

Site-specific acetylation mark on an essential chromatin-remodeling complex promotes resistance to replication stress

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Recent work has identified several posttranslational modifications (PTMs) on chromatin-remodeling complexes. Compared with our understanding of histone PTMs, significantly less is known about the functions of PTMs on remodeling complexes, because identification of their specific roles often is hindered by the presence of redundant pathways. Remodels the Structure of Chromatin (RSC) is an essential, multifunctional ATP-dependent chromatin-remodeling enzyme of *Saccharomyces cerevisiae* that preferentially binds acetylated nucleosomes. RSC is itself acetylated by Gcn5 on lysine 25 (K25) of its Rsc4 subunit, adjacent to two tandem bromodomains. It has been shown that an intramolecular interaction between the acetylation mark and the proximal bromodomain inhibits binding of the second bromodomain to acetylated histone H3 tails. We report here that acetylation does not significantly alter the catalytic activity of RSC or its ability to recognize histone H3-acetylated nucleosomes preferentially *in vitro*. However, we find that Rsc4 acetylation is crucial for resistance to DNA damage *in vivo*. Epistatic miniarray profiling of the *rsc4-K25R* mutant reveals an interaction with mutants in the INO80 complex, a mediator of DNA damage and replication stress tolerance. In the absence of a core INO80 subunit, *rsc4-K25R* mutants display sensitivity to hydroxyurea and delayed S-phase progression under DNA damage. Thus, Rsc4 helps promote resistance to replication stress, and its single acetylation mark regulates this function. These studies offer an example of acetylation of a chromatin-remodeling enzyme controlling a biological output of the system rather than regulating its core enzymatic properties.

Regulation of DNA accessibility within chromatin is enabled by the combined action of ATP-dependent chromatin-remodeling complexes and histone-modifying enzymes. Histone-modifying enzymes introduce covalent posttranslational modifications (PTMs) at specific locations. These PTMs either affect chromatin conformation directly or act as a signal for the recruitment of specific factors (1). ATP-dependent chromatin-remodeling complexes noncovalently alter chromatin conformation and composition using the energy of ATP (2). Intriguingly, these complexes also contain several different PTMs including acetylation and phosphorylation (2). However, compared with the functional effects of PTMs on histones, the functional effects of these PTMs are less well understood.

Subunits of ATP-dependent chromatin-remodeling complexes often contain domains capable of recognizing specific PTMs. This phenomenon has been best studied in the context of histone tail acetylation, which is recognized by protein modules, called “bromodomains” (1, 2). In the SWI/SNF subfamily of remodelers, many subunits contain bromodomains (2), which have been shown to facilitate recruitment of these complexes to acetylated nucleosomes (3–7). Recent work has shown that bromodomains can bind acetylation marks *in cis*. Specifically, an acetylation mark on Rsc4, a subunit of RSC, the major SWI/SNF family remodeling complex in budding yeast, is bound *in cis* by one of its two bromodomains, bromodomain 1 (BD1) (8). The interaction between the acetylated Rsc4 N-terminus and BD1 was shown to

reduce the ability of the adjacent bromodomain 2 (BD2) to bind histone H3 tail peptides acetylated at K14 (8). These observations suggested an attractive model in which occupancy of BD1 by the acetylated Rsc4 N terminus reduces the affinity of BD2, and thereby the affinity of the whole RSC complex, for acetylated nucleosomes (8). The model is consistent with observations indicating that Rsc4 contacts the nucleosome surface during remodeling (9). However, this model has not yet been tested in the context of nucleosomes. Moreover, although GCN5 deposits the acetylation mark on Rsc4, the biological role served by this mark remains unknown. Point mutations of the Rsc4 acetylation site display only mild phenotypes. Another chromatin-remodeling enzyme, *Drosophila* ISWI, has been shown similarly to be an *in vivo* and *in vitro* target of Gcn5 (10). However, the biological role of this acetylation mark also is unknown. In general, compared with our understanding of the role of histone acetylation by Gcn5, less is known about the functional impact of nonhistone modifications catalyzed by this enzyme, probably because of the challenges associated with unmasking the functions of individual PTMs in the context of redundant pathways (11).

We report here a series of studies that elucidate the impact of the single acetylation mark on Rsc4 both on RSC’s catalytic activity *in vitro* and on its biological function *in vivo*. We find that acetylation of Rsc4 does not significantly affect RSC catalytic activity or its ability to recognize acetylated nucleosomes. To uncover the biological role of Rsc4 acetylation from redundant pathways, we determined whether a point mutation of the acetylation site has a more severe phenotype in the absence of specific additional genes. We found that the K25 acetylation mark plays a key role in resistance to DNA damage, in a manner that appears to be regulated by its interaction with BD1. Further, Rsc4 acetylation acts in parallel with the INO80-remodeling complex to promote S-phase progression in cells subject to replication stress.

