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Chromatin remodelers Isw1 and Chd1 maintain chromatin structure during transcription by preventing histone exchange

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Abstract

Set2-mediated methylation of histone H3 Lys36 (H3K36) is a mark associated with the coding sequences of actively transcribed genes, yet plays a negative role during transcription elongation. It prevents trans-histone exchange over coding regions and signals for histone deacetylation in the wake of RNA polymerase II (RNAPII) passage. We have found that in *Saccharomyces cerevisiae* the Isw1b chromatin-remodeling complex is specifically recruited to open reading frames (ORFs) by H3K36 methylation through the PWWP domain of its Ioc4 subunit *in vivo* and *in vitro*. Isw1b acts in conjunction with Chd1 to regulate chromatin structure by preventing trans-histone exchange from taking place over coding regions and thus maintains chromatin integrity during transcription elongation by RNA polymerase II.

Introduction

Under physiological conditions chromatin represents a strong barrier to transcription by RNA polymerase II that has to be overcome as well as restored in its wake. Modulation of chromatin structure is achieved by the concerted actions of chromatin remodelers, histone modifying enzymes and histone chaperones. One such enzyme, the lysine methyltransferase Set2, is associated with the elongating form of RNAPII and methylates Lys36 on histone H3 over coding sequences[1,2]. H3K36 methylation is required for efficient deacetylation of histones by the Rpd3S histone deacetylase complex[3–5]. Rpd3S also associates with RNAPII[6,7] and thus maintains coding regions in a hypoacetylated state in the wake of transcription elongation. Loss of Set2 or Rpd3S activities leads to an accumulation of acetylated histones over coding regions and disruption of chromatin organization as evidenced by the accumulation of cryptic transcripts, the result of inappropriate transcription

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initiation from inside open reading frames[3,8]. Recent studies from our laboratory have shown that Set2-mediated H3K36 methylation furthermore maintains chromatin integrity by preventing histone exchange over coding regions, and thus limits the incorporation of new, acetylated histones[9]. In the current study we sought to identify mechanisms by which H3K36 methylation achieves and perpetuates the stable organization of transcribed chromatin.

Chromatin remodeling factors use the energy generated by ATP hydrolysis to slide or evict nucleosomes, or to alter their composition, thus affecting chromatin organization. They play important roles in a number of cellular processes, including gene transcription, replication and recombination. ISWI (*I*mitation *S*witch) and CHD (*C*hromodomain *H*elicase *D*NA-binding) represent two families of remodeling enzymes that are conserved from yeast to humans, although substantial diversity exists with respect to the subunit composition of individual complexes (reviewed in Clapier and Cairns[10]).

The Iswi remodeler was initially identified in *Drosophila melanogaster*[11] and has two homologs in *S. cerevisiae*[12], Isw1 and Isw2. Isw1 associates with Ioc3 (*I*sw *O*ne *C*omplex protein 3) or Ioc2 and Ioc4 to form two distinct remodeling complexes, Isw1a and Isw1b respectively[13]. Although Isw1 can interact with nucleosomes through its SANT and SLIDE domains[14,15], it is thought that the associated Ioc proteins are involved in targeting the remodeling complexes to different genomic locations. Indeed previous experiments found Ioc3 associated with the promoter of *MET16*, while Ioc2 and Ioc4 localized to the *MET16* ORF[16].

Chd1 is the sole representative of the CHD family in *S. cerevisiae* and contains a characteristic double chromodomain at its N-terminus. Chromodomain mutations result in dissociation of yeast Chd1 from chromatin[17], while no large-scale effects are observed for the fly mutant homolog[18]. However, Chd1 has been shown to interact with several known elongation factors such as the PAF complex, Spt5 and FACT[19–21], as well as to co-localize with RNAPII[19,22,23], thus linking Chd1 to transcription elongation.

Iswi and Chd1 chromatin remodelers fulfill partially redundant functions. An *isw1*Δ *isw2*Δ *chd1*Δ strain displays synthetic phenotypes[12] as well as wide-spread disruption of nucleosome positioning throughout the yeast genome[24,25]. *In vitro* both Iswi and Chd1 remodelers are particularly effective in repositioning nucleosomes and generating spaced arrays[12,26,27], a feature of remodeling enzymes often associated with transcriptional repression rather than activation. Deletion of *ISW1* or *CHD1* in a yeast reporter strain resulted in increased cryptic transcription at the *FLO8* locus[17,28], further underlining a repressive function for both remodeling factors.

In this study we determined that recruitment of Isw1b to ORFs is mediated by interaction of its Ioc4 subunit with H3K36 methylated nucleosomes both *in vivo* and *in vitro*. Furthermore, chromatin remodelers Isw1 and Chd1 were found to act synergistically within the Set2 pathway by antagonizing histone exchange and preventing increased incorporation of acetylated histones over coding regions. These results suggest a novel mechanism by which

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the chromatin remodeling activities of Isw1 and Chd1 maintain chromatin integrity in the wake of transcription elongation by RNAPII.

Results

Isw1 and Chd1 associate with H3K36me3 nucleosomes *in vivo*

Trimethylated H3K36 (H3K36me3) is a histone mark deposited on the ORFs of actively transcribed genes and signals for deacetylation of nucleosomes by the Rpd3S complex[3–5]. We were interested to determine what other factors were recruited either directly or indirectly by histone H3 K36me3. To this end we prepared a chromatin fraction from wildtype (WT) yeast cells and obtained mononucleosomes using micrococcal nuclease (MNase) digestion. Yeast mononucleosomes were immunoprecipitated using either H3K36me3 or a control IgG antibody and the eluates were analyzed by MudPIT mass spectrometry (Fig. 1a). In addition to histones, substantial amounts of RNAPII were found to be associated, as expected for nucleosomes representative of actively transcribed chromatin. Furthermore, we also identified all subunits of the Rpd3S complex as well as several histone chaperones and chromatin remodeling factors. Among the most abundant were the Isw1 remodeling complexes and Chd1. In particular we focused on the Isw1b remodeler, consisting of the catalytic Isw1 subunit complexed with Ioc2 and Ioc4, as this complex has two potential methyl-lysine interaction domains. Ioc2 contains a PHD-like domain, while Ioc4 features an N-terminal PWWP domain (Fig. 1b,c).

Ioc4 interacts directly with H3K36me3 nucleosomes

Next we wanted to determine whether Ioc2 and Ioc4 can interact directly with H3K36me3. TAP-purified Isw1b complex as well as purified recombinant Ioc2, Ioc4 and Isw1 proteins were used in histone peptide pull-down assays, but did not interact preferentially with histone H3 peptides methylated on the Lys36 residue (data not shown). However, nucleosomes might be required for binding. Thus, we used reconstituted, recombinant H3K36 methyl-lysine analogue (MLA) mononucleosomes. Histone H3 used for nucleosome reconstitutions contained a K36C mutation that was chemically modified[29] to mimic either unmethylated or trimethylated Lys36 (Fig. 2). Electromobility gel shift assays (EMSA) showed that Ioc4 had a higher affinity for trimethylated than unmethylated mononucleosomes (Fig. 2a,c). This preference is dependent on the presence of the Ioc4 PWWP domain, as Ioc4 bearing an N-terminal deletion ($Ioc4_{\Delta PWWP}$) displayed equal binding to both unmethylated and trimethylated nucleosomes (Fig. 2b,c). Overall affinity of $Ioc4_{\Delta PWWP}$ for nucleosomes was also reduced, suggesting that the PWWP domain represents a major interface for nucleosome binding. Analogous experiments for Ioc2 revealed no binding of purified, recombinant Ioc2 to either unmethylated or trimethylated mononucleosomes (Supplementary Fig. 1a). Recombinant Ioc3 also did not interact preferentially with peptides methylated on Lys36 (data not shown), while the Ioc3-containing Isw1a complex exhibited similar affinities for both unmethylated and trimethylated H3K36 MLA mononucleosomes (Supplementary Fig. 1b). Thus we conclude that Ioc4 interacts directly with H3K36me3-containing nucleosomes through its PWWP domain.

