Functional Antagonism between Sas3 and Gcn5 Acetyltransferases and ISWI Chromatin Remodelers

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Abstract

Chromatin-modifying enzymes and ATP-dependent remodeling complexes have been intensely studied individually, yet how these activities are coordinated to ensure essential cell functions such as transcription, replication, and repair of damage is not well understood. In this study, we show that the critical loss of Sas3 and Gcn5 acetyltransferases in yeast can be functionally rescued by inactivation of ISWI remodelers. This genetic interaction depends on the ATPase activities of Isw1 and Isw2, suggesting that it involves chromatin remodeling activities driven by the enzymes. Genetic dissection of the Isw1 complexes reveals that the antagonistic effects are mediated specifically by the Isw1a complex. Loss of Sas3 and Gcn5 correlates with defective RNA polymerase II (RNAPII) occupancy at actively transcribed genes, as well as a significant loss of H3K14 acetylation. Inactivation of the Isw1a complex in the acetyltransferase mutants restores RNAPII recruitment at active genes, indicating that transcriptional regulation may be the mechanism underlying suppression. Dosage studies and further genetic dissection reveal that the Isw1b complex may act in suppression through down-regulation of Isw1a. These studies highlight the importance of balanced chromatin modifying and remodeling activities for optimal transcription and cell growth.

Introduction

Two major classes of enzymes regulate the architecture of chromatin and are thereby critical for DNA-templated processes such as transcription, replication, and repair of damage. Remodeling enzymes use the energy of ATP hydrolysis to alter the structure or position of nucleosomes [reviewed in [1,2]], whereas chromatin modifying enzymes act post-translationally on multiple nuclear substrates. Prominent among these are the nucleosomal histones that are extensively modified on their N- and C-terminal tails [reviewed in [3]]. Covalent modifications of histones and other chromatin proteins are diverse, including at least six specific types of reversible and dynamic modifications that are catalyzed by multimeric enzyme complexes. Among the consequences resulting from histone modifications, two have been especially well characterized. The first is disruption of contacts between DNA and histones or between nucleosomes. In this case, lysine ε-acetylation can destabilize nucleosomal interactions since it neutralizes this otherwise charged residue. The second consequence involves recruitment of effector proteins that bind via conserved recognition domains. For example, histone acetylation can be recognized by bromodomains, whereas histone methylation is recognized by chromo-like-domains and PHD domains [reviewed in [4]]. These domains are found in many nuclear proteins, including chromatin modifying enzymes and remodeling complexes.

The simultaneous existence of multiple different marks on histones has led to the recognition that crosstalk among modifications can be a critical determinant for regulation of gene expression [5]. In addition, cooperation between histone modifiers and ATP-dependent remodeling complexes can contribute to transcriptional regulation [reviewed in [6–8]] and DNA damage repair [reviewed in [9]]. For example, the histone acetyltransferase (HAT) Gcn5 and the SWI/SNF chromatin remodeling complex were proposed early on to have cooperative functions in transcriptional activation by working in concert to modify chromatin structure [10–12]. Indeed, histone acetylation mediated by the SAGA complex containing Gcn5 stabilizes the anchoring of SWI/SNF to nucleosomes at promoters, and thus is important for SWI/SNF-dependent nucleosome remodeling and transcriptional activation in vitro and in vivo [13,14]. The SAGA complex interacts with another chromatin remodeling factor, the chromodomain protein Chd1 [15,16], which is a component of Gcn5-containing SAGA and SLIK/SALSA HAT complexes [17]. In addition, Gcn5 is functionally linked to the essential RSC chromatin remodeling complex: H3K14 acetylation is recognized by one essential tandem bromodomain of Rsc4 and contributes to RSC complex-dependent gene activation [18,19].

Acetylation of histone H3 at lysines 9 and 14 strongly correlates with transcriptional activity and peaks over start sites of active genes [20]. Gcn5 is the HAT responsible for the majority of this acetylation in vivo [21–23], consistent with the observation that Gcn5 is generally recruited to promoters of
Author Summary

In eukaryotes, essential processes such as transcription, replication, and repair of damage occur in the context of chromatin. The structure of chromatin is tightly regulated during the cell cycle by chromatin-modifying enzymes, including acetyltransferases, and ATP-dependent remodeling complexes. Although there has been extensive characterization of their individual functions, little is known about how their activities are coordinated to maintain cell viability. In this study, we show that the critical loss of Sas3 and Gcn5 acetyltransferases can be functionally rescued by inactivation of ISWI remodelers. At a molecular level, the effects on cell viability tightly correlate with the recruitment of RNA polymerase II (RNAPII) at active genes, suggesting that transcriptional regulation may be the mechanism underlying cell viability rescue. Our genetic analyses reveal distinct roles for the two Isw1a and Isw1b complexes; in particular, the antagonistic effects are mediated specifically by the Isw1a complex. These studies highlight the importance of balanced chromatin modifying and remodeling activities for optimal transcription and cell growth.

Results

Functional antagonism between Sas3 and Gcn5 acetyltransferases and ISWI chromatin remodeling enzymes

Among the major H3 acetyltransferases, either Gcn5 or Sas3 is required for cell viability: loss of both enzymes leads to death. To understand this loss of viability, we asked if other chromatin-modulating activities contributed to it, and in particular if there was a role for ATP-dependent chromatin remodeling activities.