Results

Acetylation of the RSC Complex Does Not Substantially Alter Remodeling Activity or Substrate Specificity. To determine whether acetylation affects the binding and catalytic activities of the RSC complex, RSC complexes from WT and *rsc4-K25R* mutant strains were compared (12) (Fig. 1A). We confirmed the absence of acetylation in the mutant complex using quantitative immunoblotting with an anti-acetyllysine antibody and an anti-TAP antibody (Fig. 1B). Loss of Rsc4 acetylation does not alter

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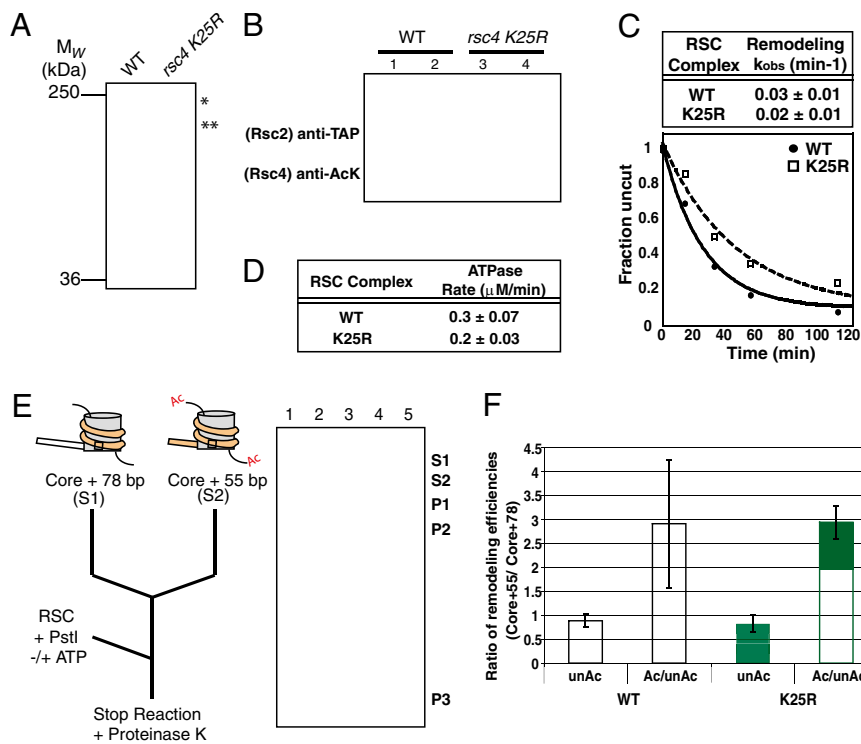


Fig. 1. Acetylated and unacetylated RSC complexes have similar biochemical properties. (A) Purified RSC complexes with TAP-tagged Rsc2. Complex was quantified based on intensity of Sth1 (*), which is the core ATPase subunit of RSC. Stoichiometry was determined based on Rsc4 intensity (**). (B) Dual-antibody Western blot with WT and *rsc4* K25R RSC complexes. Sixty- (lanes 1 and 3) and 30-nM (lanes 2 and 4) RSC complexes were loaded. IR680 (red) secondary antibody recognizes anti-TAP, and IR800 (green) secondary antibody recognizes anti-AcK antibody. (C) Representative time courses for remodeling by WT and *rsc4* K25R RSC, as assayed by restriction enzyme accessibility. Maximal rate constants (min⁻¹) and their variation from two repeats are reported. (D) ATPase rates with saturating DNA (*n* = 4; SDs shown). (E) (Left) Reaction scheme for competitive remodeling assay. PstI site is in red. (Right) Representative time course for reaction of WT RSC with a mixture of unacetylated core+78 bp (S1) and unacetylated core+55 bp (S2) nucleosomes. Proteinase K-treated substrates (uncut DNA) and products (PstI-cut DNA) are labeled "S" and "P," respectively. DNA is stained with SYBR Gold. Lane 1, 0.5 min; lane 2, 15 min; lane 3, 60 min; lane 4, 145 min; lane 5, 240 min. (F) Ratio of remodeling efficiencies for core+55 nucleosomes vs. core+78 nucleosomes (*n* = 3; error bars indicate SDs). Ac/unAc, acetylated core+55/unacetylated core+78 nucleosomes; unAc, both nucleosomes are unacetylated.

complex integrity or stoichiometry significantly (Fig. 1A and *SI Appendix*, Fig. S1A).

