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Acetylation of Rsc4p by Gcn5p Is Essential in the Absence of Histone H3 Acetylation▽

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Rsc4p, a subunit of the RSC chromatin-remodeling complex, is acetylated at lysine 25 by Gcn5p, a well-characterized histone acetyltransferase (HAT). Mutation of lysine 25 does not result in a significant growth defect, and therefore whether this modification is important for the function of the essential RSC complex was unknown. In a search to uncover the molecular basis for the lethality resulting from loss of multiple histone H3-specific HATs, we determined that loss of Rsc4p acetylation is lethal in strains lacking histone H3 acetylation. Phenotype comparison of mutants with arginine and glutamine substitutions of acetylatable lysines within the histone H3 tail suggests that it is a failure to neutralize the charge of the H3 tail that is lethal in strains lacking Rsc4p acetylation. We also demonstrate that Rsc4p acetylation does not require any of the known Gcn5p-dependent HAT complexes and thus represents a truly novel function for Gcn5p. These results demonstrate for the first time the vital and yet redundant functions of histone H3 and Rsc4p acetylation in maintaining cell viability.

Posttranslational modifications can augment protein function extending the diversity of proteins produced by the cell. For example, many thousands of proteins in a typical eukaryotic cell are modified by the covalent addition of a phosphate group (22), which can serve to either directly alter protein structure or mediate protein-protein interactions. Another well-studied modification is protein acetylation. Amino-terminal acetylation is one of the most common protein modifications in eukaryotes, occurring on ca. 85% of proteins. In addition, the acetylation of the epsilon-amino group of internal lysines occurs on α-tubulin, high-mobility group proteins, transcription factors, nuclear import factors, and histones (28).

Histones H2A, H2B, H3, and H4 are the best-characterized substrates for posttranslational acetylation of internal lysines, with the majority of histone acetylation occurring on the unstructured amino-terminal "tails" of these proteins. These modifications are proposed to have two functions: to directly alter chromatin structure by weakening histone-DNA, as well as internucleosome interactions (1, 2, 11, 33, 34), and to act as a "molecular dock" for recruitment of factors that modify chromatin structure (42). Histone acetylation is catalyzed by histone acetyltransferases (HATs), which are comprised of a catalytic subunit complexed with accessory proteins that serve to either target or potentiate HAT activity. The best-studied catalytic subunit is Gcn5p, a component of multiple histone H3-specific HAT complexes in *Saccharomyces cerevisiae*. These complexes are responsible for acetylation of lysines 9, 14, 18, 23, 27, and 36 of histone H3 (14, 26, 37). All Gcn5p-dependent HAT complexes share the accessory proteins Ada2p and Ada3p, and several studies have demonstrated that *ADA2* and *ADA3* are essential for both the nucleosomal HAT activity of Gcn5p and its incorporation into HAT complexes (5, 7, 13). Indeed, the majority of phenotypes and genetic interactions found in *gcn5* mutants generally exist in *ada2* and *ada3* mutants (19).

In addition to Gcn5p, there are two other proteins in yeast, Sas3p and Elp3p, which acetylate the histone H3 tail in vivo (15, 17, 32). Sas3p is a component of the NuA3 HAT complex (16), while Elp3p is the catalytic subunit of the elongator complex (41). Deletion of the genes encoding *GCN5*, *SAS3*, or *ELP3* results in only minor phenotypes, but deletion of *GCN5* and *SAS3* concomitantly causes synthetic lethality (15). This result suggests that histone H3 acetylation is essential for viability; however, this is surprising when one considers that the histone H3 tail is dispensable for cell growth (20, 25). Moreover, an additional inconsistency in these data is that the *gcn5*Δ *sas3*Δ synthetic lethality is not due to the loss of any of the Gcn5p-dependent HAT complexes since *ada2*Δ *sas3*Δ and *ada3*Δ *sas3*Δ strains are viable (15). This latter result suggests a novel, HAT complex-independent function for Gcn5p.

In addition to acetylating histones, Gcn5p has been shown to acetylate lysine 25 of Rsc4p, a component of the essential RSC chromatin-remodeling complex (40). Rsc4p contains two bromodomains (BD1 and BD2), the second of which (BD2) preferentially interacts with histone H3 tails acetylated at lysine 14 (H3K14ac). In vitro evidence suggests that the interaction between BD2 and H3K14ac is negatively regulated by acetylation of lysine 25 within Rsc4p. It has therefore been postulated that although the RSC complex is targeted to active genes through a direct interaction between Rsc4p and H3K14ac, the residence time of RSC at these genes is limited due to acetylation by Gcn5p (40). Mutation of Rsc4K25, however, results in only minor phenotypes, leaving the importance of this posttranslational modification for function of the essential RSC complex in question (40). In the present study, we demonstrate that the *gcn5*Δ *sas3*Δ synthetic lethality is due to a combined failure to acetylate histone H3 and Rsc4p. This indicates that acetylation of Rsc4p has an essential role in RSC function that is redun-

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dant with acetylation of histone H3. Surprisingly, however, this essential role for Rsc4p acetylation is not limited to the autoregulation of the Rsc4-H3K14ac interaction, suggesting an unexplored function for Rsc4p acetylation. Instead of revealing the function of Rsc4p acetylation, these results shed new light on the function of histone H3 acetylation by demonstrating that it is a failure to neutralize the charged lysines on the histone H3 tail that is lethal in strains with impaired RSC function. Finally, we provide evidence that acetylation of Rsc4p by Gcn5p is independent of *ADA2*, which is the first demonstrated HAT complex-independent function for Gcn5p.