There are four distinct families of biochemically defined chromatin remodeling complexes: SWI-SNF, ISWI, CHD, and INO80 [1]. The RSC and INO80 catalytic ATPases Sth1 and Ino80 are essential, although the 

\[\text{INO80}\]

lethality appears restricted to the W303 genetic background [29,30]. Individual inactivation of the other catalytic ATPases, Snf2, Isw1, Isw2 or Chd1 does not trigger marked growth defects in otherwise wild-type cells [15,31,32]. However, earlier studies reported synthetic lethality between SWI-SNF components and members of the Gcn5-SAGA complex [11].

We began by evaluating the effects of ATP-dependent chromatin remodeling activities when Sas3 and Gcn5 activities were compromised, using the temperature-sensitive \(\text{gen}^{5}\Delta\ \text{sas}^{3}\) conditional mutant described earlier [22]. We observed two types of functional interactions between chromatin remodelers and the Sas3 and Gcn5 acetyltransferases.

First, the temperature-sensitive phenotype of the \(\text{gen}^{5}\Delta\ \text{sas}^{3}\) double mutant was exacerbated upon deletion of \(\text{CHD1}\) (Figure 1A and Figure S1A). This suggested parallel functions, likely through overlap in transcriptional regulation, in agreement with previous studies [12,17]. Second, and in distinct contrast, deletion of \(\text{ISW1}\) and to a lesser extent \(\text{ISW2}\), improved growth of the \(\text{gen}^{5}\Delta\ \text{sas}^{3}\) cells (Figure 1A). Deletion of \(\text{ISW2}\) does not further restore growth of the \(\text{gen}^{5}\Delta\ \text{sas}^{3}\ \text{isw}^{1}\\text{A}\) mutant, indicating that rescue is maximal upon inactivation of Isw1 (Figure S1B).

The Isw1 and Isw2 chromatin remodeling complexes use the energy of ATP hydrolysis to alter nucleosome positioning [32–35]. To determine if the ATP-dependent catalytic activity, and not some other property of the enzymes was responsible for suppression, we analyzed the \(\text{isw}^{1}-\text{K227R}\) and \(\text{isw}^{2}-\text{K215R}\) mutants that affect ATP-binding sites to inactivate the enzymes [32]. Figure 1A shows that both catalytic mutations rescued the temperature sensitivity of the \(\text{gen}^{5}\Delta\ \text{sas}^{3}\) mutant, with \(\text{isw}^{1}-\text{K227R}\) having the stronger effect. Thus, suppression is dependent on the catalytic activities of the ISWI ATPases.

The suppression observed was unexpected since most reported interactions between chromatin modifying enzymes and chromatin remodelers describe parallel functions, often through recognition of modified nucleosomes by the remodelers [12,17,18,36–38]. Because inactivation of the ATPase function of Isw1 consistently triggers marked growth defects in otherwise wild-type cells [15,31,32], we focused on dissecting the mechanism underlying the potential antagonism between \(\text{ISW1}\) remodeling and acetyltransferase activities mediated by \(\text{SAS3}\) and \(\text{GCN5}\).

The Sas3 and Gcn5 acetyltransferases counteract Isw1 function through the Isw1a complex

The Isw1 ATPase is the catalytic subunit of two distinct complexes (Figure 1B and reviewed in [1,39]). The Isw1a complex includes the non-catalytic subunit Ioc3, whereas the non-catalytic subunits of Isw1b are Ioc2 and Ioc4 [32,35]. The in vivo functional
of these two complexes are not yet fully established. Microarray studies reveal that the Isw1a and Isw1b complexes have overlapping roles in transcriptional regulation at some genes, but distinct functions at others [35]. To determine whether one or both Isw1 complexes are involved in antagonizing Sas3 and Gcn5 function, we inactivated the complexes individually by deleting genes encoding their non-catalytic subunits. The Isw1a mutant, but neither Isw1b nor Isw1c strains, strongly rescued the gcn5Δ sas3Δ phenotype, demonstrating that inactivation of the Isw1a complex is primarily responsible for the suppression mediated by loss of Isw1 (Figure 1C). Furthermore, deletion of IOC3 exacerbated loss of viability, whereas ioc4Δ had no significant reproducible effects. This suggests an additional relationship between the Isw1b complex and the acetyltransferases Sas3 and Gcn5, and further supports the existence of distinct functions for the two Isw1 complexes. Since combined inactivation of both complexes through deletion of ISW1 rescued the temperature sensitivity, Isw1a appears to have a prominent role in antagonizing Sas3 and Gcn5 activities.

Previous studies demonstrated interactions between the SWI-SNF remodelers and Gcn5 alone, independent of Sas3 [11,12]. To determine if the antagonism between Sas3 and Gcn5 and the Isw1a complex is Gcn5-specific or acts through shared Sas3 and Gcn5 functions, we asked if inactivation of the Isw1a complex rescued temperature sensitivity associated with the single gcn5Δ mutation. Deleting IOC3 or ISW1 did not rescue, demonstrating that these acetyltransferases counteract Isw1a through shared functions of both acetyltransferases (Figure 1D).