We first assessed the extent of acetylation on the WT RSC complex. RSC complexes were purified from cells that either contained or lacked Gcn5, which is required for Rsc4 acetylation (8, 13). We then quantified whether the purified complexes could be acetylated further in vitro. Gcn5 is the core histone acetyltransferase subunit in the multisubunit acetyltransferase SAGA complex, and the Ada2 and Ada3 subunits of SAGA enhance Gcn5 activity on histone H3 in the context of nucleosomes (14). We found that this minimal SAGA complex (mSAGA), containing Gcn5, Ada2, and Ada3 acetylates Rsc4 in the *gcn5Δ* RSC complex to a level comparable to that of Rsc4 in the WT RSC complex (*SI Appendix*, Fig. S1B). In addition, mSAGA does not significantly increase the acetylation level of Rsc4 in the WT RSC complex (*SI Appendix*, Fig. S1C). These observations suggest that the majority of RSC in WT cells is acetylated on Rsc4. Interestingly, we further observed that Gcn5 alone was sufficient to acetylate Rsc4 within the RSC complex (*SI Appendix*, Fig. S1B). This result is consistent with a recent report that *ada2Δ* strains contain acetylated RSC (13). The result also suggests that the requirements for acetylation of Rsc4 and nucleosomal histone tails differ, because previous work has revealed that Gcn5 alone does not acetylate nucleosomal histone H3 tails efficiently (14).

We next determined whether the acetylation state of Rsc4 affects the catalytic activity of RSC. We compared the remodeling activities of WT and *Rsc4*-K25R mutant complexes, using a restriction enzyme accessibility assay with RSC concentrations in excess and saturating over nucleosomes. Since the *Rsc4*-K25R mutation ensures the elimination of acetylation, we can assess the complete effect of Rsc4 acetylation by this comparison. We found that both types of RSC complexes remodel nucleosomes with similar maximal rates (Fig. 1C; k_{max} = 0.03 ± 0.01 and 0.02 ± 0.01 min⁻¹, respectively, for WT and K25R RSC). In addition, no large differences were observed in the DNA-stimulated ATPase rates (Fig. 1D; 0.3 ± 0.07 and 0.2 ± 0.03 μM/min, respectively, for WT and K25R RSC). These results indicate that the remodeling and ATPase activities of RSC are not significantly affected by the Rsc4 acetylation mark.

The RSC complex contains 8 of the 15 of bromodomains in *Saccharomyces cerevisiae*, suggesting that recognition of acetyllysine plays a major role in the regulation of this complex. Consistent with this observation, it has been reported that RSC binds preferentially to histone H3-acetylated nucleosomes rather than unacetylated nucleosomes (5, 15–17). Moreover, studies of the Rsc4 subunit suggest that occupancy of BD1 by acetylated K25 is mutually exclusive with occupancy of BD2 by acetylated histone H3 peptides when the latter ligand is added in vitro (8). One proposed model from these observations is that the occupancy of BD1 by acetylated K25 would reduce the ability of BD2 to bind nucleosomes acetylated on histone H3 (8). A simple prediction from this model is that unacetylated RSC would display a larger preference than acetylated RSC for acetylated nucleosomes.

To test this prediction, we measured the functional preference of RSC for acetylated compared with nonacetylated nucleosomes, using a competitive remodeling assay. In this assay, equal concentrations of mSAGA-acetylated and -unacetylated nucleosomes were added in excess to limiting concentrations of RSC (Fig. 1E and F) (14). Completion of the histone H3 acetylation was determined by a histone downshift assay and Western analysis using an anti-acetyllysine-histone H3 antibody (*SI Appendix*, Fig. S1D–F). Under the conditions of the assay, equal concentrations of acetylated and unacetylated nucleosomes must compete for a limiting concentration of RSC. The enzyme partitions between the two types of nucleosomes in proportion to its K_M for each type of nucleosome and, once bound, remodels each type of nucleosome with its respective maximal rate. Thus, the competition assay measures the combined effects of RSC acetylation on binding and remodeling of acetylated nucleosomes relative to unacetylated nucleosomes. Further, we reasoned that the competition conditions may help recapitulate in vivo conditions in which limiting RSC partitions among high effective concentrations of different types of nucleosomes. Changes in restriction enzyme accessibility of acetylated and unacetylated nucleosomes were monitored on the same gel using nucleosomes containing two different lengths of flanking DNA in addition to the 147-bp core DNA, respectively named "core+78" and "core+55." Both the WT and mutant RSC complexes showed a small preference for core+78 nucleosomes over core+55 nucleosomes (~1.1-fold and 1.2-fold for acetylated

and mutant RSC, respectively) (Fig. 1 *E* and *F*). Despite this slight preference, the acetylated core+55 nucleosomes were preferred over unacetylated core+78 nucleosomes by both types of RSC complexes (2.91 ± 1.34 -fold and 2.94 ± 0.34 -fold for acetylated and mutant RSC, respectively) (Fig. 1*F*). This result reflects an overall 3.2- and 3.5-fold preference for mSAGA-acetylated nucleosomes for acetylated and mutant RSC, respectively. The preference of WT RSC for acetylated nucleosomes is consistent with previously reported binding studies using histone H3-acetylated peptides (16) and acetylated nucleosomes (5, 15). Overall, the above results suggest that the absence of acetylation on Rsc4 does not significantly increase the preference of the RSC complex for SAGA-acetylated nucleosomes.