MATERIALS AND METHODS

Yeast strains, plasmids, and genetic methods. All strains used in the present study are isogenic to S288C. Yeast culture and genetic manipulations were carried out by using standard protocols (3, 21, 31). Genomic deletions were verified by PCR analysis. The strains carrying the histone H3 mutations were derived from FY2162, which has deletions of the *HHT1-HHF1* and *HHT2-HHF2* genes, and carries *HHT2-HHF2* on a *URA3* plasmid (8). Due to the fact that this strain and all strains derived from it carry a wild-type version of *HHF2* on a plasmid, for simplicity, the genotypes of these strains will be referred to as *hht1*Δ *hht2*Δ in the figures. The *TRP1* plasmid expressing wild-type *HHT2* and *HHF2* (pLH305) was constructed by ligation of the SpeI restricted fragment from pDM18 (8) into the SpeI site of pRS414. Plasmids expressing lysine-to-arginine (K14R [pLH307], K9,14,18,23R [pLH311], K9,14,18,23,27R [pLH353], K9,14,18,23,27,36R [pLH354]), and lysine to glutamine (K9,14,18,23Q [pLH434]) mutant versions of histone H3 were described previously (23) or prepared for the present study by ligating annealed oligonucleotides into the BamHI and AgeI sites of pLH305. The gene encoding *RSC4*, including 122 bp of upstream and 250 bp of downstream sequences, was cloned into the SalI and BamHI sites of both pRS416 (pLH372) and pRS415 (pLH373). Mutation of Rsc4p lysine 25 to alanine was done by megaprimer-based mutagenesis to generate pLH374. For simultaneous expression of *RSC4*, *HHT2*, and *HHF2* from the same plasmid, the SpeI restricted fragments from pLH305, pLH307, pLH311, and pLH434 were ligated into the SpeI sites of pLH373 and pLH374. Plasmids expressing wild-type Gcn5p (pLH185), $Gcn5p_{1\text{-}261}$ (pLH385), wild-type Sas3p (pLH141), and temperature-sensitive Sas3C357Y/P375A (pLH157) were described previously (7, 15).

Calmodulin affinity purification and Western blot analysis. Strains expressing Rsc2p with a tandem affinity purification (TAP) tag (31) were cultured in yeast extract, peptone, and dextrose (YPD) media to mid-log phase. Lysates from cells were prepared in extraction buffer (50 mM HEPES [pH 7.5], 350 mM NaCl, 2 mM $CaCl_2$, 0.1% Tween 20, 10% glycerol, protease inhibitor cocktail [P8215; Sigma-Aldrich Co.]) by bead beating. Approximately 75 mg of extract was incubated with 10 μl of calmodulin affinity resin (Stratagene) for 2 h at 4°C. The resin was washed three times with 40 volumes of extraction buffer, and the bound proteins were eluted by boiling in 3 volumes of sodium dodecyl sulfate-polyacrylamide gel electrophoresis loading buffer. The samples were Western blotted and probed with antibodies specific to the TAP tag (P2026; Sigma-Aldrich Co.), anti-acetylated histone H3 (catalog no. 06-599; Millipore), anti-histone H3 (ab1791; Abcam, Inc.), or anti-acetyl-lysine (ab409; Abcam, Inc.).

RESULTS AND DISCUSSION

The *gcn5*Δ *sas3*Δ synthetic lethality is not due to loss of histone H3 acetylation by Gcn5p. We previously demonstrated that strains with deletions of *GCN5* and *SAS3* are inviable (15). A temperature-sensitive *gcn5*Δ *sas3C357Y/P375A* strain fails to recover after growth at a nonpermissive temperature, indicating that disruption of both *GCN5* and *SAS3* results in lethality (15; data not shown). Although plasmids expressing wild-type HATs rescue the viability of a *gcn5*Δ *sas3*Δ strain, plasmids expressing Gcn5p and Sas3p with substitutions of conserved amino acids within the acetyl coenzyme A binding domains do not (Fig. 1A). While this confirms that the synthetic lethal phenotype is a result of loss of the acetyltransferase activities of these proteins, a major paradox is that while *GCN5* or *SAS3* is essential for viability, the histone H3 tail, which is the major target of these enzymes, is not (20, 25). Both Gcn5p and Sas3p have been shown to acetylate histone H3 in vivo (15, 18, 32, 44); however, whether the *gcn5*Δ *sas3*Δ inviability is due to loss of acetylation of a substrate other than histone H3 has not been explored. To determine whether this phenotype is due to loss of histone H3 acetylation by either Gcn5p or Sas3p, we sought to determine whether mutation of target lysines within histone H3 could recapitulate the *gcn5*Δ *sas3*Δ synthetic lethality in either a *gcn5*Δ or *sas3*Δ strain. To this end, we generated wild-type, *gcn5*Δ, and *sas3*Δ strains that expressed the sole copy of the histone H3 gene from a *URA3* plasmid. We next introduced *TRP1*-based plasmids expressing histone H3 with arginine substitutions of acetylatable lysines and examined the synthetic phenotypes on 5-fluoroorotic acid (5-FOA). Simultaneous mutation of lysines 9, 14, 18, 23, and 27 to arginines caused a noticeable growth defect in a wild-type strain but resulted in lethality in a *gcn5*Δ strain (Fig. 1B). This phenotype can be recapitulated by mutation of lysines 14 and 23 alone (data not shown), which are the sites targeted by the NuA3 complex (15). These data are consistent with the fact that in the absence of *GCN5*, the acetylation of histone H3 by Sas3p is essential. In contrast to the synthetic phenotypes observed upon mutation of histone H3 in a *gcn5*Δ strain, deletion of *SAS3* in the K9,14,18,23,27R mutant did not result in any additional phenotype (Fig. 1B). In addition to lysines 9, 14, 18, 23, and 27, Gcn5p has recently been shown to acetylate lysine 36 of histone H3 (26). However, deletion of *SAS3* in a strain with concomitant mutations of lysines 9, 14, 18, 23, 27, and 36 to arginines did not result in loss of viability of this mutant. The fact that we were unable to phenocopy the *gcn5*Δ *sas3*Δ synthetic lethality by mutating all of the known Gcn5p-targeted sites on histone H3 in a *sas3*Δ background suggests that Gcn5p