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Ioc4 localizes to ORFs in a Set2-dependent manner

Since Ioc4 interacts with H3K36me3-containing nucleosomes *in vitro*, we wanted to investigate whether this interaction also occurred *in vivo*. We used chromatin immunoprecipitation coupled with microarrays (ChIP-chip) to determine the genome-wide localization of Flag-tagged Ioc3 and Ioc4 *in vivo* for both wildtype and *set2*Δ yeast strains. Using average gene analysis (Supplementary Fig. 2a,b) we determined that Ioc4 localized primarily to the mid- and 3′ coding regions of genes (Fig. 3a), which are also generally associated with H3K36me3 (refs.8,30). By contrast, Ioc3 localized mostly to the regions surrounding the transcription start and end sites (Fig. 3b). Deletion of *SET2* resulted in an almost complete abrogation of Ioc4 occupancy over ORFs (Fig. 3a). We also observed reductions in the association of Flag-tagged Ioc2 and Isw1 over ORFs in a *set2*Δ background, as determined by ChIP-qPCR and ChIP-chip respectively (Supplementary Fig. 2c–f). Taken together these data suggest that the H3K36me3 mark is involved in mediating Isw1b occupancy over coding regions. Ioc3 localization at intergenic regions was not affected by deletion of *SET2*, although Ioc3 occupancy over ORFs increased slightly in a *set2*Δ mutant background (Fig. 3b), perhaps as a consequence of Ioc4 delocalization. Based on the reduction of Ioc4, Ioc2 and Isw1 occupancy over ORFs in the *set2*Δ mutant we conclude that Isw1b occupancy depends on Set2 and H3K36 methylation.

Isw1 and Chd1 prevent intragenic transcription

Previous experiments have shown that Set2-dependent H3K36 methylation is vital for the catalytic activity of the Rpd3S histone deacetylase complex in order to maintain chromatin in a hypoacetylated state following transcription[7]. In the absence of H3K36 methylation nucleosomes in ORFs become hyperacetylated, resulting in aberrant transcription initiation from cryptic promoters inside ORFs[3]. Association of the Isw1b remodeling complex with H3K36 methylated nucleosomes (Fig. 1a) suggested its potential involvement in maintaining ordered chromatin structure following transcription elongation. Thus, we wanted to assess the involvement of Isw1 in suppressing cryptic transcription, as well as its involvement in the Set2 pathway. Indeed, previous studies using a yeast reporter strain for cryptic transcription showed that deletion of *ISW1* did result in a selectable phenotype[28]. Deletion of *ISW1* alone did cause the production of low to moderate amounts of cryptic transcripts at most genes tested when assessed by Northern blotting (Fig. 4 and Supplementary Fig. 3). The same phenotype is observed for a strain containing the *ISW1*^{K227R} catalytic mutant (Fig. 4e–h), suggesting that Isw1 remodeling activity is required for the prevention of cryptic initiation in wildtype cells. Previous reports have indicated that Isw1, Isw2 and Chd1 can have overlapping functions[12]. While we have not observed cryptic transcription in an *isw2*Δ strain (Supplementary Fig. 3, data not shown), deletion of *CHD1* alone caused low levels of cryptic transcripts to accumulate in some instances (Supplementary Fig. 3a,c)[28]. However, the *isw1*Δ *chd1*Δ double deletion strain did exhibit a substantially stronger cryptic transcript phenotype that was completely unaffected by the additional deletion of *ISW2* (Fig. 4a–d) in agreement with previously published data[17]. These results suggest that Isw1 and Chd1 but not Isw2 affect chromatin structure during transcription. Deletion of *SET2* in an *isw1*Δ *chd1*Δ background further exacerbates the cryptic transcript phenotype of this strain. However, the total levels of cryptic transcripts produced when *SET2* is deleted in either the

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single deletion (Supplementary Fig. 3e) or double deletion backgrounds (Fig. 4) are similar to those obtained for *set2*Δ alone. In all instances the *ISW1*^{K227R} catalytic mutant phenocopies an *isw1*Δ mutant (Fig. 4e–h). These results implicate *ISW1*, *CHD1* and *SET2* as being involved in the same pathway.

Isw1 and Chd1 prevent wide-spread cryptic transcription

It has been shown recently that deletion of *ISW1* and *CHD1* results in large-scale changes in chromatin structure[24]. Thus we wanted to investigate further whether deletion of both remodelers resulted in the appearance of cryptic transcripts genome-wide. For this purpose we purified mRNA from both wildtype and *isw1*Δ *chd1*Δ yeast cells, directly labeled the mRNA using fluorescent compounds and carried out competitive hybridizations to strand-specific yeast genome tiling arrays. These experiments allowed us to obtain strand-specific gene expression data as described previously[8]. While regularly transcribed genes show uniform signals all across open reading frames, cryptic transcripts transcribed from the sense strand cause a signal increase towards the 3′ end of genes. The reverse is true for cryptic transcripts initiated from the antisense strand. Previously, our laboratory has used K-means clustering to analyze this type of data[8]. While we found that this approach worked well for the antisense transcription data, regular transcriptional background proved problematic in the analysis of the sense data set. Instead, we directly compared the probe signals from the 3′ to the 5′ ends of open reading frames and selected genes that exhibited at least a 1.5-fold signal increase at the 3′ end for the sense data set. In order to validate our methodology we reanalyzed data for sense cryptic transcripts in a *set2*Δ mutant. This approach proved to be more stringent than K-means cluster analysis and underestimates the number of cryptic transcript genes for *set2*Δ, as only 327 out of 621 genes were identified. However, these genes retain the same characteristics, being predominantly long (89% > 1 kb) and infrequently transcribed (95% < 5 mRNA per hour), and include known cryptic transcript genes for *set2*Δ, such as *STE11* and *PCA1*. We used the same approach for the data analysis of gene expression in the *isw1*Δ *chd1*Δ mutant, which revealed wide-spread cryptic initiation. A large number of genes with sense (646 genes) and/or antisense (962 genes) cryptic transcripts were identified (Fig. 5a). Thus, even though the approach used to identify sense cryptic transcript genes likely led to an underestimate, a substantial number of genes were identified indicating that both sense and antisense cryptic transcription is widespread in the *isw1*Δ *chd1*Δ mutant. Again, both *STE11* and *PCA1* were identified, in agreement with our Northern analysis (Fig. 4).

Recent experiments from our laboratory have shown that the presence of H3K36 methylated nucleosomes is important for the retention of the original histones at the ORFs of transcribed genes by preventing trans-histone exchange, ie. the replacement of existing histones with histones from the free cellular pool[9]. Histone exchange affects many more genes in a *set2*Δ mutant when compared to the number of cryptic transcript genes (Supplementary Fig. 4a,b), presumably because many genes possess no cryptic promoters. Hence, we wanted to compare the genes that exhibit cryptic initiation in either the *set2*Δ or *isw1*Δ *chd1*Δ mutants to the group of genes with altered histone exchange over ORFs in a *set2*Δ background. Figure 5b shows that ca. 60% of genes with cryptic transcripts in an *isw1*Δ *chd1*Δ mutant also exhibit increased histone exchange over open reading frames in a *set2*Δ strain.