Synthetic Interaction with INO80 Uncovers a Role for Rsc4 Acetylation in Resistance to DNA Damage. The lack of a large direct effect on RSC activity suggested that the acetylation instead could play another type of biological role. To uncover such a role, we mutated the acetylated residue instead of mutating GCN5, because GCN5 has several other cellular targets. Previous work has shown that mutation of the Rsc4 K25 residue by itself has very mild growth phenotypes (8). We therefore applied the epistatic miniarray profiling (E-MAP) approach (18–20) to investigate whether the combination of the Rsc4 K25R point mutation with mutations in other factors might uncover the pathways in which the Rsc4 K25Ac mark participates. The minimal media conditions used in the E-MAP approach can further sensitize the system to reveal phenotypes not apparent under growth in rich media. We created a set of double-mutant strains by crossing *rsc4-K25R* to a library of 368 mutants involved in various aspects of chromosome biology, including chromatin remodeling, transcription, and DNA repair (20). Analysis of the resulting genetic data in the context of protein complexes and biological processes derived from Gene Ontology functional annotations (21, 22) revealed strong genetic connections to several processes, including histone acetylation and chromatin remodeling, as well as, to the INO80 and SWR1 complexes (Fig. 2*A* and *SI Appendix*, Table S2). Biochemically, the INO80-remodeling complex is thought principally to alter nucleosome conformation, whereas the SWR1-remodeling complex alters nucleosome composition via histone H2AZ variant exchange (23–25). Interestingly, both complexes have been functionally implicated in DNA-damage responses (26–31).

In addition to analyzing the *rsc4-K25R* allele, we applied the same screens with two other previously characterized RSC4 alleles (Fig. 2*B* and *D*). The first, *rsc4-2*, is a previously studied double mutant that alters a key residue in each bromodomain (8, 32) (Fig. 2*B* and *D*). The second, *rsc4-Δ4*, is a C-terminal deletion mutant that disrupts RSC association with all three RNA polymerases (33) (Fig. 2*B* and *D*). The correlation of the genetic interaction scores revealed that the profile of interactions for the *rsc4-K25R* mutant was more similar to that of the double bromodomain point mutant, *rsc4-2*, than to that of the C-terminal deletion, *rsc4-Δ4* ($R^2 = 0.31$ versus $R^2 = 0.05$, respectively) (Fig. 2*D* and *SI Appendix*, Table S2). For comparison, in previous work using a similar library of gene deletions, the average R^2 for any two pairs of genes is ~ 0.0004 , whereas the average R^2 for pairs of genes that are in the same complex is ~ 0.06 (20). These results suggest that Rsc4 acetylation plays a functional role that is more similar to the role of the bromodomains than to the role of the C terminus.

Given the enriched genetic interactions of the Rsc4 mutants with two complexes implicated in DNA damage responses, we assayed whether *rsc4-K25R* and *rsc4-2* displayed growth sensitivity in the presence of methyl methanesulfonate (MMS). MMS treatment of cells causes DNA base methylation that subsequently can lead to single- and double-stranded breaks in DNA and replication fork stalling (34, 35). Both mutants displayed a dose-dependent MMS sensitivity (Fig. 2*C*). For comparison, the phenotype of cells lacking *RAD9*, a critical DNA-damage checkpoint component, is shown also (Fig. 2*C*). These results indicate a role for the Rsc4 acetylation mark in resistance to DNA damage. Substantial previous work has shown that subunits of the RSC complex participate in various stages of DNA-damage