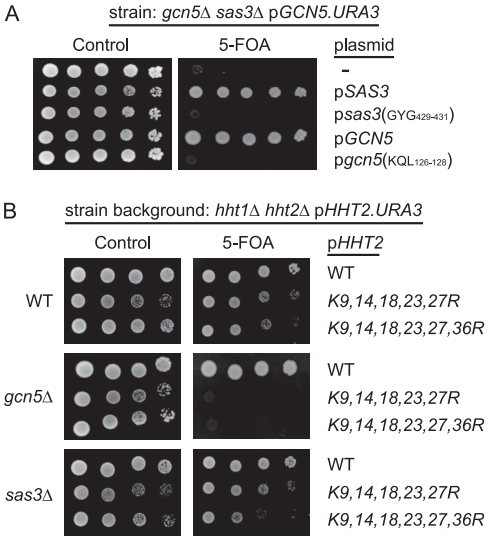

FIG. 1. The *gcn5*Δ *sas3*Δ synthetic lethality is not due to loss of histone H3 acetylation by Gcn5p. Tenfold serial dilutions of the indicated strains with the specified plasmids were plated on synthetic complete media without (Control) and with 5-FOA and incubated at 30°C for 3 days for WT and *sas3*Δ strains and 4 days for *gcn5*Δ strains. GYG: *sas3* with a triple alanine substitution of amino acids $GYG_{429\text{-}431}$; KQL, *gcn5* with a triple alanine substitution of amino acids $KQL_{126\text{-}128}$.

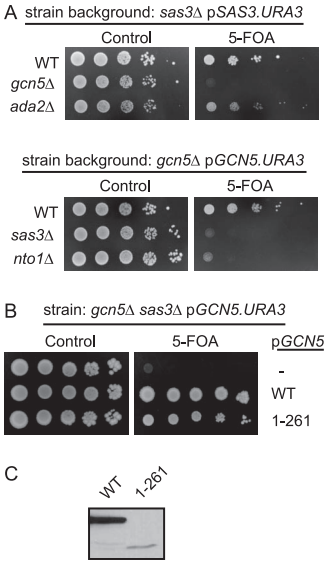

FIG. 2. The *gcn5*Δ *sas3*Δ synthetic lethality is not due to loss of the known Gcn5p-dependent HAT complexes. (A) The indicated strains were plated in 10-fold serial dilutions on synthetic complete media without (Control) and with 5-FOA. (B) Strains transformed with vector alone (−), or plasmids expressing either full-length Gcn5p (WT) or $Gcn5p_{1\text{-}261}$ (1-261) were plated on synthetic complete media without (Control) and with 5-FOA and incubated at 30°C for 3 days. (C) WCE prepared from cell expressing triple HA-tagged full-length (WT) and C-terminal truncated (1-261) Gcn5p were blotted and probed for the HA tag.

is required for a function other than acetylating the amino-terminal tail of histone H3. It is loss of this acetylation that is lethal in the absence of histone H3 acetylation.

Gcn5p has a HAT complex-independent function. Our phenotype analysis strongly suggests that Gcn5p is acetylating a substrate other than the histone H3 tail. In addition to histone H3, Gcn5p has been shown to acetylate lysines 11 and 16 of histone H2B in vivo (37). Moreover, the possibility that Gcn5p is acetylating unidentified sites within any of the core histones cannot be excluded. To determine whether the *gcn5*Δ *sas3*Δ phenotype is due to loss of Gcn5p's ability to acetylate histones, we sought to determine whether any of the Gcn5p-dependent HAT complexes are essential in a *sas3*Δ strain. Gcn5p is the catalytic subunit of at least three different HAT complexes, including SAGA, SLIK/SALSA, and ADA (9, 13, 30, 36). In addition to Gcn5p, these HATs also share Ada2p, and experimental evidence suggests that Ada2p is required for Gcn5p HAT activity (5, 38). Furthermore, phenotypes associated with deletions of *GCN5* are indistinguishable from those associated with deletions of *ADA2* (12). Thus, both in vitro and in vivo evidence supports the fact that Ada2p is required for the function of Gcn5p as a HAT. However, despite the requirement of Ada2p for Gcn5p HAT activity, *ada2*Δ *sas3*Δ strains are viable (Fig. 2A) (15). In contrast, deletion of *NTO1*, a gene encoding a structural component of NuA3, is lethal in a *gcn5*Δ strain (Fig. 2A). These data indicate that although the *gcn5*Δ *sas3*Δ synthetic lethality is due to loss of NuA3 in a *gcn5*Δ strain, the Gcn5p-dependent HAT complexes are dispensable in a *sas3*Δ strain. To further confirm that the *gcn5*Δ *sas3*Δ synthetic lethality is not due to loss of any of the Gcn5p-dependent HATs, we sought to determine whether a mutation in Gcn5p that disrupts the incorporation of this protein into a HAT complex is lethal in a *sas3*Δ strain. It has been previously demonstrated that although the first 261 amino acids of Gcn5p are sufficient for histone HAT activity in vitro, additional residues located carboxyl-terminal to this HAT domain are required for the incorporation of this protein into HAT complexes (7). We sought to determine whether a mutant version of Gcn5p, lacking the HAT interaction domain, could rescue the *gcn5*Δ *sas3*Δ synthetic lethality. Figure 2B demonstrates that a *TRP1* plasmid expressing $Gcn5p_{1\text{-}261}$ rescues the growth of a *gcn5*Δ *sas3*Δ p*GCN5.URA3* strain on 5-FOA, further confirming that the *gcn5*Δ *sas3*Δ synthetic lethality is not due to loss of any of the Gcn5p-dependent HAT complexes. This strain does show a growth defect compared to an isogenic strain expressing full-length Gcn5p. To determine whether this is due to decreased stability of truncated Gcn5p, we fused full-length and truncated Gcn5p to carboxyl-terminal triple hemagglutinin (HA) tags and examined levels of Gcn5p in whole-cell extracts (WCE) by Western blotting. Figure 2C shows that Gcn5p(1-261) is significantly less abundant than full-length Gcn5p, suggesting that the growth defect shown in Fig. 2B is due to lower levels of Gcn5p. The fact that the HAT interaction domain is not required to rescue the *gcn5*Δ *sas3*Δ synthetic phenotype and that Gcn5p must be incorporated into a HAT complex to acetylate nucleosomal histones (5, 13) suggests that the *gcn5*Δ *sas3*Δ synthetic lethality is a result of a failure of Gcn5p to acetylate a nonhistone substrate in a strain lacking histone H3 acetylation.