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Similarly, ca. 60% of *set2*Δ cryptic transcript genes also display intragenic initiation in an *isw1*Δ *chd1*Δ mutant. However, an appreciable number of cryptic transcript genes in *isw1*Δ *chd1*Δ show no overlap with genes associated with either increased histone exchange or cryptic transcription in *set2*Δ and are presumably regulated by Isw1 and Chd1 independently of Set2. In summary, these data show that deletion of *ISW1* and *CHD1* causes wide-spread intragenic transcription initiation in the yeast genome.

Isw1 and Chd1 prevent histone exchange over ORFs

Given the substantial overlap of genes displaying cryptic initiation in an *isw1*Δ *chd1*Δ background and Set2-regulated genes, and since the Isw1b complex is a reader of the H3K36 methyl mark in particular, we sought to investigate whether these proteins were involved in trans-histone exchange (Fig.6). Histone exchange was studied as described before[31], using a yeast strain containing constitutively expressed, Myc-tagged H3 as well as Flag-tagged H3 under the control of the *GAL1* promoter. In order to study transcription-coupled exchange, yeast were arrested in G1 with α-factor and expression of Flag-H3 was induced briefly by growth in galactose-containing media. Using microarray and average gene analysis, transcription-coupled histone dynamics can be followed by comparing occupancy levels for Flag-H3 relative to Myc-H3. In agreement with previously published work, histone exchange in wildtype yeast is high at the promoters of genes and low over coding regions (Supplementary Fig. 5a,b)[31,32]. We focused our analysis primarily on genes known to exhibit increased exchange over ORFs in a *set2*Δ background (n=3,728) (Supplementary Fig. 4a)[9], although the profiles for the whole-genome data are very similar (Fig. 6a and Supplementary Fig. 5). Comparing data for both *isw1*Δ and *chd1*Δ relative to the wildtype, both mutants showed increased levels of histone exchange from mid-ORF to the 3′ ends of genes (Fig. 6a), areas particularly associated with H3K36me3 (Fig. 7a)[8,30]. Furthermore, the effect of each gene deletion was clearly additive (Fig. 6a), in agreement with the results obtained for cryptic transcription by Northern blotting (Fig. 4 and Supplementary Fig. 3a–d). We also assessed the effect of deletion of *IOC4* on histone exchange and saw an increase in exchange over coding regions, similar to that observed for *isw1*Δ (Fig. 6b). Slight differences in the exchange profiles between *isw1*Δ and *ioc4*Δ presumably result from the fact that both the Isw1a and Isw1b remodelers are affected in the *isw1*Δ background. Genes that show increased exchange over ORFs in either an *ioc4*Δ, *isw1*Δ or *chd1*Δ background overlap to large extents with those known to be affected in a *set2*Δ mutant (Fig. 6c,d). The proportion of genes affected in *isw1*Δ and *chd1*Δ further increases when assessing increases in histone exchange specifically for the 3′ halfs of ORFs (Supplementary Fig. 4c). Also, genes exhibiting cryptic transcription in an *isw1*Δ *chd1*Δ background largely overlap with those genes that are affected by increased histone exchange over ORFs in both *isw1*Δ *chd1*Δ as well as *set2*Δ (Fig. 6g).

In order to confirm that deletion of *ISW1* and *CHD1* increases histone exchange over ORFs we also investigated incorporation of histone H3 acetylated on Lys56 (H3K56ac). Acetylation of H3K56 is mediated specifically by the Rtt109 acetyltransferase (KAT11), which acts on soluble histones, but not on chromatin[33]. Distribution of H3K56ac has been shown previously to correlate with both replication-dependent and -independent histone exchange[32,34] and thus can be used as a marker for genomic regions undergoing trans-

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histone exchange. Using ChIP-chip we determined the distribution of H3K56ac and histone H3 in G1-arrested strains in a BY4741 background to confirm that increases in histone exchange in the mutant strains was not due to overexpression of histone H3. We observed increased levels of H3K56ac from mid-ORF to the 3′ ends of genes (Fig. 6e) for *isw1*Δ and *chd1*Δ as seen also for the histone exchange strains (Fig. 6a). Similarly, the effect of each gene deletion was additive (Fig. 6a,e). The increases in histone exchange for *isw1*Δ, *chd1*Δ and *isw1*Δ *chd1*Δ, whether represented as Flag/Myc in the histone exchange strains or by H3K56ac/H3, are very highly correlated (Fig. 6h). The *ISW1*^{K227R} catalytic mutant strain also showed increased levels of H3K56ac over coding regions (Fig. 6f), similar to exchange in an *ioc4*Δ background (Fig. 6b). This indicates that the catalytic activity of Isw1 is required to suppress histone exchange and maintain ordered chromatin structure in wildtype yeast, in agreement with results on cryptic transcription obtained by Northern blotting (Fig. 4e–h).

Clustering all yeast genes according to transcription rates, we determined that exchange increased over the ORFs of lowly transcribed genes in an *isw1*Δ background relative to the wildtype, but did not affect exchange in highly transcribed genes (Fig. 6i). These results conform with expectations as genes known to rely on Set2 for accurate transcription are generally long and transcribed at low levels[8]. Interestingly, deletion of *CHD1* increased exchange over both lowly and highly transcribed genes when compared to the wildtype (Fig. 6j). Similar analysis of H3K56ac levels over ORFs for both *isw1*Δ and *chd1*Δ show the same distributions (Fig. 6k,l), hinting at the complementary action of these two chromatin remodelers (see Discussion). From these experiments we conclude that the Isw1b and Chd1 chromatin remodeling factors both prevent co-transcriptional trans-histone exchange at largely overlapping, yet somewhat distinct groups of genes.

Deletion of *ISW1* and *CHD1* increases AcH4 over ORFs

The appearance of cryptic transcripts in a *set2*Δ background is associated with a lack of H3K36 methylation and hyperacetylation of ORF nucleosomes[3]. Also, we have recently shown that increased trans-histone exchange in a *set2*Δ mutant results in greater levels of histone acetylation over ORFs[9]. Therefore, we sought to determine whether deletion of *ISW1* or *CHD1* affected either of these histone marks. For purposes of direct comparison, we focused our analysis on the same group of genes that show increased histone exchange in *set2*Δ. However, the trends seen for each deletion are the same genome-wide (Supplementary Fig. 6).