Loss of Sas3 and Gcn5 acetyltransferases modestly alters Isw1a recruitment at some active genes

Isw1 contains a SANT domain (reviewed in [40]) that is critical for binding to chromatin at regulated genes in vivo [41]. Biochemical studies indicate that the SANT domains from Ada2 and SMRT preferentially bind unacetylated histone H3 tails [42,43]. To determine whether H3 acetylation antagonizes Isw1 recruitment to chromatin, we evaluated Ioc3-Myc occupancy in gcn5Δ sas3Δ cells by chromatin immunoprecipitation (ChIP) at transcriptionally active target genes (Figure 2). Indeed, Sas3 and Gcn5 acetyltransferases are recruited to a similar set of actively transcribed genes, which correlate with H3K14 acetylation [26]. We selected the PIK1 gene for analysis since H3 acetylation at PIK1 was reduced upon inactivation of Sas3 and Gcn5, as revealed by H3 occupancy (Figure 2E).

Of note, nucleosome density increased in the coding region of PIK1 upon inactivation of Sas3 and Gcn5, as revealed by H3 occupancy (Figure 2F).

Inactivation of the Isw1a complex does not rescue defects in H3 acetylation, nor nucleosome occupancy

As the combined loss of Sas3 and Gcn5 resulted in a dramatic loss of H3 acetylation, we asked if suppression mediated by inactivation of the Isw1a complex might rescue this defect. Although the chromatin remodeling ATPase Isw1 has not been reported to regulate histone H3 acetylation, we hypothesized that nucleosome repositioning resulting from Isw1a inactivation might rescue gcn5Δ sas3Δ defects. Since K14 is the major and common target of Sas3 and Gcn5 acetyltransferases [42,26,45], we assayed global levels of H3K14 acetylation in wild-type, gcn5Δ sas3Δ and gcn5Δ sas3Δ ioc3Δ cells by protein immunoblotting. As previously described [22], H3K14 acetylation decreased in the gcn5Δ sas3Δ strain, however there was no significant difference in acetylation in the gcn5Δ sas3Δ ioc3Δ strain (Figure 3A).

Because global restoration of H3K14 acetylation did not occur, we tested the idea that locus-specific changes might be responsible for rescue of the gcn5Δ sas3Δ mutant by assaying the local levels of H3K14 acetylation at the Isw1-responsive PIK1 gene under suppressing conditions. H3K14 acetylation levels were impaired over the whole PIK1 gene (promoter and coding regions) in the gcn5Δ strain, and more dramatically in the gcn5Δ sas3Δ strain (Figure 3B), demonstrating that Sas3 and Gcn5 are responsible for H3K14 acetylation at this locus. Further, as revealed by the levels of H3K14 acetylation in the gcn5Δ sas3Δ strain, Sas3 markedly contributed to H3K14 acetylation in the promoter and 3′ regions of the PIK1 gene (Figure 3B). Yet, no further difference in H3K14 acetylation levels was observed upon deletion of IOC3 or ISW1 (Figure 3B). Similarly, the elevated H3 occupancy observed in the gcn5Δ sas3Δ strain at the PIK1 gene remained unaffected by inactivation of Ioc3 (Figure 2F). Because inactivation of Isw1a does not suppress Sas3 and Gcn5 defects by directly restoring K14 acetylation either globally or locally, or by decreasing nucleosome occupancy, suppression must occur through some other mechanism.

Isw1a and the acetyltransferases Sas3 and Gcn5 regulate the recruitment of RNAPII to active genes

We asked if the interaction between Isw1a and H3 HATs is related to transcription as reflected by RNAPII occupancy. Indeed, genome-wide analyses revealed that Sas3 and Gcn5 are recruited to actively transcribed genes and that their occupancies correlate with transcriptional rates [20,24,26]. Based on the fact that Isw1 also regulates transcriptional activation [35,44], we assayed RNAPII occupancy at the PIK1 gene.

In agreement with the proposed roles of Sas3 and Gcn5 in transcriptional activation, we observed that loss of these HATs resulted in defective RNAPII recruitment at PIK1 (Figure 3C and Figure S4A). Loss of Gcn5 slightly impaired occupancy at the 3′ region yet did not affect the promoter and 5′ regions, highlighting a role for Sas3 in RNAPII recruitment and in transcriptional...
Figure 2. Loss of the Sas3 and Gcn5 H3 acetyltransferases modestly alters Isw1a recruitment to chromatin. (A) Schematic representation of the PYK1 locus and positions of primer pairs used for ChIP analysis in (B), (E) and (F). loc3-Myc occupancy was assayed by ChIP in wild-type and gcn5Δ sas3 cells grown in SC medium at 34°C over the PYK1 gene (B), at promoter (C) and 5’ coding regions (D) of RPL10, CDC25 and UBP7 genes. (E) Histone H3K14 acetylation occupancy was analyzed by ChIP over the PYK1 gene in wild-type and gcn5Δ sas3 cells grown in SC medium at 34°C. (F) Histone H3 occupancy over the PYK1 gene was assayed by ChIP in wild-type, gcn5Δ sas3, and gcn5Δ sas3 loc3Δ cells grown in SC medium at 34°C. loc3-Myc occupancy was normalized to the telVI control region. H3K14Ac and H3 levels were normalized to the promoter region of PYK1. The values represent the means from two or more independent experiments, with error bars reflecting standard deviations.