The acetylation of Rsc4p and that of histone H3 are redundant. Gcn5p has been shown to acetylate numerous proteins in yeast in addition to the canonical core histones, including Rsc4p, Sin1p, and Htz1p (4, 24, 29, 40). Whether acetylation of these proteins requires the Gcn5p-dependent HAT complexes has never been tested. To determine whether the *gcn5*Δ *sas3*Δ phenotype is due to a failure to acetylate Rsc4p in a *sas3*Δ strain, we sought to determine whether we could recapitulate the *gcn5*Δ *sas3*Δ synthetic lethality by mutating the Rsc4p acetylation site (lysine 25) in a strain lacking histone H3 acetylation. Since histone H3 is acetylated by both Sas3p and multiple Gcn5p-dependent HATs, we disrupted H3 acetylation by concomitant deletions of *SAS3* and *ADA2*. We generated *rsc4*Δ, *rsc4*Δ *sas3*Δ, *rsc4*Δ *ada2*Δ, and *rsc4*Δ *sas3*Δ *ada2*Δ strains that expressed *RSC4* from a *URA3*-based plasmid. A plasmid shuffle experiment was performed using plasmids expressing wild-type Rsc4p (WT), and Rsc4p with an alanine substitution of lysine 25 (K25A). Figure 3A demonstrates that while *rsc4*K25A mutants are viable in wild-type, *sas3*Δ, and *ada2*Δ backgrounds, mutation of Rsc4K25 in an *ada2*Δ *sas3*Δ strain is lethal. The phenocopy of the *gcn5*Δ *sas3*Δ synthetic lethality by mutation of Rsc4K25 in a mutant lacking histone H3-specific HATs confirms that this phenotype is due to redundancy in acetylation of histone H3 and Rsc4K25. As a further confirmation, we tested whether mutation of K25 of Rsc4p results in lethality in a strain with point mutations of the acetylatable lysines within the H3 tail. Figure 3B shows that while *rsc4*K25A *HHT2* and *rsc4*K25A *hht2*K14R strains are viable, simultaneous mutation of histone H3 lysines 9, 14, 18, and 23 to arginines is lethal in a strain lacking Rsc4K25, further confirming the redundant function of these residues in maintaining cell viability.

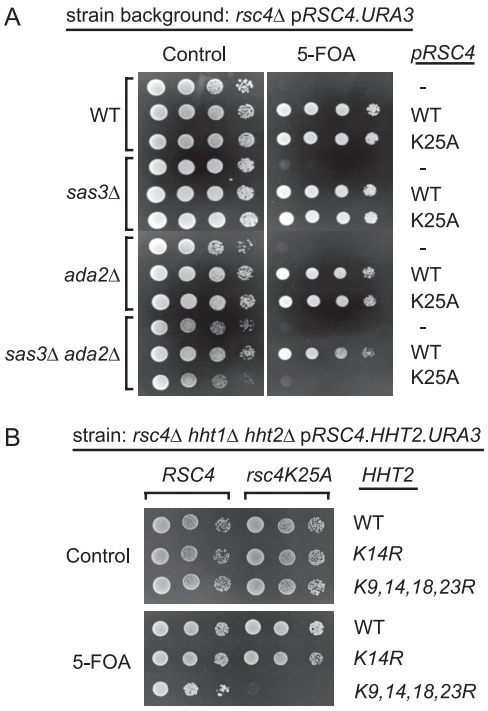

FIG. 3. The *gcn5*Δ *sas3*Δ synthetic lethality is due to redundancy between acetylation of histone H3 and Rsc4p. Tenfold serial dilutions of the indicated strains transformed with the specified plasmids were plated on synthetic complete media without (Control) and with 5-FOA and incubated at 30°C for 3 days.