Microarray analysis of H3K36me3 ChIPs clearly showed that the distribution and occupancy levels of H3K36me3 in an *isw1*Δ strain are highly similar compared to the wildtype (Fig. 7a). In contrast, deletion of *CHD1* clearly affected the overall distribution but not the absolute levels of H3K36me3. Using histone H3 ChIP-chip data we determined the overall distribution of nucleosomes for both the *isw1*Δ and *chd1*Δ mutants. Histone H3 occupancy was slightly reduced in an *isw1*Δ background relative to the wildtype. However, deletion of *CHD1* caused an overall redistribution of nucleosomes towards the 5′ end of genes (Fig. 7b), suggesting that Chd1 prevents loss of nucleosomes from the 3′ end of genes. Since deletion of *ISW1* or *CHD1* did not seem to affect absolute H3K36me3 levels *in vivo*,

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we asked whether deletion of either gene could cause an increase in co-transcriptional ORF acetylation, thought to result in sustained decompaction of chromatin. In wildtype cells histone H4 acetylation (AcH4) is high at promoters and low over the bodies of genes (Supplementary Fig. 6a). Promoter acetylation was unaffected in the *isw1*Δ mutant, but reduced in the *chd1*Δ background, indicating a secondary role for Chd1 at promoters. Importantly, deletion of either *ISW1* or *CHD1* resulted in a rise of ORF histone H4 acetylation with small additive effects in the *isw1*Δ *chd1*Δ mutant (Fig. 7c). We also assessed the effects of simultaneous deletion of *ISW1* or *CHD1* in a *set2*Δ background on histone H4 acetylation levels. As expected for proteins that act within the same pathway, AcH4 levels and distribution of either *isw1*Δ *set2*Δ or *chd1*Δ *set2*Δ mimic those of *set2*Δ alone (Fig. 7d), which also agrees with our results on cryptic transcription for those mutants (Supplementary Fig. 3e). Taken together, our results show that in the wildtype Isw1 and Chd1 function to suppress trans-histone exchange, thereby preventing the incorporation of soluble, highly acetylated histones over ORFs.

Discussion

We have shown here that the Isw1b chromatin remodeler is recruited to coding sequences by association of its Ioc4 subunit with H3K36 methylated nucleosomes, as interaction of Ioc4 with chromatin was reduced both *in vivo* and *in vitro* in the absence of H3K36 methylation (Figs. 2,3). By contrast, Ioc3, representative of the Isw1a complex was found preferentially at intergenic regions. Interestingly, its association with coding sequences increased in a *set2*Δ background, perhaps as a consequence of Ioc4 delocalization. Increased Ioc3 occupancy in a *set2*Δ mutant, taken together with the overall genome-wide distributions of Ioc3 and Ioc4 in the wildtype (Fig. 3), point towards the existence of a dynamic equilibrium between the Isw1a and Isw1b complexes in the cell. This is further supported by the observation that estimates on protein abundance imply that Isw1 is the least abundant of all ISWI subunits[35].

In an earlier study, Isw1 was reported to preferentially pull down histone H3 Lys4 trimethylated (H3K4me3) nucleosomes[36]. Based on our ChIP-chip data for Ioc3 (Fig. 3b), we speculate that Isw1a may preferentially bind to H3K4me3-containing nucleosomes that are generally associated with promoters. In our hands TAP purifications of Isw1 result in near equal amounts of Ioc3 and Ioc2–Ioc4 being pulled down. Furthermore, *in vitro* gel shift experiments on mononucleosome binding by Isw1a and Isw1b have shown that Isw1a displays a much higher affinity for mononucleosomes due to its high affinity for DNA when compared to Isw1b[13,27]. Taken together, in the absence of crosslinks, any nucleosomes isolated from Isw1 pull-down experiments are more likely to be associated with Isw1a rather than Isw1b. Previous ChIP-qPCR experiments at the inducible *MET16* locus suggested that Isw1a is recruited to the promoter only when the gene is inactive[16]. However, we observed Ioc3 occupancy at a large number of genes known to be actively transcribed, implying that Isw1a may play a more wide-spread role than previously anticipated.

Chd1 is known to associate with elongating RNAPII through interaction with the PAF complex and/or other RNAPII-associated proteins such as Spt5 and FACT[19–21]. Previous experiments using ChIP-qPCR could not establish differential association of Chd1 with

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chromatin when comparing wildtype cells to a *set2*Δ background[17]. Neither did Chd1 preferentially bind to H3K36 methylated nucleosomes *in vitro*[37]. While it is possible that Chd1 preferentially remodels H3K36 methylated nucleosomes, it is likely that its association with RNAPII mediates localization of its activity at coding sequences. This is in agreement with the observation that both histone exchange and H3K56ac in a *chd1*Δ but not an *isw1*Δ background are increased over genes with higher transcription rates (Fig. 6i–l).

Recent ChIP-seq experiments by Gkikopoulos et al.[24] on nucleosome positioning in strains bearing single deletions of *ISW1* or *CHD1*, as well as the combination of *isw1*Δ *chd1*Δ or *isw1*Δ *isw2*Δ *chd1*Δ show that nucleosome positioning is greatly perturbed across the genome in the double and triple deletion strains. The coding regions were severely affected in an *isw1*Δ *chd1*Δ background, in agreement with our own results showing that deletion of *ISW1* and *CHD1* has additive effects with respect to both cryptic transcription (Figs. 4,5) and histone exchange (Fig. 6a,e) and leads to wide-spread intragenic transcription initiation. Examining each deletion individually, single deletion of *CHD1* had more severe effects overall compared to deletion of *ISW1* alone[24,38], also in agreement with our own data (Fig. 6a,e). These results presumably reflect the fact that Chd1 can compensate for the loss of Isw1 better than vice-versa. Chd1 association with RNAPII may well account for this effect; conversely, even though highly transcribed genes contain high levels of H3K36me3, their lower nucleosome density overall may translate as a reduced “interaction surface” for Isw1b recruitment.

Previous studies have shown that histone exchange of H3/H4 tetramers generally only takes place over promoters, while it is limited to highly transcribed genes over ORFs[31,32,39]. In contrast, exchange of H2A/H2B dimers is generally prevalent over transcribed regions[39,40]. This observation together with *in vitro* experiments lead to the suggestion that at low to moderately transcribed genes, RNAPII can transcribe through a hexasomal template following the eviction of a single H2A/H2B dimer, thus resulting in the retention of the H3/H4 tetramer on the DNA[41,42]. In contrast, the passage of multiple RNAPII molecules reminiscent of the transcription of highly transcribed genes causes complete dissociation of histone octamers from the DNA, which are subsequently reassembled behind the polymerase. We hypothesize that Isw1b and Chd1 may exert their overlapping, yet distinct functions in this context. Studies on *Drosophila* Chd1 and the Iswi containing ACF complex have shown that both enzymes can catalyze the transfer of histones from the histone chaperone Nap1 onto DNA and generate regularly spaced nucleosomal arrays *in vitro*[26]. Further experiments in *Drosophila* established that Chd1 together with the Hira chaperone is important for the replication-independent deposition of histone variant H3.3 in male fly pronuclei[43]. The yeast Chd1 and Isw1 chromatin remodelers are similarly known to efficiently space nucleosomes[13,27,44]. This opens up the intriguing possibility that yeast Isw1 and Chd1 may differ functionally in preventing histone exchange and promoting chromatin integrity at lowly and highly transcribed genes respectively: Isw1 in the form of the Isw1b complex by remodeling nucleosomes that were retained on the DNA in spite of transcription, and Chd1 by reassembling nucleosomes in cis in the wake of RNAPII (Fig. 8). In agreement with this hypothesis we see that in the wildtype Isw1 catalytic activity is required to suppress both cryptic transcription (Fig. 4e–h) as well as histone exchange (Fig.