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A

promoter  5' region  3' region

B

H3K14Ac/H3 enrichment at PYK1

Max. occupancy

promoter  5' region  3' region  tel VI

WT  gcn5Δ  gcn5Δ sas3  gcn5Δ sas3 ioc3Δ  gcn5Δ sas3 isw1Δ

C

RNAPII occupancy at PYK1

Max. occupancy

promoter  5' region  3' region

WT  gcn5Δ  gcn5Δ sas3  gcn5Δ sas3 ioc3Δ  gcn5Δ sas3 isw1Δ

D

RNAPII occupancy at active genes

Max. occupancy

PMA1  RPL10  RPS5  CDC25  UBP7

WT  gcn5Δ  gcn5Δ sas3  gcn5Δ sas3 ioc3Δ  gcn5Δ sas3 isw1Δ
Figure 3. Sas3 and Gcn5 acetyltransferases and Isw1a antagonistically regulate RNAPII recruitment to active genes. (A) Schematic representation of the PYK1 locus and positions of primer pairs used for ChIP analysis in (B) and (C). (B) Histone H3K14 acetylation (Ac) levels and (C) RNAPII occupancy over the PYK1 gene were assayed by ChIP analysis of cells grown in SC medium at 34°C. H3K14Ac ChIP samples were normalized to total H3. (D) RNAPII occupancy was assayed by ChIP as in (C), but at the 5′ regions of PMA1, RPL10, RPS5, CDC25 and UB17 genes. H3K14Ac levels were further normalized to the promoter region of PYK1, and RNAPII occupancies were normalized to WT for each locus, arbitrarily set to 1. The values represent the means from two or more independent experiments, with error bars reflecting standard deviations. doi:10.1371/journal.pgen.1002994.g003

activation. Significantly, deletion of IO23 and ISW1 in a gen5Δ sas3 mutant partially rescued RNAPII occupancy at the promoter and coding region of PYK1 (Figure 3C and Figure S4A).

We evaluated RNAPII at the other active genes described above to determine how general the suppressive effects were on occupancy. For this study, we also included the additional Isw1 and Sas3 target genes PMA1 and RPS5, respectively [26,44]. As shown for PYK1, we found that Sas3 and Gcn5 contributed to the recruitment of RNAPII at PMA1, RPL10, RPS5, CDC25 and UB17 coding regions (Figure 3D and Figure S4B). Furthermore, deletion of IO23 improved RNAPII occupancy at these genes, more than the modest effects observed with isw1A. We asked if these differences in the recruitment of RNAPII influenced transcription (Figure S4C). We assayed the steady state levels of PYK1, PMA1 and RPL10 mRNAs by RT-qPCR, and observed no significant changes in the gen5Δ sas3 and gen5Δ sas3 ioc3Δ mutants when compared to the wild-type strain (Figure S4C). Together these results demonstrate that Sas3 and Gcn5 acetyltransferases and the Isw1a complex have antagonistic roles in chromatin as reflected by recruitment of RNAPII.

Isw1-dependent rescue of RNAPII at PYK1 is independent of nucleosomal repositioning

RNAPII function and regulated recruitment during transcription are critically dependent on chromatin architecture. Given that Sas3 and Gcn5 acetyltransferases and the Isw1a complex have opposing effects on RNAPII recruitment to transcriptional target genes, we asked whether they also antagonistically regulate nucleosomal occupancy at the PYK1 locus. It has been suggested that PYK1 chromatin structure is dependent on Isw1 since the ATPase is associated with this coding region [44].

We examined nucleosomal organization at PYK1 by comparing micrococcal nuclease (MNs)e cleavage patterns of chromatin prepared from wild-type and mutant strains (Figure S5). We observed no significant differences in MNase cleavage patterns in gen5Δ sas3 ioc3Δ cells when compared to gen5Δ sas3 or wild-type cells (Figure S5). One possible explanation was that the MNase assay might not detect the Isw1 remodeling activities at the PYK1 gene. For example, the ISW1-dependent rescue may involve nucleosome repositioning at a level too subtle to be detected using the MNase mapping assay. Indeed, Isw1 and Isw2 remodeling activities increase genome-wide nucleosome occupancy at mid-coding regions and intergenic regions, respectively, to prevent cryptic transcription [46,47]. In order to map nucleosome location with high resolution at the PYK1 gene, we took advantage of nucleosome-scanning analysis [48]. This method couples isolation of mononucleosomal DNA by MNase digestion with qPCR analysis using a set of overlapping primer pairs spanning the region of interest [48]. Nucleosome scanning analysis of the PYK1 region revealed the presence of four positioned nucleosomes, one located in the promoter region and three others positioned in the 5′ coding region (Figure 4). Further, a 150 bp region highly sensitive to MNase digestion has been identified in the promoter region (−350 to −200 from the start codon), indicating the presence of a nucleosome depleted region (NDR) (Figure 4). This organization is characteristic of “open” promoters which favor the binding of transcription factors at the expense of nucleosomes [49]. Open promoters are a common property of constitutive genes, such as the conditionally essential gene PYK1. No major changes in nucleosome positioning were observed upon inactivation of Gcn5 and Sas3, or further depletion of Io3 (Figure 4).

A dual role for the ATPase Isw1 revealed by functional interactions between the Isw1a and Isw1b complexes and the acetyltransferases Sas3 and Gcn5

Multiprotein complexes can be altered both by mutation and by changing subunit abundance through gene dosage. We took advantage of gene overexpression [reviewed in (50–52)] as an independent approach to probe the relationship of ISW1 to SAS3 and GCN5.