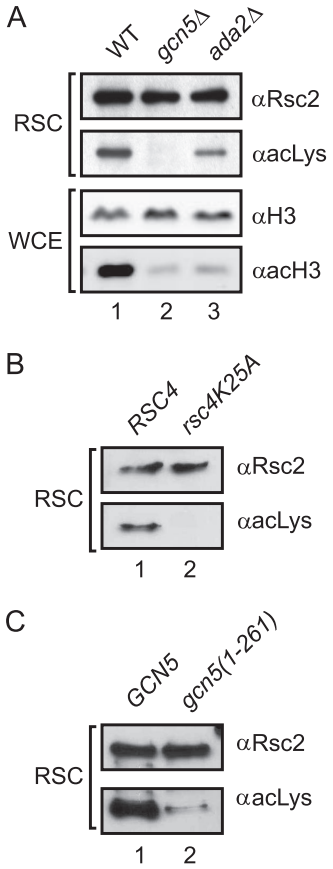

FIG. 4. The acetylation of Rsc4p by Gcn5p does not require any of the Gcn5p-dependent HAT complexes. WCE and calmodulin affinity-purified RSC (RSC) from the specified strains were blotted and probed with the antibodies indicated.

The synthetic phenotype observed upon loss of Rsc4p and histone H3 acetylation is surprising when one considers the proposed function of Rsc4p acetylation. Recent data suggest that within Rsc4p, K25ac binds to bromodomain 1, inhibiting the interaction of H3K14ac with bromodomain 2 (40). Considering the opposing roles of H3K14ac and Rsc4K25ac in the binding and release of RSC from chromatin, respectively, clearly mutation of Rsc4K25 should not result in an enhanced phenotype in a strain with a mutation of H3K14. Instead, these results suggest an additional function for Rsc4p acetylation that is independent of regulating the bromodomain 2-H3K14ac interaction. The impact of Rsc4p acetylation on RSC function could be at the level of complex integrity, remodeling activity, or DNA or histone binding. The data presented in this study underscore the need for further study into the function of Rsc4p acetylation, which obviously has a role in RSC that is essential in the absence of histone H3 acetylation.

Distinct proteins are required for Rsc4p and H3 acetylation by Gcn5p. The fact that *ada2*Δ *sas3*Δ strains are viable (Fig. 2A) (15) suggests that *ADA2* is not required for acetylation of Rsc4p. This represents the first example of a Gcn5p function that is independent of the known HAT complexes. To verify that acetylation of Rsc4p is independent of the Gcn5p-containing HAT complexes, we tested whether we could detect Rsc4p acetylation in an *ada2*Δ strain. To this end, we purified RSC from a Rsc2TAP strain using calmodulin affinity purification and subjected the coprecipitating proteins (RSC) to Western blot analysis with anti-TAP (αRsc2) and anti-acetyl-lysine (αacLys) antibodies. Figure 4A shows that, as observed by others, purified RSC contains an acetylated protein that comigrates with Rsc4p. Mutation of lysine 25 of Rsc4p results in loss of this signal (Fig. 4B), demonstrating that the acetylated-lysine signal is indeed from Rsc4K25. As shown by others, acetylation of Rsc4p is disrupted by deletion of *GCN5* (Fig. 4A, compare αacLys signal in lanes 1 and 2) (40). Interestingly, deletion of *ADA2* has only a minimal effect on the levels of acetylated Rsc4p (lane 3), suggesting that acetylation of Rsc4p is independent of any of the known Gcn5p-dependent HAT complexes. As a control we performed Western blot analysis of WCE from each strain using an anti-acetyl H3 antibody (αacH3) to verify that deletion of *ADA2* resulted in the same loss of histone H3 acetylation seen in a *gcn5*Δ strain (Fig. 4A, compare lanes 1, 2, and 3). Although these data clearly demonstrate that Ada2p is not required for Rsc4p acetylation, we have not ruled out the possibility that Rsc4p is acetylated by Gcn5p that is complexed with Ada2p. Indeed, we have found that, similar to the HAT-interacting domain of Gcn5p, Ada2p is required for the stability of Gcn5p in vivo (unpublished observation). It is therefore probable that the majority of Gcn5p in the cell is associated with Ada2p. As a result, although Ada2p is not required for the Rsc4p acetyltransferase activity of Gcn5p, it may contribute through stabilizing the protein. In support of this, we see reduced levels of acetylated

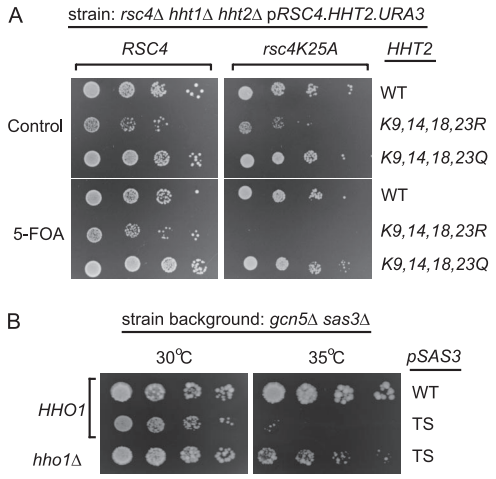

FIG. 5. Histone acetylation is important for neutralizing the positive charge of the histone H3 tail. (A) Strains carrying the indicated mutations within Rsc4p and/or histone H3 were plated on synthetic complete media without (Control) and with 5-FOA and incubated at 30°C for 2 days. (B) *gcn5*Δ *sas3*Δ strains with the indicated plasmids were plated in 10-fold serial dilutions on YPD and incubated at 30 and 35°C for 3 days. WT, a wild-type *SAS3*; TS, temperature-sensitive *sas3 C357Y/P375A*.