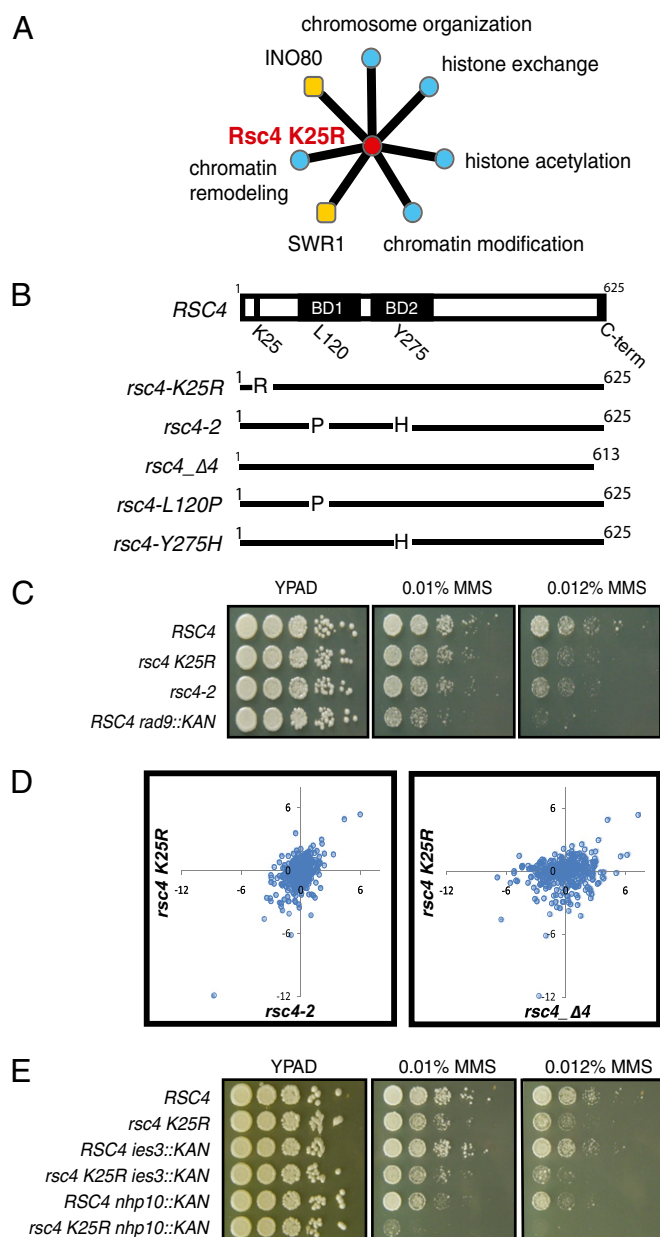


Fig. 2. Rsc4 acetylation and bromodomain function are required for resistance to DNA damage. (A) Pathway analysis of E-MAP synthetic genetic interactions. Strongest interactions to complexes (yellow) and biological processes (blue) are displayed for the *rsc4 K25R* mutant ($P < 0.005$). (B) Schematic of Rsc4 mutants. (C) Serial growth assays on YPAD medium and YPAD with varying MMS concentrations. Cells were diluted to $OD_{600} = 0.6$ and pinned onto agar plates containing drugs (48–72 h, 30 °C). Strains contain plasmid-expressed WT or mutant *rsc4*, as indicated. (D) S-score values for *rsc4 K25R* genome interactions (y axis) are correlated with those of *rsc4-2* (Left) and *rsc4-Δ4* (Right) mutants (x axis). $R^2 = 0.31$ for *rsc4-2* and $R^2 = 0.05$ for *rsc4-Δ4*. (E) Synthetic interaction between Rsc4 K25 and INO80 complex components IES3 and NHP10. Serial growth assays were performed as in *C*.

detection and repair (27, 36–41). The results in Fig. 2 extend these observations by providing evidence for a functional role of the Rsc4 subunit in resistance to DNA damage. The sensitivity of the *rsc4* mutants to DNA damage does not seem to arise from large defects in the transcription of specific gene sets as assessed from microarray experiments (*SI Appendix*, Fig. S2). However, subtle gene-expression effects cannot be excluded.

To dissect further the role of the Rsc4 acetylation mark, we focused on its interaction with the INO80 complex rather than with the SWR1 complex, because the roles of the INO80 complex in DNA-damage responses and replication have been studied more extensively (26–31, 42–44). We explored whether a reduction of INO80 function in the context of DNA damage could uncover a larger role for the Rsc4 acetylation mark in resistance to DNA damage. We investigated the MMS sensitivity of cells harboring the *rsc4-K25R* mutation that also were deleted for the gene encoding IES3 or NHP10, two unique subunits of the INO80 complex (28) that regulate INO80 participation in DNA-damage and replication-stress responses. The *nhp10Δ* mutant shows sensitivity to MMS, similar to that of the *rsc4-K25R* mutant. The MMS sensitivity of the NHP10 mutant appears somewhat greater than previously reported for this mutation (42, 44), but this difference could be a result of the specific yeast strain background (*SI Appendix, Table S1*). Although, we observed little or no increase in the MMS sensitivity of the *rsc4-K25R* mutant in the absence of *IES3*, we observed enhanced MMS sensitivity in the absence of *NHP10* (Fig. 2E). In the context of the E-MAP analysis, a stronger synthetic phenotype was obtained with the *IES3* mutant than with the *NHP10* mutant (*SI Appendix, Table S2*). However, because the E-MAP analysis was done in the absence of MMS and was used mainly to uncover the pathways in which Rsc4 acetylation participates, we reason that the data shown in Fig. 2E uncover the DNA damage-specific genetic interactions between INO80 subunits and the Rsc4 acetylation site. In this context, the stronger synthetic phenotype with the *NHP10* mutant is consistent with two previous observations. Specifically, Nhp10 is required for Ies3 association in the INO80 complex (45). Nhp10 also is required for INO80 association with γ -H2AX, the modified histone that signals damaged DNA (26, 30). These results indicate that, in cells in which the ability of the INO80 complex to mediate DNA-damage responses is strongly compromised, the Rsc4 acetylation mark plays a major role in resistance to DNA damage.