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6f) and thus maintains chromatin integrity. Such different functional roles for Isw1 and Chd1 could also account for the reduced capacity of Isw1 or Ioc4 to compensate for the loss of Chd1 activity, assuming that Isw1b is less able to recapture histones displaced by transcription. A number of histone chaperones have been implicated in RNAPII transcription elongation and it will be interesting to determine which may function in concert with these remodelers *in vivo*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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a

Protein	K36me3		IgG Control		Description
	Spectra[a]	SC (%)[b]	Spectra[a]	SC (%)[b]	
H2A	34	45.5	3	17.4	
H2B	21	50.4	1	6.9	
H3	11	44.1	2	5.9	Histones
H4	727	63.1	139	51.5	
H1	10	14.3	2	5.4	
Rpb1	376	33.3	79	11.3	
Rpb2	242	38.0	45	11.2	
Rpb3	26	19.5	0	0.0	
Rpb4	7	28.1	1	3.6	
Rpb5	116	21.9	29	13.0	RNA polymerase II
Rpb7	12	26.9	2	7.6	
Rpb8	21	18.5	0	0.0	
Rpb10	21	37.1	0	0.0	
Rpb11	26	55.8	3	9.2	
Rpb12	17	40.0	3	28.6	
Eaf3	12	15.7	3	6.7	
Rco1	11	14.9	3	5.6	
Rpd3	23	24.7	4	6.5	RPD3S
Sin3	82	20.1	2	1.2	
Ume1	14	21.5	0	0.0	
Isw1	185	40.8	4	3.6	
Ioc2	124	32.5	18	7.8	
Ioc3	138	36.0	19	8.9	
Ioc4	60	24.4	0	0.0	
Chd1	108	20.0	2	1.8	Chromatin remodelers
Isw2	39	6.2	0	0.0	
Dpb4	6	20.4	0	0.0	
Dep1	12	13.3	0	0.0	
Itc1	5	6.2	0	0.0	
Spt16	397	46.5	26	12.6	
Pob3	141	39.0	7	4.9	
Spt6	42	16.3	0	0.0	
Hir1	5	5.6	0	0.0	Histone chaperones
Hir2	26	21.7	0	0.0	
Hir3	69	17.7	0	0.0	
Rtt106	31	25.3	0	0.0	

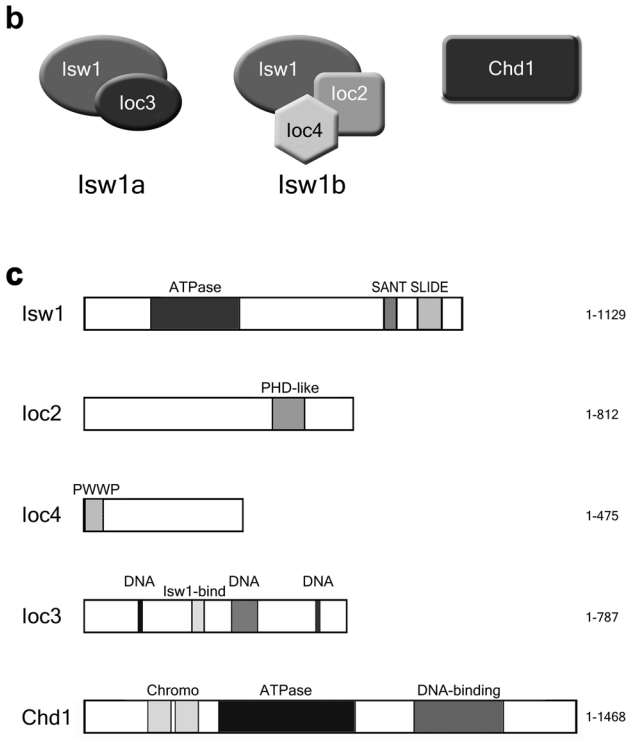

Figure 1. Proteins associated with H3K36me3-containing mononucleosomes

(a) MudPIT mass spectrometry analysis of proteins co-immunoprecipitated with H3K36me3-containing mononucleosomes from wildtype yeast. IgG was used as a negative control. [a] Spectral count, total spectra matching peptides detected by tandem mass spectrometry for the indicated protein. [b] Sequence coverage, percentage of protein sequence represented in peptides identified by tandem mass spectrometry. **(b)** Subunit composition of Isw1 and Chd1 remodelers. Isw1 is the catalytic subunit of two chromatin-remodeling complexes: It associates with Ioc3 to form Isw1a, while Ioc2 and Ioc4 are part of the Isw1b complex. Chd1 is thought to exist largely as a monomer. **(c)** Schematic representations of the domain organizations for the Isw1 and Chd1 chromatin remodelers.

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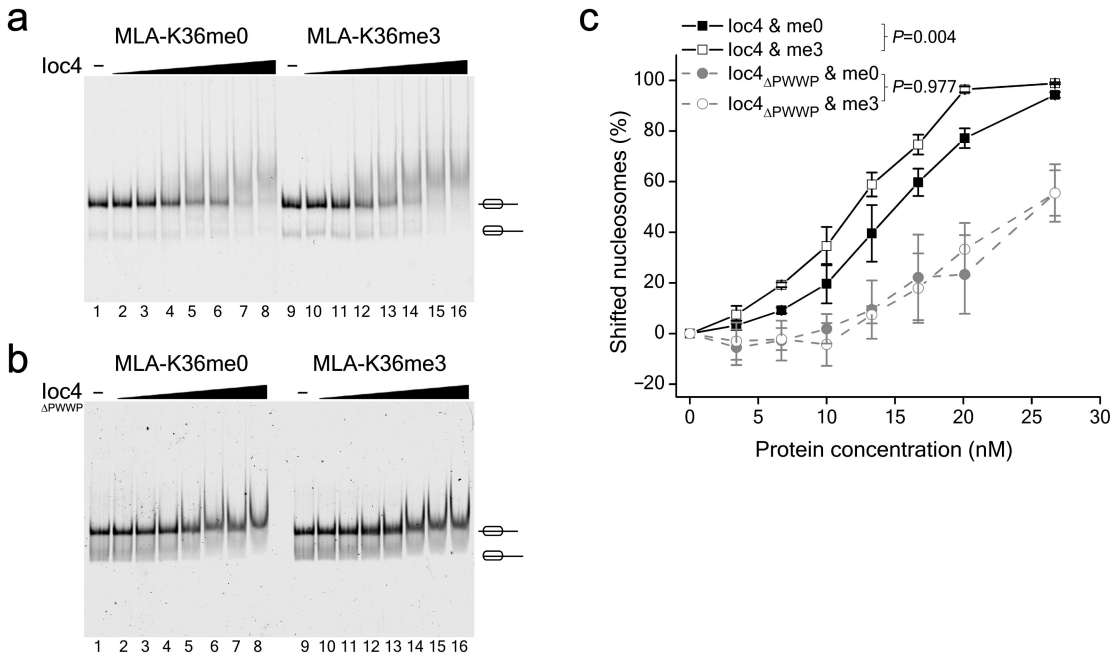

Figure 2. Ioc4 preferentially binds H3K36me3 nucleosomes

(a,b) Recombinant, reconstituted H3K36 MLA nucleosomes (5 fmol) were incubated with increasing concentrations (0, 3, 7, 10, 13, 17, 20 and 27 nM) of Ioc4 **(a)** or Ioc4 with its N-terminal PWWP domain deleted ($Ioc4_{\Delta PWWP}$) **(b)** and analyzed using EMSAs. Mononucleosome bands are indicated. **(c)** Quantitation of Ioc4 and $Ioc4_{\Delta PWWP}$ binding to tri- and unmethylated H3K36 MLA mononucleosomes. Mononucleosome bands were quantitated for each lane. Lanes 2–8 were normalized against input lane 1, while lanes 10–16 were normalized to input lane 9. The percentage of nucleosomes bound by Ioc4 or $Ioc4_{\Delta PWWP}$ was expressed as 100 – % mononucleosomes for each lane. Mean values ± SEM were plotted for at least three independent experiments. *P*-values were calculated using Student's t-test.