Rsc4p in strains lacking *ADA2* consistent with the fact that there is less Gcn5p in these cells. As a final confirmation that the acetylation of Rsc4p is independent of the Gcn5p HAT complexes, we examined the levels of Rsc4p acetylation in a strain lacking the HAT interaction domain of Gcn5p. As shown in Fig. 4C, Rsc4p is still acetylated in strains expressing Gcn5p(1-261). The level of acetylation is severely reduced compared to strains expressing full-length Gcn5p consistent with the fact that there is less Gcn5p in these cells (see Fig. 2C). These results confirm a novel function for Gcn5p that is independent of the accessory proteins found in the SAGA, ADA, and SLIK/SALSA HAT complexes.

Histone acetylation is important for neutralizing the positive charge of the histone H3 tail. The results in Fig. 3B suggest that Rsc4p acetylation has a role that is independent of regulating the Rsc4p bromodomain 2-H3K14ac interaction. Rsc4p acetylation therefore has multiple roles in RSC function and, based on what we know about Rsc4p acetylation alone, it is difficult to speculate as to why the loss of histone H3 and Rsc4p acetylation results in loss of viability. Fortunately, unlike the recently discovered Rsc4p acetylation, histone acetylation has been the focus of intense study for many decades. To date, histone H3 acetylation has been proposed to function in two, non-mutually exclusive manners: to act as a molecular "tag" for the recruitment of chromatin-modifying complexes (42) and to directly alter chromatin structure by weakening histone-DNA contacts (10, 39). We hypothesized that if the loss of H3 acetylation is disrupting the binding of a chromatin-modifying complex to the H3 tail, then mutation of the acetylatable lysines to glutamine should recapitulate the phenotypes of arginine substitutions and be lethal in a *rsc4*K25A mutant. In contrast, if histone H3 acetylation is required to weaken histone-DNA contacts, then substitution of acetylatable lysines with uncharged glutamines should be tolerated in a strain lacking Rsc4p acetylation Fig. 5A shows that unlike a *rsc4*K25A strain with arginine substitutions of lysines 9, 14, 18, and 23 of histone H3, strains with glutamine substitutions are viable. These results are reminiscent of observations made by Zhang (44), which showed that, while simultaneous mutation of several sites on histones H3 and H4 to arginine in a *gcn5*Δ background results in lethality, mutation of the same sites to glutamine bypasses the need for *GCN5* for transcriptional activation by Gal4-VP16. These data strongly suggest that the *rsc4*K25A *hht2*K9,14,18,23R inviability, and hence the *gcn5*Δ *sas3*Δ synthetic lethality, is due to a failure to neutralize the positive charge on the histone H3 tail. This charge neutralization is required to weaken histone-DNA contacts, which is essential when RSC function is impaired due to loss of Rsc4p acetylation.

The direct effects of histone acetylation on chromatin structure have been the focus of intense study. The majority of these studies have examined the impact of simultaneous acetylation of all four core histones, although two studies have directly examined the effect of histone H3 acetylation alone. First, chemical acetylation of histone H3 has been shown to result in a transient unwrapping of DNA from the octamer, as shown by measuring distances between the linker DNA ends using FRET analysis (39). Second, tetra-acetylation of histone H3 using a peptide ligation strategy results in a twofold increase in the rate of intrinsic mono-nucleosome sliding in vitro (10). Thus, histone H3 acetylation may weaken histone-DNA contacts resulting in both enhanced nucleosome "breathing" and increased octamer mobility. To confirm that it is the loss of these events that results in lethality in a *rsc4*K25A strain, we sought to determine whether we could bypass the requirement for *GCN5* and *SAS3* by deleting a gene that inhibits both nucleosome breathing and octamer mobility. Linker histones associate with the linker DNA that extends between nucleosomal core particles and are essential for condensation of nucleosome arrays into the 30-nm fibers. Incorporation of histone H1 into a nucleosome causes the linker DNA to contact as a "stem" at the nucleosome edge (6, 39) and restricts passive nucleosome movement (24, 25). *S. cerevisiae* encodes a single linker histone, Hho1p, which, unlike linker histones in higher eukaryotes, has two globular domains. However, Hho1p binds nucleosomes in vitro (27), is expressed during S phase coordinately with the core histones (35), and colocalizes with the four core histones in vivo (43). Moreover, regions of the yeast genome with high Hho1p levels tended to be underacetylated at lysines 9 and 14 of histone H3 (43), and thus an intriguing hypothesis is that, in addition to weakening histone-DNA contacts, histone H3 acetylation may also prevent the binding of histone H1 to chromatin. To determine whether loss of *HHO1* rescues growth of a *gcn5*Δ *sas3*Δ mutant, we generated a *gcn5*Δ *sas3*Δ *hho1*Δ strain that expressed *SAS3* from a *URA3*-based plasmid. This strain failed to grow on 5-FOA (data not shown); however, when using a conditional *gcn5*Δ *sas3*Δ mutant that expressed a temperature-sensitive version of Sas3p (TS), we found that deletion of *HHO1* could rescue growth of this strain at the nonpermissive temperature (Fig. 5B). When taken together with the fact that Rsc4p acetylation is essential in strains that fail to neutralize the positively charged lysines within the H3 tail, these results suggest that histone H3 acetylation is required to disrupt histone-DNA contacts, and loss of this activity is lethal in strains with impaired RSC function. We envision a model whereby both RSC and histone acetylation function to destabilize chromatin structure, and concomitant

deletions of *GCN5* and *SAS3* hinder both events resulting in loss of viability.