Increased gene dosage of IO2C or IO24 restored viability at elevated temperature, whereas overexpression of IO23 exacerbated sickness of the gen5Δ sas3 mutant (Figure 5A). Increased ISW1 also interfered with growth, confirming its generally antagonistic function. Furthermore, increased gene dosage of IO2C did not rescue thermosensitivity of the gen5Δ single mutant (Figure S6A), nor did it serve as a bypass suppressor of the gen5Δ sas3Δ strain. Together these results suggest that increasing the stoichiometry of the Isw1b complex can ameliorate the negative effects of the Isw1a complex in the gen5Δ sas3 mutant.

To determine if the Isw1a and Isw1b complexes act in parallel or in the same pathway, we performed a series of analyses to dissect the relative contribution of each complex. We first evaluated increased gene dosage of the Isw1b complex components in a strain depleted for the Isw1a complex. Of note, deletion of IO23 partially rescues the gen5Δ sas3Δ temperature sensitivity. Therefore, although the gen5Δ sas3Δ ioc3Δ strain is still somewhat temperature sensitive (Figure 5B), and provides the possibility for a dynamic range in which growth enhancement or inhibition could be observed. We found that overexpression of IO2C or IO24 did not rescue the residual gen5Δ sas3Δ temperature sensitivity in an ioc3Δ background, demonstrating that the Isw1a complex is required for Isw1b-mediated dosage suppression (Figure 5B).

We next evaluated whether Isw1a overexpression enhanced the gen5Δ sas3 phenotype in a strain depleted for Isw1b by deleting IO2C and IO24. Overexpression of IO23 still exacerbated the gen5Δ sas3Δ temperature sensitivity in a strain depleted for Isw1b (Figure 5C and Figure 5D). These results support the idea that increasing the relative balance of the Isw1b complex suppresses the gen5Δ sas3 phenotype by counteracting Isw1a function. In addition, we observed that deletion of ISW1 interfered with the phenotypes resulting from overexpression of the IO2C genes in the gen5Δ sas3 strain, underscoring the critical role for the Isw1 catalytic ATPase itself (Figure S6B).

Discussion

Since the acetyltransferase activities of Sas3 and Gcn5 were initially reported to be essential [22], understanding the molecular mechanisms underlying this synthetic lethality has remained incomplete. Indeed, whereas disruption of these H3-specific
acetyltransferases results in cell death, mutation of lysine residues in histone H3 that are known to be targeted by Sas3 and Gcn5 has only modest phenotypes [27,28]. This discrepancy and the growing number of validated non-histone acetylation targets [53,54] strongly suggest that the Sas3-Gcn5 essential function may reside in acetylation of histone and/or non-histone targets.

Our previous work demonstrated that the Sas3 and Gcn5 acetyltransferases are jointly required for viability and are responsible for the majority of histone H3 acetylation at K9 and K14 in vivo [22]. Here we show that both HATs contribute to in vivo acetylation of H3K14 at an actively transcribed gene, and furthermore we correlate the dramatic decrease in H3K14 acetylation with defective recruitment of RNAPII to promoter and coding regions. This reinforces the view that H3K14 acetylation is an epigenetic mark associated with transcriptional activation [20]. Moreover, the joint contributions of Sas3 and Gcn5 provide a molecular explanation for previous results showing that loss of Gcn5 only modestly affects acetylation of H3K14 at various genes [23,55].

We found that inactivation of ISWI family remodelers alleviates gen5Δ sas3 cell death. In deep contrast, inactivation of the Chd1 remodeler exacerbates the sickness of the gen5Δ sas3 strain. Unlike other chromatin remodeler families, most ISWI complexes are required for formation of repressed structures [32,44,46,56,57] and chromosome compaction in vivo [58–60]. Furthermore, inactivation of the linker histone H1, another critical player in chromatin condensation, also rescues the growth defect of a gen5Δ sas3 mutant [28]. Thus, destabilizing chromatin compaction,
repressed structures, or higher-order chromatin structures through inactivation of ISWI complexes or histone H1 partially reduces growth defects in gen5Δ sas3 cells. This supports the view that Sas3 and Gcn5 acetyltransferase activities counterbalance negative effects of repressed structures and condensed chromatin. In agreement with this model, we observed that inactivation of the Isw1a complex rescues RNApolII recruitment at active genes in a gen5Δ sas3 mutant, to levels similar to those observed in the gen5Δ mutant. This correlates with the degree of rescue observed for cell viability, strongly suggesting that Isw1-dependent rescue is mediated at least partially through the recruitment of RNApolII at actively transcribed genes. Similarly, the H4 HAT Esa1 can overcome the repressing function of Isw1 on transcription [61]. Conditional inactivation of Esa1 leads to defects in RNApolII recruitment at the MET16 gene upon induction, as well as impaired accumulation of MET16 transcript. Deletion of ISW1 was also seen to restore MET16 RNA levels and RNApolII distribution, which is very similar to our observations with RNApolII recruitment at active genes in the gen5Δ sas3 mutant. However, the outcomes of these genetic interactions are clearly different: whereas inactivation of Isw1 rescues the sickness of gen5Δ sas3 cells, it exacerbates cell growth defects of esal1 mutants [62,63]. These differences are likely to reflect the distinct biological functions and substrates of the Sas3/Gcn5 and Esa1 acetyltransferases.

