the structure of chromatin (RSC) complex, which is both abundant and essential in *S. cerevisiae* (Cairns et al., 1996) and is involved in multiple chromosomal processes including transcriptional regulation, DNA repair, stress response, and chromosome cohesion and segregation (Angus-Hill et al., 2001; Baetz et al., 2004; Cairns et al., 1999; Chai et al., 2005; Chang et al., 2005; Yukawa et al., 1999). Importantly, RSC subunits contain 8 of the 15 bromodomains in *S. cerevisiae*, indicating that histone acetylation likely plays a central role in recruiting RSC to chromatin and/or in regulating its remodeling activity. Consistent with this notion, acetylation of histones promotes nucleosome remodeling by RSC and the passage of RNA polymerase II through chromatin in vitro (Carey et al., 2006).

The Rsc4 subunit of the RSC complex, which contributes to RSC-activated Pol II transcription in vivo (Kasten et al., 2004; Soutourina et al., 2006), contains a pair of bromodomains, termed BD1 and BD2, that are essential for cell viability (Kasten et al., 2004). BD1 and BD2 are adjacent in the primary protein sequence and together form the Rsc4 tandem bromodomain (TBD, residues 56–304). Our previous studies indicated that the Rsc4 TBD binds H3K14ac (Kasten et al., 2004). For example, the Rsc4 TBD preferentially binds histone H3 N-terminal peptides acetylated at K14, but not at several other positions tested on both the H3 and the H4 tails. Furthermore, conditional *rsc4* alleles are lethal in combination with *gcn5Δ* (Gcn5 acetylates H3K14) or with *h3*K14 replacements (at 33°C), but not in combination with mutation of other lysine residues in the H3 and H4 tails (Kasten et al., 2004). However, it was unresolved which of the bromodomains (or both) bound H3K14ac, and the clear possibility remained of alternative ligands. Furthermore, it was not known whether other modifications occurring near H3K14ac, such as H3S10 phosphorylation, might affect binding.

To better understand Rsc4 recognition of chromatin, we performed biochemical and genetic studies that showed that only BD2 binds to H3K14ac peptides, and we visualized the acetyl lysine component of this interaction using X-ray crystallography. Serendipitously, crystal structure determination of protein prepared by in vitro acetylation with Gcn5 revealed that an acetylated lysine of Rsc4(K25ac) binds to its own BD1. This interaction was shown to be important in vivo, and the modifying enzyme was identified as Gcn5, the same acetyltransferase that modifies the H3K14 ligand of BD2. Importantly, peptide-binding data showed that binding of Rsc4 K25ac to BD1 impairs the ability of BD2 to bind an H3K14ac peptide, thereby indicating an autoregulation mechanism for recognition of a histone modification.

RESULTS AND DISCUSSION

Structure of the Rsc4 Tandem Bromodomain

Several crystal structures of Rsc4 constructs were determined (Figure 1). The first, Rsc4(36–340), was determined by the SAD method using selenomethionine-substituted protein and refined to a free R value of 21.8% against native data to 1.8 Å resolution. This structure is ordered from residue 36 to residue 320 with no disordered internal loops. Structures of other constructs were subsequently determined by molecular replacement and refined to resolutions of 1.75–2.35 Å and R_{free} values of 21.9%–24.8%. While the structures varied significantly in their last ordered residue (313–320), only minor differences were seen for residues 36–312, with root-mean-square deviations (rmsds) of 0.5–1.0 Å following least-squares overlap on 275 pairs of Cα atoms.

The Rsc4 TBD is a compact structure in which each of the individual bromodomains (BD1 and BD2) resembles bromodomains from other proteins (Mujtaba et al., 2002; Owen et al., 2000; Sun et al., 2007). In keeping with standard nomenclature, we name the four bundle helices Z, A, B, and C, with a -1 or -2 suffix to indicate if it is from the first or second bromodomain (Figure 1C). The acetyl-lysine binding pockets are formed primarily by residues within the BC and ZA loops including the short helix Z′. Both BD1 and BD2 conserve two tyrosine residues within Z′ and an asparagine residue within B that are characteristic of bromodomain binding sites. Overlap on Cα atoms with the bromodomain from Gcn5, which shares 19% and 33% sequence identity with Rsc4 BD1 and BD2, respectively, and whose crystal structure has been determined at 1.9 Å resolution (Owen et al., 2000), gives rmsds of 1.8 Å (100 pairs of Cα atoms) for BD1 and 1.6 Å (106 pairs of Cα atoms) for BD2.

The Rsc4 TBD reveals important differences with the previously reported structure of the double bromodomain of $TAF_{II}1$ (formerly termed $Taf_{II}250$) (Jacobson et al., 2000), the largest subunit of the TBP-associated factors for RNA Pol II transcription. We note that $TAF_{II}1$ was only crystallized in the absence of ligand. Notably, the relative positions and orientations of the two bromodomains in Rsc4 are very different to those in $TAF_{II}1$ (see Figure S1 in the Supplemental Data available with this article online). Also, Rsc4 is substantially more compact, with extensive BD1-BD2 interactions (mainly through αC-1 and αB-2) that bury a total of 1621 $Å^2$ of solvent accessible surface area at the bromodomain interface (Figure S2). In comparison, 1122 $Å^2$ are buried at the BD1-BD2 interface in the $TAF_{II}1$ structure. This supports the impression from genetic data that the Rsc4 TBD functions as a single structural unit (Kasten et al., 2004). In contrast, the two bromodomains of $TAF_{II}1$ appear to be relatively independent. As a result of these differences, the two acetyl-lysine binding sites of Rsc4 face the same side of the structure in the same relative orientation and are separated by just 20 Å.

Whereas BD1 conforms to the standard bromodomain architecture, BD2 includes an additional "wing" helix (W) inserted between Z-2 and A-2. Because it is adjacent to the presumed binding surface, we hypothesized that W might play an important functional role. We therefore designed a deletion variant that replaced residues 187–206 with a short Ser-Ser-Gly linker, termed *rsc4*Δ187–206. Although the *rsc4*Δ187–206 allele does not confer a strong phenotype in isolation, a temperature-sensitive (ts^-)