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REFERENCES

1. **Anderson, J. D., P. T. Lowary, and J. Widom.** 2001. Effects of histone acetylation on the equilibrium accessibility of nucleosomal DNA target sites. J. Mol. Biol. **307:**977–985.
2. **Ausió, J., and K. E. van Holde.** 1986. Histone hyperacetylation: its effects on nucleosome conformation and stability. Biochem. **25:**1421–1428.
3. **Ausubel, F. M.** 1987. Current protocols in molecular biology. Greene Publishing Associates/Wiley-Interscience, New York, NY.
4. **Babiarz, J. E., J. E. Halley, and J. Rine.** 2006. Telomeric heterochromatin boundaries require NuA4-dependent acetylation of histone variant H2A. Z in *Saccharomyces cerevisiae*. Genes Dev. **20:**700–710.
5. **Balasubramanian, R., M. G. Pray-Grant, W. Selleck, P. A. Grant, and S. Tan.** 2002. Role of the Ada2 and Ada3 transcriptional coactivators in histone acetylation. J. Biol. Chem. **277:**7989–7995.
6. **Bednar, J., R. A. Horowitz, S. A. Grigoryev, L. M. Carruthers, J. C. Hansen, A. J. Koster, and C. L. Woodcock.** 1998. Nucleosomes, linker DNA, and linker histone form a unique structural motif that directs the higher-order folding and compaction of chromatin. Proc. Natl. Acad. Sci. USA **95:**14173–14178.
7. **Candau, R., J. X. Zhou, C. D. Allis, and S. L. Berger.** 1997. Histone acetyltransferase activity and interaction with ADA2 are critical for GCN5 function in vivo. EMBO J. **16:**555–565.
8. **Duina, A. A., and F. Winston.** 2004. Analysis of a mutant histone H3 that perturbs the association of Swi/Snf with chromatin. Mol. Cell. Biol. **24:**561–572.
9. **Eberharter, A., D. E. Sterner, D. Schieltz, A. Hassan, J. R. Yates III, S. L. Berger, and J. L. Workman.** 1999. The ADA complex is a distinct histone acetyltransferase complex in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **19:**6621–6631.
10. **Ferreira, H., A. Flaus, and T. Owen-Hughes.** 2007. Histone modifications influence the action of Snf2 family remodeling enzymes by different mechanisms. J. Mol. Biol. **374:**563–579.
11. **Garcia-Ramirez, M., F. Dong, and J. Ausio.** 1992. Role of the histone "tails" in the folding of oligonucleosomes depleted of histone H1. J. Biol. Chem. **267:**19587–19595.
12. **Georgakopoulos, T., N. Gounalaki, and G. Thireos.** 1995. Genetic evidence for the interaction of the yeast transcriptional coactivator proteins GCN5 and ADA2. Mol. Gen. Genet. **246:**723–728.
13. **Grant, P. A., L. Duggan, J. Côté, S. M. Roberts, J. E. Brownell, R. Candau, R. Ohba, T. Owen-Hughes, C. D. Allis, F. Winston, S. L. Berger, and J. L. Workman.** 1997. Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. Genes Dev. **11:**1640–1650.
14. **Grant, P. A., A. Eberharter, S. John, R. G. Cook, B. M. Turner, and J. L. Workman.** 1999. Expanded lysine acetylation specificity of Gcn5 in native complexes. J. Biol. Chem. **274:**5895–5900.
15. **Howe, L., D. Auston, P. Grant, S. John, R. G. Cook, J. L. Workman, and L. Pillus.** 2001. Histone H3 specific acetyltransferases are essential for cell cycle progression. Genes Dev. **15:**3144–3154.
16. **John, S., L. Howe, S. T. Tafrov, P. A. Grant, R. Sternglanz, and J. L. Workman.** 2000. The something about silencing protein, Sas3, is the catalytic subunit of NuA3, a yTAF(II)30-containing HAT complex that interacts with the Spt16 subunit of the yeast CP (Cdc68/Pob3)-FACT complex. Genes Dev. **14:**1196–1208.
17. **Kristjuhan, A., J. Walker, N. Suka, M. Grunstein, D. Roberts, B. R. Cairns, and J. Q. Svejstrup.** 2002. Transcriptional inhibition of genes with severe histone h3 hypoacetylation in the coding region. Mol. Cell **10:**925–933.
18. **Kuo, M.-H., J. Zhou, P. Jambeck, M. E. A. Churchill, and C. D. Allis.** 1998. Histone acetyltransferase activity of yeast Gcn5p is required for the activation of target genes in vivo. Genes Dev. **12:**627–639.
19. **Lin, Y. Y., Y. Qi, J. Y. Lu, X. Pan, D. S. Yuan, Y. Zhao, J. S. Bader, and J. D. Boeke.** 2008. A comprehensive synthetic genetic interaction network governing yeast histone acetylation and deacetylation. Genes Dev. **22:**2062–2074.
20. **Ling, X., T. A. A. Harkness, M. C. Schultz, G. Fisher-Adams, and M. Grunstein.** 1996. Yeast histone H3 and H4 amino termini are important for nucleosome assembly in vivo and in vitro: redundant and position-independent functions in assembly but not in gene regulation. Genes Dev. **10:**686–699.
21. **Longtine, M. S., A. McKenzie III, D. J. Demarini, N. G. Shah, A. Wach, A. Brachat, P. Philippsen, and J. R. Pringle.** 1998. Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. Yeast **14:**953–961.