In contrast with Sas3 and Gcn5 acetyltransferases, functional characterization of ISWI enzymes in transcriptional regulation reveals significant roles in gene repression [35,46,47,56]. Future studies should determine if Sas3 and Gcn5 acetyltransferases and ISWI family remodelers act in the same pathway.

In Drosophila, H4-K16 acetylation regulates chromatin compaction by reducing the ability of ISWI to bind chromatin [64,65]. Such a mechanism has not been described for H4 acetylation in yeast, and we show here that loss of the main H3 acetyltransferases Sas3 and Gen5 only modestly affects Isw1a recruitment to chromatin at some active genes (Figure 2). Thus it appears that Sas3 and Gen5 acetyltransferase activities may counteract Isw1 function independently of chromatin binding. Such regulation has been reported for the Chd1 and Isw2 remodeling enzymes: H4 acetylation is reported to antagonize nucleosome remodeling by lowering the catalytic turnover of ATP hydrolysis without affecting nucleosome binding [38]. Further, Sas3 and Gen5 acetyltransferases might control Isw1 function directly through acetylation. In Drosophila, Gen5 acylates Isw1 at a single lysine in vitro and in vivo [66]. This acetylation occurs in a region similar to the N-terminal tail of H3, at a residue corresponding to K14. We also detected low levels of Isw1 acetylation in wild-type cells, with a two-fold decrease in the gen5Δ sas3 mutant (data not shown).

Although loss of the ATP-dependent remodeling activity of Isw1 is required to restore cell viability, we observed no significant effects on nucleosome positioning upon inactivation of Isw1a at the PTK1 gene. Of note, nucleosome occupancy appears slightly reduced in the gen5Δ sas3 mutant, at the predicted nucleosomes +1 and +3 (Figure 4). This appears in contrast with the increased H3 occupancy observed at the PTK1 promoter and coding region by ChIP (Figure 2F). However, it should be noted that nucleosome occupancy assayed by the MNase scanning method was normalized to the PHO5 promoter while the ChIPs for H3 were not, which is likely to account for this discrepancy. Alternatively, these results may suggest a local change in nucleosome assembly or disassembly in gen5Δ sas3 mutants that results in accumulation of incomplete nucleosomes. Addition and removal of the H3/H4 tetramer is the first and last step of nucleosome assembly and disassembly, respectively [reviewed in [67]]. Tetrasomes protect around 80 base pairs of DNA, below the resolution of our MNase-qPCR study, which may contribute to the loss of nucleosome signal in the MNase assay. This possibility can be explored in future studies by evaluating H2B occupancy at the 5' region of PTK1 and by testing genetic interactions between gen5Δ sas3 and histone chaperones.

An additional possibility for the ISWI-dependent chromatin remodeling rescue in gen5Δ sas3 mutants is that it may be mediated through alteration of higher order chromatin structures. Indeed, the Drosophila ISWI-containing remodeler ACF can assemble regularly spaced arrays of H1-containing nucleosomes and can further catalyze repositioning of chromatosomes (nucleosome+H1) in chromatin fibers [68,69]. Furthermore, inactivation of histone H1 also rescues the growth defect of a gen5Δ sas3 mutant [29]. Therefore, restoration of RNApolII upon inactivation of Isw1a complex might rely on different states of chromatin that differ in the periodicity of chromatosome arrays.

An unexpected finding uncovered by our genetic analysis is the opposing functions of Isw1a and Isw1b complexes in relation to the H3 HATs. Specifically, we observed that rescue mediated by loc2 and loc4 requires Ioc3, but this requirement is not reciprocal. This suggests that, at least in the context of gen5Δ sas3, the Isw1b complex antagonizes the function of the Isw1a complex. Early on, functional characterization of these two complexes revealed distinct roles [35]. Notably they differ in their biochemical activities to bind and space nucleosomes [35,70,71], their nucleosome remodeling properties in vitro [46,47], as well as their roles in transcriptional regulation [35,44]. Similarly in other eukaryotes, ISWI complexes that share the same catalytic subunit have distinct biological functions, specified by their associated proteins [reviewed in [1,39]]. The observations reported here bring new understanding toward defining their differences by showing that one ISWI complex may counteract the function of another ISWI complex.

Together, the results from this study deepen understanding of the essential roles for H3 HATs. Not only do the HATs positively promote gene-specific transcriptional activation, as has been well established, they also have a critical role in balancing the activities of ATP-driven chromatin remodelers. The functional antagonism between Isw1a and the Sas3 and Gen5 acetyltransferases further
defines biological distinctions between the Isw1 enzyme’s catalytic activities in its two structurally distinct complexes. Thus, in addition to interactions between histone modifications defining transcriptional output (reviewed in [6–8]), it is likely that future studies will reveal increasingly diverse interactions between the modifying machines and the complexes that dynamically define chromatin architecture through its remodeling.

