derivative, thereby indicating that binding of K25ac to BD1 is coupled with inhibition of H3K14ac binding to BD2 (Figure 5B). The mechanism for this inhibition is not immediately obvious, because BD1 and BD2 are separated by 20 Å. Our preferred model is that N-terminal residues that become ordered upon binding of K25ac to BD1 overlap the site of H3 residues that bind cooperatively with the K14ac - BD2 interaction, although an allosteric propagation of a subtle conformational change is also possible.

Models for Biological Mechanism

Our structural, biochemical, and genetic data have established that H3K14ac binds Rsc4 BD2 and that additional interactions with flanking residues in the H3 tail (not resolved in the structure) also contribute to binding affinity. The interaction is inherently weak, but it is specific and biologically important, and binding in the biological context is presumably enhanced by cooperative effects involving the additional DNA- and nucleosome-interacting domains, such as the SWIRM, SANT, and additional bromodomains of RSC subunits (Boyer et al., 2002; Da et al., 2006; Yu et al., 2003). Weak interactions are appropriate components of such regulatory switches, because they are reversible and avoid saturation with incorrect binding partners, such as other acetylated lysine residues. Our surprising finding that Rsc4 K25 is acetylated by Gcn5 both in vitro and in vivo reveals that yeast Gcn5 is both a HAT and a protein acetyltransferase. This is consistent with the roles of related enzymes such as PCAF and p300 in vertebrates (Gu and Roeder, 1997; Mujtaba et al., 2002). Also surprising was our discovery that acetylated Rsc4 K25 bound to BD1. The biological relevance of the interaction is indicated by our growth competition and transcriptional profiling data. The magnitude of the selection coefficients obtained shows that prolonged stress conditions such as those examined would cause the loss of the mutant population (8.7% loss per generation). The likely mechanistic basis for the biological loss of fitness is our finding that this interaction inhibits binding of H3K14ac peptides to BD2. Another direction that merits future study is that BD1 and/or BD2 likely has additional binding partners; these bromodomains are essential for viability, whereas their currently identified ligands are not. To find them, we have tried many other histone tail peptide substrates in our in vitro binding assays, including H3 K9ac, H3 K23ac, H3 K9ac K18ac, and H3 K18ac K27ac in addition to the series tested previously (Kasten et al., 2004). However, none of these provided acetyl-enhanced binding. In principle, the binding of Rsc4 TBDs to alternative ligands might be regulated by the mechanisms revealed here. For example, the binding of Rsc4 K25ac to BD1 might enhance the binding of another ligand to BD2. Alternatively, BD1 may bind an alternative ligand on a histone tail when K25 is not acetylated, leaving the BD1 pocket available and perhaps enabling cooperativity with H3K14ac bound in BD2.

Gcn5 acetylates ligands for both BD1 and BD2, and these ligands compete for binding to Rsc4. This raises the possibility that Gcn5 activity serves as a switch; Gcn5 acetylation of H3K14 would favor RSC-nucleosome binding, and this interaction would be countered by acetylation of Rsc4 K25. One attractive possibility is that this mechanism regulates the residence time of RSC to sites of remodeling (Figure 5C). In this model, activators recruit Gcn5 to promoter regions where it acetylates H3K14. This recruits RSC to promote nucleosome sliding and enhance promoter accessibility and also places Rsc4 in the vicinity of Gcn5, which then acetylates K25 and triggers release of RSC from the now-remodeled nucleosome. Finally, autoregulatory mechanisms, in which a binding protein or enzyme adopts a repressed conformation in response to intramolecular binding of a posttranslationally attached group, have been extensively characterized for kinases (reviewed in Kuriyan and Cowburn, 1997) and recently described for ubiquitylation (Hoeller et al., 2006). Our demonstration that RSC function is optimized by an analogous approach raises the possibility that mechanisms of this type might be widespread for acetylation and other modifications associated with chromatin dynamics.

EXPERIMENTAL PROCEDURES

See the Supplemental Data for details of media, strain lists, plasmid lists, protein expression and purification, in vitro acetylation, and crystallographic methods.

Histone Tail Binding Assay

Biotinylated histone tail peptides were bound to streptavidin beads (Invitrogen) as previously described (Kasten et al., 2004) and resuspended in a 50% slurry. Binding assays were conducted by rotating 15 μl of the peptide/bead slurry (20 nmol peptide/100 μl bed volume beads) with 500 ng purified Rsc4 protein in peptide binding buffer (PBB) (20 mM Tris [pH 7.5], 150 mM NaCl, 5% glycerol, 0.05% Tween-20, 1 mM EDTA, 1 mM β-mercaptoethanol, protease inhibitors) at 4°C for 3 hr. Typically, the beads were washed twice with PBB and twice with PBB containing 250 mM NaCl or the alternative NaCl concentrations indicated, followed by elution with 4 × SDS sample buffer.

RSC Purification, Extract Preparation, Immunoprecipitation, and Antibodies

RSC was purified as previously described (Saha et al., 2002). Whole-cell extracts were prepared as previously described (Cairns et al., 1999). Partially purified RSC was analyzed for Rsc4 acetylation in the *gcn5Δ* and *rsc4* K25A mutant and was derived from whole-cell extracts lysed in 3 × lysis buffer (60 mM HEPES [pH 7.6], 30% glycerol, 750 mM NaCl, 0.3% Tween-20, 30 mM EDTA, 1.5 mM DTT, protease inhibitors), which were then bound to IgG beads, washed three times with IPP150 (20 mM HEPES [pH 7.6], 10% glycerol, 150 mM NaCl, 0.1% Tween-20, 10 mM EDTA, 0.5 mM DTT, protease inhibitors), and eluted by boiling in 4 × SDS sample buffer prior to western blot analysis. The anti-Rsc4 antibody was made to the full-length protein and was previously described (Kasten et al., 2004). The anti-acetyl lysine antibody was from Cell Signaling.

Affinity Purification of Rsc4(1–340) from Yeast

Rsc4(1–340) preceded by 10 × HIS and 2 × NLS sequences expressed in yeast was nickel affinity purified prior to examining acetylation. Whole-cell extracts were prepared as previously described (Saha et al., 2005) except in modified breaking buffer (12% glycerol, 50 mM Tris [pH 7.5], 0.1% Triton X-100, 500 mM NaCl, 1.5 mM β-mercaptoethanol, protease inhibitors). Extracts (3 mg) were incubated with