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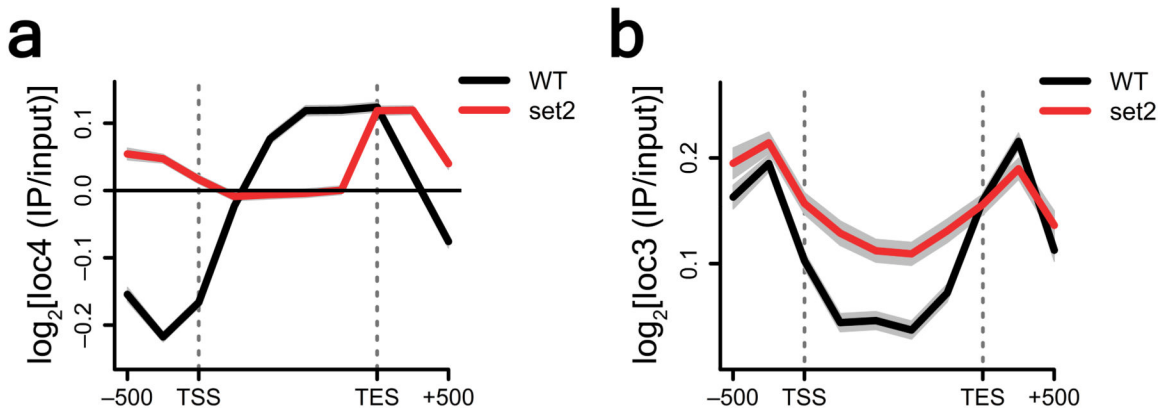

Figure 3. Deletion of *SET2* abrogates localization of Ioc4 to coding regions

(a,b) ChIP-chip experiments were performed using yeast genomic tiling arrays. The $\log_2$ ratios of IP over input were subjected to average gene analysis (Supplementary Fig. 2a). Whole-genome average data were calculated and plotted as mean ± SEM. SE of mean are shown in grey and represent three independent experiments. The *T*ranscription *S*tart *S*ite (TSS) and *TE*rmination *S*ite (TES) are indicated. Flag-tagged Ioc4 **(a)** and Ioc3 **(b)** were immunoprecipitated from wildtype and *set2*Δ yeast strains.

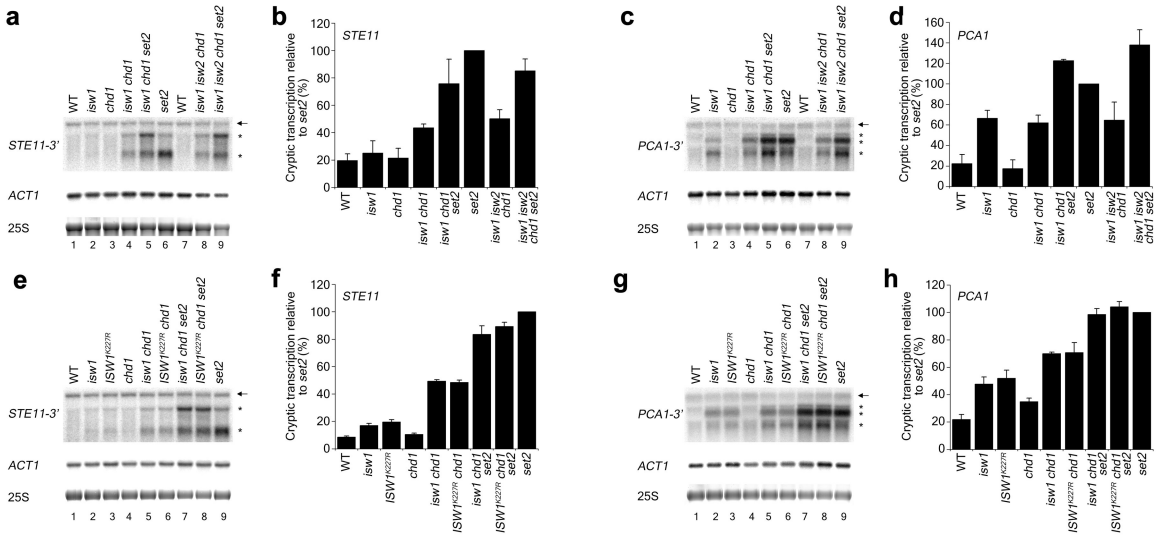

Figure 4. *ISW1* and *CHD1* have overlapping functions during transcription within the Set2 pathway

(a,c,e,g) Total RNA for each strain was isolated and used for Northern blot analysis. The probes used were directed against the 3′ ends of the *STE11* and *PCA1* genes. *ACT1* and rRNA were used as loading controls. The full-length (←) and cryptic transcripts (*) are indicated. **(b,d,f,h)** For each lane all cryptic transcript bands were quantitated and normalized against the *ACT1* loading control. Total levels of cryptic transcription were expressed relative to *set2*Δ. Data were plotted as mean ± SEM for three independent experiments.

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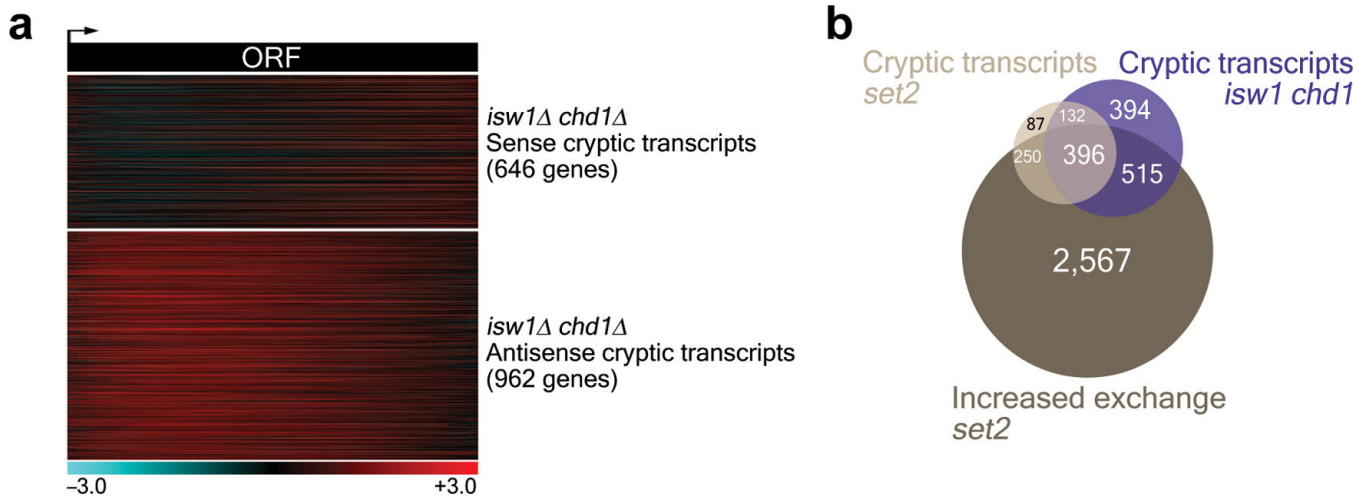

Figure 5. Deletion of *ISW1* and *CHD1* causes wide-spread intragenic transcription

(a) Heatmap of cryptic transcript genes in an *isw1*Δ *chd1*Δ mutant. Probe intensities for the 5′ and 3′ ends of all genes were determined. Resulting 3′/5′ ratios with a cutoff value of $\log_2 > 0.5$ were used to define sense cryptic transcripts (n=646). K-means clustering of gene expression profiles was used to identify antisense cryptic transcripts (n=962). Only cryptic transcript genes are shown. **(b)** Venn diagram showing the overlaps between genes displaying increased histone exchange over ORFs in *set2*Δ (mean $\log_2 > 0$; n=3,728) and genes with cryptic transcription in *set2*Δ (n=865) and/or *isw1*Δ *chd1*Δ (n=1,437). Cryptic transcript sets contain genes with sense and/or antisense cryptic transcripts, whereby 68 and 171 genes show intragenic initiation in both the sense and antisense direction for *set2*Δ and *isw1*Δ *chd1*Δ, respectively. For the purpose of direct comparison we selected cryptic transcript genes for *set2*Δ according to the same criteria applied to *isw1*Δ *chd1*Δ (a), rather than the previously published data[8].