Materials and Methods

Yeast strains and plasmids

Strains used are listed in Table S1 and are in the W303 background. Gene deletions and other standard procedures were performed as described [72]. The *gcn5*Δ *sa3* conditional mutant was constructed with a chromosomal allele of the *sa3*-C357Y, P375A double point mutation. As described for the plasmid conditional mutant [22], the chromosomal version of the mutant grows well at 30°C, but dies at 37°C. All strains carrying *isw1Δ::kanMX, *isw2Δ::kanMX, *isw3Δ::matMX, *isw4Δ::pHMX, *isw1*-K227R and *isw2*-K215R-3FLAG-kanMX alleles were derived from the strains YTT441, YTT823, YTT825, YTT855 [35], YTT1223 and YTT1996 respectively, generous gifts from T. Tsukiyama. The *gcn5Δ::matMX* allele was obtained by marker swapping using p4339 (generous gift from C. Boone) on *gcn5Δ::kanMX*. The strains expressing Ioc3-13Myc and Isw1-13Myc from their chromosomal loci were constructed as described in [73]. All plasmids were derived from Yerp351 (2μ LEU2). pLP645 was constructed by inserting a BamHI-Sall fragment containing *ΔSA3* into Yerp351 opened with Sall and BamHI (J. Lowell). pLP1524 was obtained from a genomic library generously provided by S. Roeder [74]. It contains a 3.9 kb fragment encompassing *GCN5* gene (Chr.VII 995,188 to 998,784 bp). To construct Yerp351-1OC2 (pLP2234), a HindIII fragment (4.6 kb) containing *1OC2* was subcloned from pLP2170 (containing genomic fragment from Chr.XII 328,038 to 332,847 bp) into Yerp351. To create Yerp351-BSW1 (pLP2256), a BamHI-PstI fragment was subcloned from pRS416-BSW1 (a generous gift from T. Tsukiyama) into Yerp351. Plasmid Yerp351-1OC4 (pLP2260) was constructed by PCR amplification of *1OC4* (~395 bp from start codon to ~412 bp from stop codon), and cloned into Yerp351 using HindIII-PstI restriction sites. Plasmid Yerp351-1OC3 (pLP2266) was constructed by PCR amplification of *1OC3* (~700 bp from start codon to ~600 bp from stop codon), and cloned into Yerp351 using HindIII-PstI restriction sites. Integrity of the constructs was confirmed by DNA sequencing.

Temperature sensitivity assays

 Cultures were grown for 2 days in SC or appropriate selective medium at permissive temperature. Cells were diluted to an A600 of 1 and plated in fivefold serial dilutions onto SC or selective medium, supplemented with 1 M sorbitol where indicated, and incubated for 4 days at the indicated temperatures prior to data collection.

ChIP

Chromatin immunoprecipitation assays were performed as described previously [75] with minor modifications. Cultures were grown in SC medium at 34°C to an A600 of 0.7–0.9. Then ~2×10⁷ cells were harvested, washed in 1 ml sorbitol 1 M, resuspended in 1 ml of zymolyase solution (sorbitol 1.1 M, 20 mM KPO4, pH7, 0.5 mM CaCl2, β-mercaptoethanol 0.5 mM, zymolyase 100T 1 mg/ml) and incubated for 1.5 min at room temperature. Spheroplasts were then washed twice in 1 M sorbitol and gently resuspended in 1.6 ml of cold buffer A (1 M sorbitol, 50 mM NaCl, 10 mM Tris-HCl, pH 7.4, 5 mM MgCl2, 0.5 mM spermidine, 0.075% NP40 and 1 mM β-mercaptoethanol). The cell slurry was diluted into 400 μl aliquots, and each was added to a microfuge tube containing the MNase (0, 60, 150 and 400 U/ml final concentrations) and incubated at 37°C for 4 minutes. The reaction was stopped by addition of 40 μl of stop buffer (250 mM EDTA, 5% SDS). DNA purification was performed as described in [76]. MNase digested DNA was run out on a 1.5% agarose gel and mononucleosome sized fragments were excised and purified using Qiagen’s Gel Extraction kit. Purified mononucleosomes were analyzed by real-time PCR using the MJ Research Opticon2 system. Primer sequences are listed in Table S2. For quantification of Mnnenase digested samples, standard curves were generated for each set of primers. Digested and input DNAs were assayed for each strain in triplicate real-time PCR reactions. Each IP sample was normalized to the control IP (i.e. no epitope or no antibody) by subtraction, divided by the input sample, and expressed as percent of input (% IP/input). The % IP/input values for H3K14Ac were further normalized to % IP/input values for total H3. The % IP/input values for Ioc3-Myc and RNAPII were normalized to telVI and RNA5S control regions, respectively. Data represent averages from two or more independent experiments.