Rsc4 BD1 Function Promotes Maintenance of Acetylation and Resistance to DNA Damage in Vivo. Because both the *rsc4-K25R* mutant, which lacks acetylation, and the *rsc4-2* mutant display sensitivity to MMS, we next asked whether the observed sensitivity is correlated with the acetylation state of these mutants. To determine the acetylation levels, we used quantitative immunoblotting to measure the level of Rsc4 acetylation relative to the level of a TAP-tagged Rsc2 subunit of the RSC complex. Interestingly, in the double-bromodomain point mutant *rsc4-2*, Rsc4 acetylation is reduced to 52% of the Rsc4 acetylation levels in WT cells (Fig. 3A and B). To dissect the effects of BD1 from the effects of BD2, we separately analyzed the single point mutations *rsc4-L120P* and *rsc4-Y275H* (Fig. 3A). The BD1 mutant *rsc4-L120P* has Rsc4 acetylation levels that are 45% of WT, similar to those observed for the *rsc4-2* mutant (Fig. 3A and B). In contrast, the BD2 mutant *rsc4-Y275H* does not show reduced RSC acetylation (Fig. 3A and B). Reduction of Rsc4 acetylation does not have a large impact

on the integrity and relative subunit composition of the purified complex (*SI Appendix, Fig. S14*). Additionally, the Rsc4 mutants display similar levels of Rsc2, suggesting that the mutations do not alter overall RSC levels significantly (*SI Appendix, Fig. S3*). These results suggest that BD1, but not BD2, helps maintain the level of Rsc4 acetylation in vivo.

To investigate further the correlation between the acetylation level of Rsc4 and resistance to DNA damage, the MMS sensitivity of each of these *rsc4* mutants was compared. The *rsc4-L120P* mutant, which shows reduced Rsc4 acetylation comparable to *rsc4-2*, also displays MMS sensitivity similar to that of *rsc4-2* (Fig. 3Ci). Contrastingly, the Y275H mutation, which does not affect the acetylation level, showed the same MMS sensitivity as WT (Fig. 3Ci). We next explored whether the extent of Rsc4 acetylation correlates with the extent of synthetic interaction with INO80 in the presence of DNA damage. The *rsc4-L120P* and *rsc4-2* mutants, with dampened but not absent acetylation, displayed a milder synthetic MMS sensitivity in the *nhp10Δ* background as compared with double mutants lacking Rsc4 acetylation (Fig. 3Cii). Consistent with data showing that *ies3Δ* has less severe effects on INO80 function, in comparison with *nhp10Δ*, in the *ies3Δ* background, the *rsc4-2* and *rsc4-L120P* mutations did not display large synthetic phenotypes (Fig. 3Cii and iii). Again, the *rsc4-Y275H* strain, which displays an Rsc4 acetylation level similar to that of WT cells, when combined with *nhp10Δ* or *ies3Δ* conferred little or no extra MMS sensitivity (Fig. 3C). Together, these data suggest that Rsc4 acetylation and BD1 functionality promote tolerance to DNA damage.

Rsc4 Acetylation Promotes S-Phase Progression in the Presence of Replication Stress. The INO80 complex is implicated directly in the regulation of replication progression in the presence of DNA damage (26–31, 42–44). We, therefore, next investigated whether, in the absence of NHP10, Rsc4 acetylation helps promote DNA replication under conditions of DNA damage. Cells were arrested in the G1 phase, using mating pheromone, and were released into the cell cycle in the presence of 0.03% MMS (Fig. 4A). DNA content was measured by flow cytometry at various time points. For comparison, asynchronous profiles for the single and double mutants without MMS are shown also (Fig. 4B).

In the presence of MMS, the single mutants *rsc4-K25R* or *nhp10Δ* progressed through S-phase like WT cells (Fig. 4A). The cell-cycle behavior of the *nhp10Δ* strain in MMS is similar to that previously reported (42, 44). However, the double mutant displayed a delayed progression through S-phase, most evident at the 105-min time point after release from the G1-arrest (Fig. 4A). Budding indices, a marker of cell-cycle progression, reveal that the delay is not caused by a defect in entry into S-phase (Fig. 4C). In the absence of MMS, the double mutant progressed through S-phase with rates very similar to those of the WT strain (*SI Appendix, Fig. S4*). Intriguingly, for reasons that we do not fully understand, in the absence of MMS the single mutants appear to progress through S-phase slightly more slowly than the WT strain (*SI Appendix, Fig. S4*). These data suggest a role for Rsc4 acetylation in promoting S-phase progression during replication stress.