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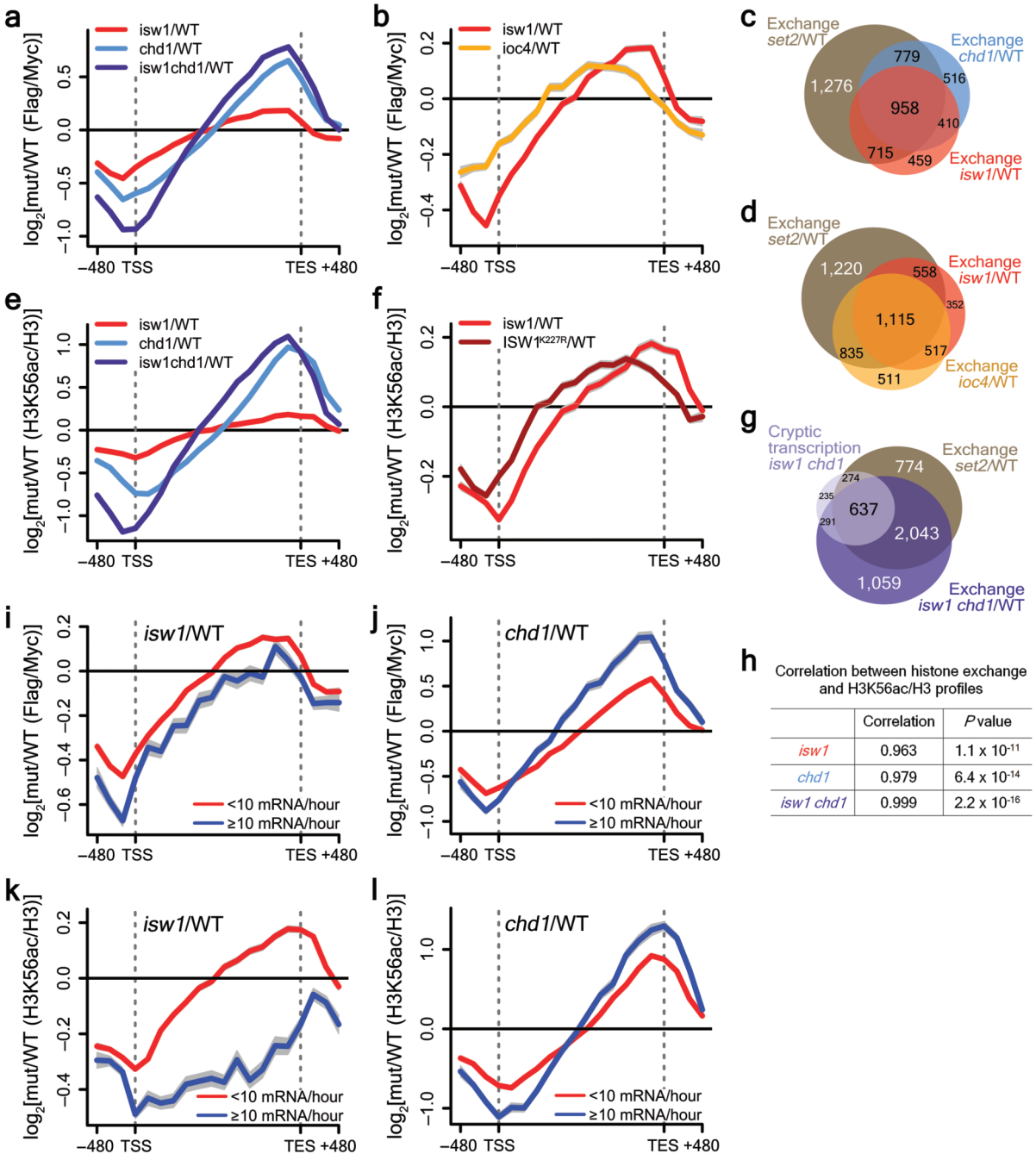

Figure 6. Deletion of *ISW1* and *CHD1* increases histone exchange over 3′ ends of ORFs

(a–b,e–f) ChIP-chip experiments were performed using yeast genomic tiling arrays. Only genes known to display increased exchange over ORFs in a *set2*Δ mutant (n=3,728) (Supplementary Fig. 4a) were used for average gene analysis and plotted as mean ± SEM (grey) for three independent experiments. The *T*ranscription *S*tart *S*ite (TSS) and *TE*rmination *S*ite (TES) are indicated. **(a–b)** Histone exchange (Flag-H3/Myc-H3) was determined for wildtype, *isw1*Δ, *chd1*Δ, *isw1*Δ *chd1*Δ **(a)** and *ioc4*Δ **(b)** strains and presented as difference profiles for mutant (mut) over wildtype. **(c–d)** Genes were clustered

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into two groups based on their average histone exchange signals for mutant relative to wildtype profiles over ORFs (mean $\log_2 < 0$, mean $\log_2 > 0$). Venn diagrams show overlaps between genes displaying increased histone exchange over ORFs (mean $\log_2 > 0$) for the mutants indicated. **(e–f)** Average gene analysis for acetylated K56 (H3K56ac) immunoprecipitated from wildtype, *isw1*Δ, *chd1*Δ, *isw1*Δ *chd1*Δ **(e)** and the *ISW1*^{K227R} catalytic mutant **(f)** strains. H3K56ac occupancy was normalized to histone H3 levels. Difference profiles are shown for mutant over wildtype. **(g)** Venn diagram showing the overlap between genes that exhibit cryptic transcription in an *isw1*Δ *chd1*Δ mutant (Fig. 5a,b) and genes that exhibit increased histone exchange over ORFs in an *isw1*Δ *chd1*Δ and *set2*Δ background. **(h)** Pearson correlation coefficients and *P*-values were calculated for histone exchange **(a)** and H3K56ac/H3 **(e)** profiles for each mutant background using R[45]. **(i–l)** For whole-genome analysis genes (n=4,894) were clustered into two groups (< 10 mRNA per hour (4,250 genes), ≥ 10 mRNA per hour (644 genes)) based on transcription rates published by Holstege et al.[46]. Average gene analysis for histone exchange **(i–j)** or H3K56ac/H3 **(k–l)** difference profiles clustered according to transcription rates are shown. Data were plotted for *isw1*Δ **(i,k)** or *chd1*Δ **(j,l)** relative to the wildtype.