Nucleosome scanning analysis

Extracts from MNase digestions were prepared as described [76,77]. Briefly, cultures were grown in SC medium at 34°C to an A600 of 0.7–0.9. Then ~2×10⁷ cells were harvested, washed in 1 ml sorbitol 1 M, resuspended in 1 ml of zymolyase solution (sorbitol 1.1 M, 20 mM KPO4, pH7, 0.5 mM CaCl2, β-mercaptoethanol 0.5 mM, zymolyase 100T 1 mg/ml) and incubated for 1.5 min at room temperature. Spheroplasts were then washed twice in 1 M sorbitol and gently resuspended in 1.6 ml of cold buffer A (1 M sorbitol, 50 mM NaCl, 10 mM Tris-HCl, pH 7.4, 5 mM MgCl2, 0.5 mM spermidine, 0.075% NP40 and 1 mM β-mercaptoethanol). The cell slurry was diluted into 400 μl aliquots, and each was added to a microfuge tube containing the MNase (0, 60, 150 and 400 U/ml final concentrations) and incubated at 37°C for 4 minutes. The reaction was stopped by addition of 40 μl of stop buffer (250 mM EDTA, 5% SDS). DNA purification was performed as described in [76]. MNase digested DNA was run out on a 1.5% agarose gel and mononucleosome sized fragments were excised and purified using Qiagen’s Gel Extraction kit. Purified mononucleosomes were analyzed by real-time PCR using the MJ Research Opticon2 system. Primer sequences are listed in Table S2. For quantification of MNase digested samples, standard curves were generated for each set of primers. Digested and input DNAs were assayed for each strain with each primer set in triplicate PCR reactions. MNase digested samples were divided by the input value for each primer set to generate percent of input. This was further normalized to the PHO3 TATA region [78].

The protein immunoblotting, mRNA quantification, and chromatin analysis techniques used in Figures S2, S3, S4, S5 are described in Supporting Methods (Text S1).

Supporting Information

Figure S1 Functional interactions between Sas3 and Gcn5 acetyltransferases and chromatin remodeling enzymes ISWI and Chd1. (A) Inactivation of the Chd1 chromatin remodeling enzyme exacerbates the growth defects of the *gcn5*Δ *sa3* mutant. Strains were plated onto SC medium supplemented with 1 M sorbitol, and grown at the indicated temperatures (see also Figure 1A). (B) Inactivation of the chromatin remodeling enzyme Isw2 does not further rescue the growth defects of the *gcn5*Δ *sa3* *isw1A* mutant. Strains were plated onto SC medium supplemented with 1 M sorbitol, and grown at the indicated temperatures. (TIF)

Figure S2 Overexpression of Isw1b subunits does not affect Ioc3 protein levels. Increased gene dosage of *IWC2* and *IWC4* does not affect *IWC3* expression. WT and *gcn5*Δ *sa3* cells expressing Ioc3-Myc were transformed with *IWC2* or *IWC4* in the 2 μ plasmid
and grown at 37°C. Ioc3-Myc levels were determined by immunoblotting using anti-Myc, normalized using anti-tubulin and further normalized to empty vector control for relative quantification. Shown is a representative blot of three experiments.

Figure S3 Deletion of IOC3 does not restore bulk levels of H3K14 acetylation in gen5Δ sas3 cells. Whole cell protein extracts from wild-type, gen5Δ sas3, gen5Δ sas3 ioc3Δ cells were immunoblotted with anti-H3K14ac, and anti-H3 as a control for histone levels. Quantification of H3K14Ac was normalized to H3 levels with WT level set to 1.

(EPS)

Figure S4 Sas3 and Gcn5 acetyltransferases and Islw1 antagonistically regulate RNAPII recruitment to active genes, but do not alter gene expression. RNAPII occupancy over the PTK1 gene (A) or at the 5′ regions of PMA1, RPL10, RPS5, CDC25 and UBP7 genes (B) were assayed by ChIP analysis of cells grown in SC medium at 34°C. RNAPII occupancies in (A) and (B) were normalized to 5′ region of PTK1 and the PMA1 gene respectively, arbitrarily set to 1. The values represent the means from two or more independent experiments, with error bars reflecting standard deviations. (C) cDNAs from WT, gen5Δ sas3, and gen5Δ sas3 ioc3Δ cells grown at 34°C were analyzed by quantitative PCR. Expression values are relative to ACT1 and normalized to WT. The values represent the means from three independent experiments, with error bars reflecting standard deviations.

(EPS)

Figure S5 Loss of H3 HATs does not lead to major changes in nucleosome positioning at the PTK1 gene. MNase analysis of PTK1-specific chromatin in wild-type, gen5Δ sas3 and gen5Δ sas3 ioc3Δ cells. Chromatin was probed for PTK1 following digestion with MNase at concentrations of 0, 60, 150 and 400 U/ml, and EcoRI digestion. Marker restriction digests (Marker) are positioned relative to schematic maps of the PTK1 gene. Restriction site positions are relative to the transcriptional start site of PTK1. The black line corresponds to the probe used for indirect end labeling. Densitometric scans of chromatin digested with 60 and 150 U/ml of MNase for the wild-type and mutant strains, respectively, were generated using ImageJ software (National Institutes of Health, USA).

(EPS)

Figure S6 Requirements of Islw1 complex subunits for restoration of gen5Δ and gen5Δ sas3 growth defects. (A) Increased gene dosage of IOC2 did not rescue the temperature sensitivity of the gen5Δ mutant. The gen5Δ mutant was transformed with the indicated plasmids. Strains were plated on SC-Leu medium and grown for 4 days. (B) Suppression and exacerbation of temperature sensitivity mediated by overexpression of Islw1a and Islw1b complexes, respectively, require the Islw1 ATPase. The gen5Δ sas3 ioc3Δ mutant was transformed with the indicated plasmids. Transformed strains were plated onto SC-Leu medium and grown for 4 days at the indicated temperatures.

(EPS)

Table S1 Yeast strains used in this study.

(DOC)

Table S2 ChIP primers used in this study.

(DOCX)

Text S1 Supporting Methods.

(DOCX)

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Author Contributions

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