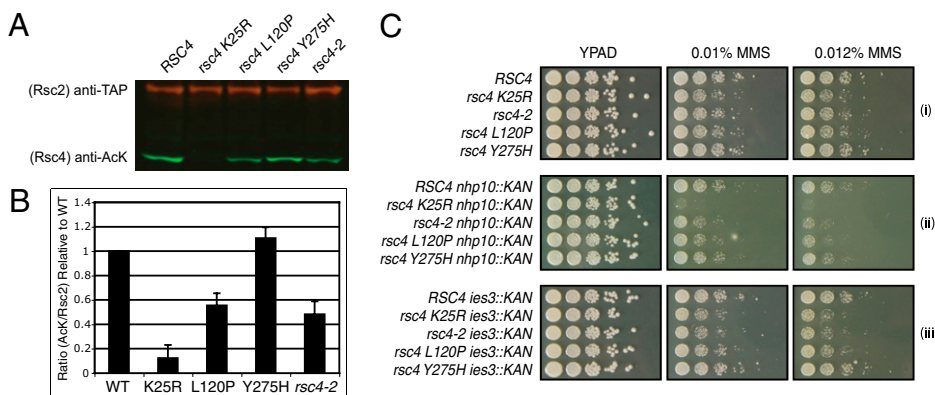


Fig. 3. BD1 is required for maintenance of Rsc4 acetylation in vivo and DNA-damage resistance. (A) Mutation of BD1 (L120P) is sufficient to reduce Rsc4 acetylation in vivo. (B) Quantification of in vivo Rsc4 acetylation levels relative to WT. In each strain acetylation levels are normalized to Rsc2 levels by the IR800/IR680 (AcRsc4/Rsc2-CBP) ratio ($n = 3$ independent experiments; error bars indicate SDs). (C) MMS sensitivities of BD1 and BD2 mutants and their synthetic interactions with INO80 components. Assays were performed as in Fig. 2C.

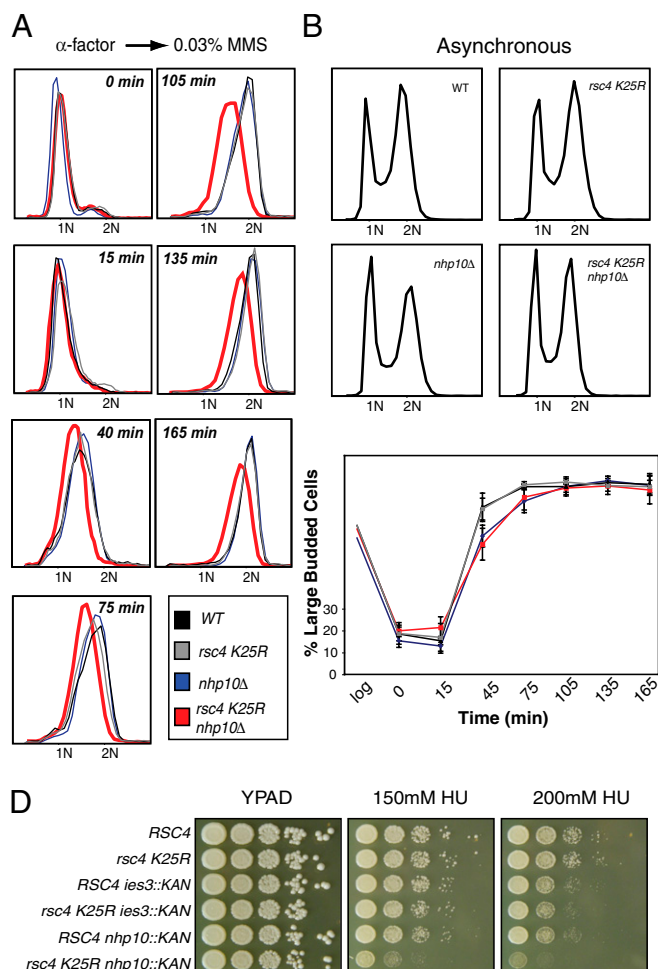


Fig. 4. Requirement for Rsc4 acetylation in the context of replication stress. (A) The double-mutant *rsc4K25R nhp10Δ* has delayed S-phase progression in MMS. Shown are representative cytometry profiles ($n = 3$ experiments). N, DNA copy number. (B) Representative profiles for midlog (asynchronous) cultures used in A. (C) Budding indices for cells treated as in A ($n = 3$; error bars indicate SDs). Cells with a bud diameter $<50\%$ of that of the mother cell were scored as small-budded cells, and cells with a diameter $>50\%$ of that of the mother cell were scored as large-budded cells. (D) Rsc4 K25Ac and NHP10 are synthetically required for growth in the presence of HU. Assays were performed as in Fig. 2C with various HU concentrations.

To investigate the above hypothesis further, we tested the sensitivity of these mutants to hydroxyurea (HU), an inhibitor of ribonucleotide reductase that reduces deoxyribonucleotide triphosphate levels. The *rsc4-K25R* single mutant did not show HU sensitivity, while *nhp10Δ* displayed a mild HU sensitivity. Strikingly, the double mutant *rsc4-K25R nhp10Δ* showed a strong sensitivity to 150 mM and 200 mM HU (Fig. 4D). The *rsc4-K25R ies3Δ* double mutants did not display HU sensitivity significantly increased over that of the *ies3Δ* single mutant, consistent with the previous observations that Nhp10 has a more critical role in the functionality of the INO80 complex (28) (Fig. 4D). Together, these data support a role for Rsc4 acetylation in resistance to DNA damage during replication.

