/ml guanine (in 0.1 M NaOH) and 150 ug/ml 6 azauracil (−6AU) or Hc lacking methionine, or supplemented with 1 mM methionine. Rich medium was YPD (10 g/l Difco yeast extract, 10 g/l Bacto peptone, 10 g/l glucose).

RNA Analysis, Transcription Run On, and the Transcriptional Interference Assay

These experiments were conducted exactly as described in Morillon et al. (2003).

In Vivo Chromatin Analysis

Experiments were conducted exactly as described in (Kent et al., 2001) and repeated at least twice. The isolated DNA was restricted with EcoRI. The probes were prepared by PCR amplification of a 1.2 kb fragment that was restricted with EcoRI to generate a 400 bp fragment for the end label.

High Resolution MNase Nuclease Mapping

This method was based on the procedure of Teng et al. (1997), modified as described in Supplemental Data available on *Cell* website.

ChIP Analysis

Antibodies for chromatin immunoprecipitation and the real-time PCR procedure are described in (Morillon et al., 2003) except that DNA was purified using spin columns rather than phenol extraction and precipitation. Positions of PCR primers are shown in Supplemental Data available on *Cell* website. H3 Tri Me K4 (ab8580), H3 Di Me K4 (ab7766), and H3 Di Me K36 (ab9049) antibodies were obtained from Abcam.

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