22. **Mann, M., S. E. Ong, M. Gronborg, H. Steen, O. N. Jensen, and A. Pandey.** 2002. Analysis of protein phosphorylation using mass spectrometry: deciphering the phosphoproteome. Trends Biotechnol. **20:**261–268.
23. **Martin, D. G. E., D. E. Grimes, K. Baetz, and Howe, L.** 2006. Methylation of histone H3 mediates the association of the NuA3 histone acetyltransferase with chromatin. Mol. Biol. Cell **26:**3018–3028.
24. **Millar, C. B., F. Xu, K. Zhang, and M. Grunstein.** 2006. Acetylation of H2AZ Lys 14 is associated with genome-wide gene activity in yeast. Genes Dev. **20:**711–722.
25. **Morgan, B. A., B. A. Mittman, and M. M. Smith.** 1991. The highly conserved N-terminal domains of histones H3 and H4 are required for normal cell cycle progression. Mol. Cell. Biol. **11:**4111–4120.
26. **Morris, S. A., B. Rao, B. A. Garcia, S. B. Hake, R. L. Diaz, J. Shabanowitz, D. F. Hunt, C. D. Allis, J. D. Lieb, and B. D. Strahl.** 2007. Identification of histone H3 lysine 36 acetylation as a highly conserved histone modification. J. Biol. Chem. **282:**7632–7640.
27. **Patterton, H. G., C. C. Landel, D. Landsman, C. L. Peterson, and R. T. Simpson.** 1998. The biochemical and phenotypic characterization of Hho1p, the putative linker histone H1 of *Saccharomyces cerevisiae*. J. Biol. Chem. **273:**7268–7276.
28. **Polevoda, B., and F. Sherman.** 2002. The diversity of acetylated proteins. Genome Biol. **3:**reviews0006.
29. **Pollard, K. J., and C. L. Peterson.** 1997. Role of ADA/GCN5 products in antagonizing chromatin-mediated transcriptional repression. Mol. Cell. Biol. **17:**6212–6222.
30. **Pray-Grant, M. G., D. Schieltz, S. J. McMahon, J. M. Wood, E. L. Kennedy, R. G. Cook, J. L. Workman, J. R. Yates III, and P. A. Grant.** 2002. The novel SLIK histone acetyltransferase complex functions in the yeast retrograde response pathway. Mol. Cell. Biol. **22:**8774–8786.
31. **Puig, O., F. Caspary, G. Rigaut, B. Rutz, E. Bouveret, E. Bragado-Nilsson, M. Wilm, and B. Seraphin.** 2001. The tandem affinity purification (TAP) method: a general procedure of protein complex purification. Methods **24:**218–229.
32. **Rosaleny, L. E., A. B. Ruiz-Garcia, J. Garcia-Martinez, J. E. Perez-Ortin, and V. Tordera.** 2007. The Sas3p and Gcn5p histone acetyltransferases are recruited to similar genes. Genome Biol. **8:**R119.
33. **Shogren-Knaak, M., H. Ishii, J. M. Sun, M. J. Pazin, J. R. Davie, and C. L. Peterson.** 2006. Histone H4-K16 acetylation controls chromatin structure and protein interactions. Science **311:**844–847.
34. **Simpson, R. T.** 1978. Structure of chromatin containing extensively acetylated H3 and H4. Cell **13:**691–699.
35. **Spellman, P. T., G. Sherlock, M. Q. Zhang, V. R. Iyer, K. Anders, M. B. Eisen, P. O. Brown, D. Botstein, and B. Futcher.** 1998. Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. Mol. Biol. Cell **9:**3273–3297.
36. **Sterner, D. E., R. Belotserkovskaya, and S. L. Berger.** 2002. SALSA, a variant of yeast SAGA, contains truncated Spt7, which correlates with activated transcription. Proc. Natl. Acad. Sci. USA **99:**11622–11627.
37. **Suka, N., Y. Suka, A. A. Carmen, J. Wu, and M. Grunstein.** 2001. Highly specific antibodies determine histone acetylation site usage in yeast heterochromatin and euchromatin. Mol. Cell **8:**473–479.
38. **Syntichaki, P., and G. Thireos.** 1998. The Gcn5-Ada complex potentiates the histone acetyltransferase activity of Gcn5. J. Biol. Chem. **273:**24414–24419.
39. **Toth, K., N. Brun, and J. Langowski.** 2006. Chromatin compaction at the mononucleosome level. Biochemistry **45:**1591–1598.
40. **VanDemark, A. P., M. M. Kasten, E. Ferris, A. Heroux, C. P. Hill, and B. R. Cairns.** 2007. Autoregulation of the rsc4 tandem bromodomain by gcn5 acetylation. Mol. Cell **27:**817–828.
41. **Wittschieben, B. O., G. Otero, T. de Bizemont, J. Fellows, H. Erdjument-Bromage, R. Ohba, Y. Li, C. D. Allis, P. Tempst, and J. Q. Svejstrup.** 1999. A novel histone acetyltransferase is an integral subunit of elongating RNA polymerase II holoenzyme. Mol. Cell **4:**123–128.
42. **Yang, X. J.** 2004. Lysine acetylation and the bromodomain: a new partnership for signaling. Bioessays **26:**1076–1087.
43. **Zanton, S. J., and B. F. Pugh.** 2006. Full and partial genome-wide assembly and disassembly of the yeast transcription machinery in response to heat shock. Genes Dev. **20:**2250–2265.
44. **Zhang, W., J. R. Bone, D. G. Edmondson, B. M. Turner, and S. Y. Roth.** 1998. Essential and redundant functions of histone acetylation revealed by mutation of target lysines and loss of the Gcn5p acetyltransferase. EMBO J. **17:**3155–3167.