Discussion

Using a combination of in vitro and in vivo approaches, we investigated the role of a recently discovered acetylation mark on the Rsc4 subunit of the essential, multifunctional chromatin-remodeling complex, RSC. The role for the acetylation mark does not involve a change in the fundamental enzymatic properties of the RSC complex, is regulated by interactions with a

linked bromodomain, and is partially redundant with the function of the INO80 chromatin-remodeling complex. Below, we discuss the bioregulatory implications of these findings.

A Bromodomain That Promotes Steady-State Acetylation of Its Ligand.

Previous work demonstrated that, in the context of a recombinant Rsc4 fragment, BD1 binds acetylated K25 in a manner that is mutually exclusive with BD2 binding of acetylated histone H3 peptides (8). This observation suggested a model in which acetylation of RSC reduces the ability of BD2 to bind nucleosomes acetylated on histone H3. In one version of this model, the loss of Rsc4 acetylation increases RSC's preference for acetylated nucleosomes during remodeling. In a competitive remodeling assay, however, we found that unacetylated RSC does not display a larger preference than acetylated RSC for SAGA-acetylated nucleosomes.

How then can the prior results showing antagonism between BD1 and BD2 occupancy be reconciled? There probably are many classes of possible models; below we suggest one type of preliminary model. This model draws on the observation that the BD1 mutant reduces acetylation, raising the possibility that the BD1-K25Ac interaction also protects the acetyl mark from removal or further regulation. BD2 may interact with nucleosomes primarily in a regulatory context that entails an orientation of RSC on the nucleosome that is different from the orientation adopted during nucleosome remodeling. In such a context, occupancy of BD2 by acetylated histone residues could cause the acetylation mark on Rsc4 to flip out from BD1, consistent with the previous work (8, 32). The "out" state then could act as a transient regulatory signal in the context of RSC function in DNA-damage resistance (*SI Appendix, Fig. S4B*). The antagonism between BD1 and BD2 occupancy thus could regulate the remodeling of acetylated nucleosomes.

Gcn5-Mediated Acetylation of RSC Promotes Replication in the Presence of DNA Damage.

In the absence of Nhp10, the acetylated state of Rsc4 promotes DNA replication under DNA-damage stress. In general, acetylation of nonhistone factors appears to be important for the regulation of critical DNA-replication steps (46, 47). In particular, Gcn5 has been shown recently to acetylate a key regulator of S-phase progression in higher eukaryotes (47). In addition, GCN5 and other acetyl transferases have been implicated in regulating DNA replication and repair via acetylation of specific histone residues (48–51). Thus, to extend the model in *SI Appendix, Fig. S4B*, it is possible that there is a damage- or replication-specific histone mark that is recognized specifically by either BD1 or BD2. Recognition of this mark may promote the out state of Rsc4's N terminus (*SI Appendix, Fig. S4B*). In such a model, the out state would have to be very transient, because under steady-state conditions most Rsc4 is acetylated. If, however, the transient out state is important for coordinating DNA replication with DNA-damage responses, a constitutively out state of the Rsc4 N-terminus would disrupt the coordination. Although this preliminary model is consistent with the current data, it clearly will require substantial further testing using integrated in vivo and in vitro approaches. Interestingly, we also found that the *rsc4-K25R* single mutant is sensitive to MMS, but not to HU. This observation raises the possibility that Rsc4 acetylation has additional roles in DNA-repair pathways, outside of S-phase, consistent with the different roles played by RSC components as well as by Nhp10 in the context of DNA damage (27, 36–41).

Regulation of Chromatin-Remodeling Factors by Posttranslational Modifications.

Our understanding of the role of posttranslational modifications in chromatin-remodeling complexes is only beginning to emerge. During mitosis, human SWI/SNF is phosphorylated, and this modification inhibits its remodeling activity, a mechanism that may promote global repression of chromatin remodeling during mitosis (52). Upon exposure to DNA damage, the yeast INO80 complex is phosphorylated on the Ies4 subunit in a Mec1/Tel1-dependent manner (31). Ies4 phosphorylation regulates Rad53 phosphorylation and DNA-damage checkpoint

responses (31). Whether and how this phosphorylation alters the biochemical activity of INO80 is not known. Comparatively less has been understood about the impact of acetylation on the activity and biological function of ATP-dependent chromatin remodeling complexes. Effects of acetylation on other chromatin-associated factors, such as histone acetyltransferases and the p53 transcription factor, are better understood. Acetylation of p53 modulates DNA-binding activity and enhances transcriptional activation (53), whereas active site autoacetylation of different histone acetyltransferases can reduce or promote catalytic activity (54). Our work suggests an additional role for acetylation marks by describing one that impacts a specific biological function of a multipurpose and essential chromatin-remodeling complex rather than altering its core enzymatic properties. In the future, it will be interesting to determine how acetylation might control the biological outputs of other multifunctional chromatin-remodeling complexes.

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