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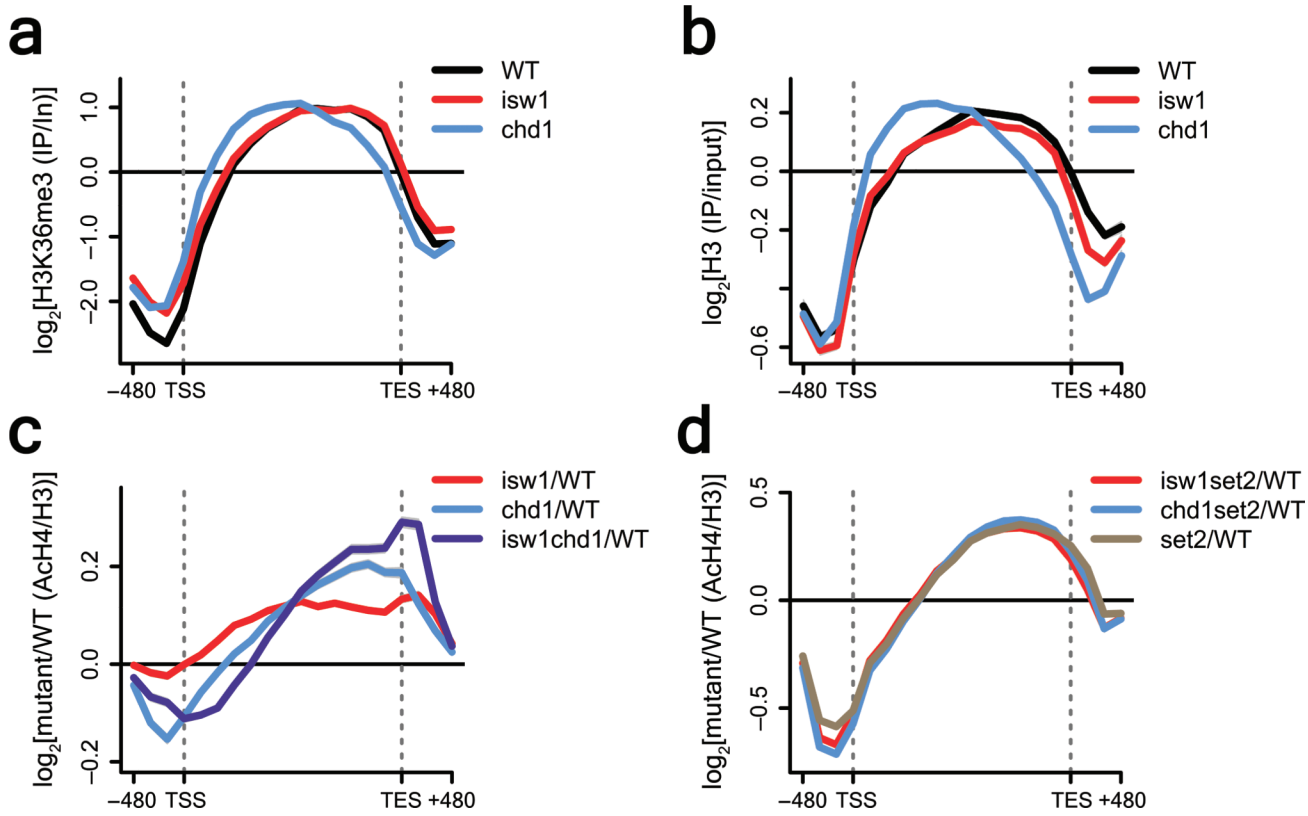

Figure 7. Deletion of *ISW1* and *CHD1* increases histone acetylation over coding regions

(a–d) ChIP-chip experiments were performed as described in Fig. 6. Only genes known to display increased exchange over ORFs in a *set2*Δ mutant (n=3,728) (Supplementary Fig. 4a) were used for average gene analysis and plotted as mean ± SEM (grey) for three independent experiments. The *T*ranscription *S*tart *S*ite (TSS) and *TE*rmination *S*ite (TES) are indicated. **(a)** Average gene analysis for H3K36me3 immunoprecipitated from wildtype, *isw1*Δ and *chd1*Δ yeast strains. **(b)** Average gene analysis for histone H3 immunoprecipitated from wildtype, *isw1*Δ and *chd1*Δ yeast strains. **(c–d)** Average gene analysis for acetylated H4 (AcH4) immunoprecipitated from wildtype, *isw1*Δ, *chd1*Δ, *isw1*Δ *chd1*Δ **(c)** as well as *set2*Δ, *isw1*Δ *set2*Δ and *chd1*Δ *set2*Δ **(d)** yeast strains. AcH4 occupancy was normalized to histone H3 levels. Difference profiles are shown for mutant over wildtype.

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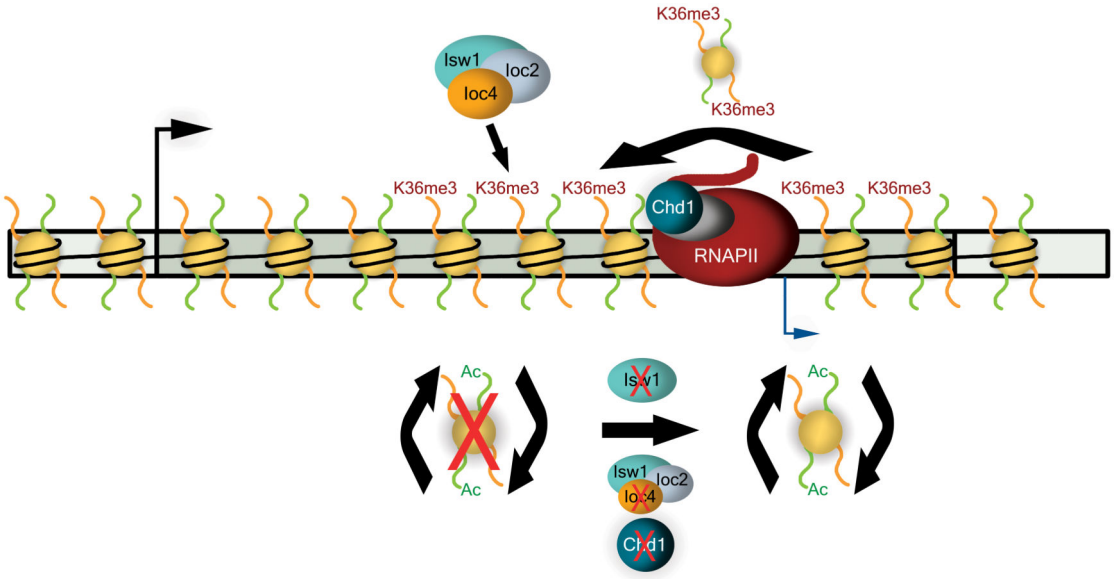

Figure 8. Isw1b and Chd1 maintain chromatin integrity over coding regions

Isw1b is recruited to ORFs by interaction of its Ioc4 subunit with H3K36me3, while Chd1 is thought to co-localize with RNAPII due to its interaction with known transcription elongation factors (grey). In the wildtype both remodelers cooperate to preserve chromatin structure by preventing histone exchange over ORFs. Deletion of *ISW1*, *IOC4* or *CHD1*, or abrogation of Isw1 catalytic activity allows for trans-histone exchange to occur over coding sequences and results in increased levels of histone acetylation over ORFs. This perturbation of ORF chromatin structure exposes normally hidden cryptic promoters and results in the production of internal, cryptic transcripts.