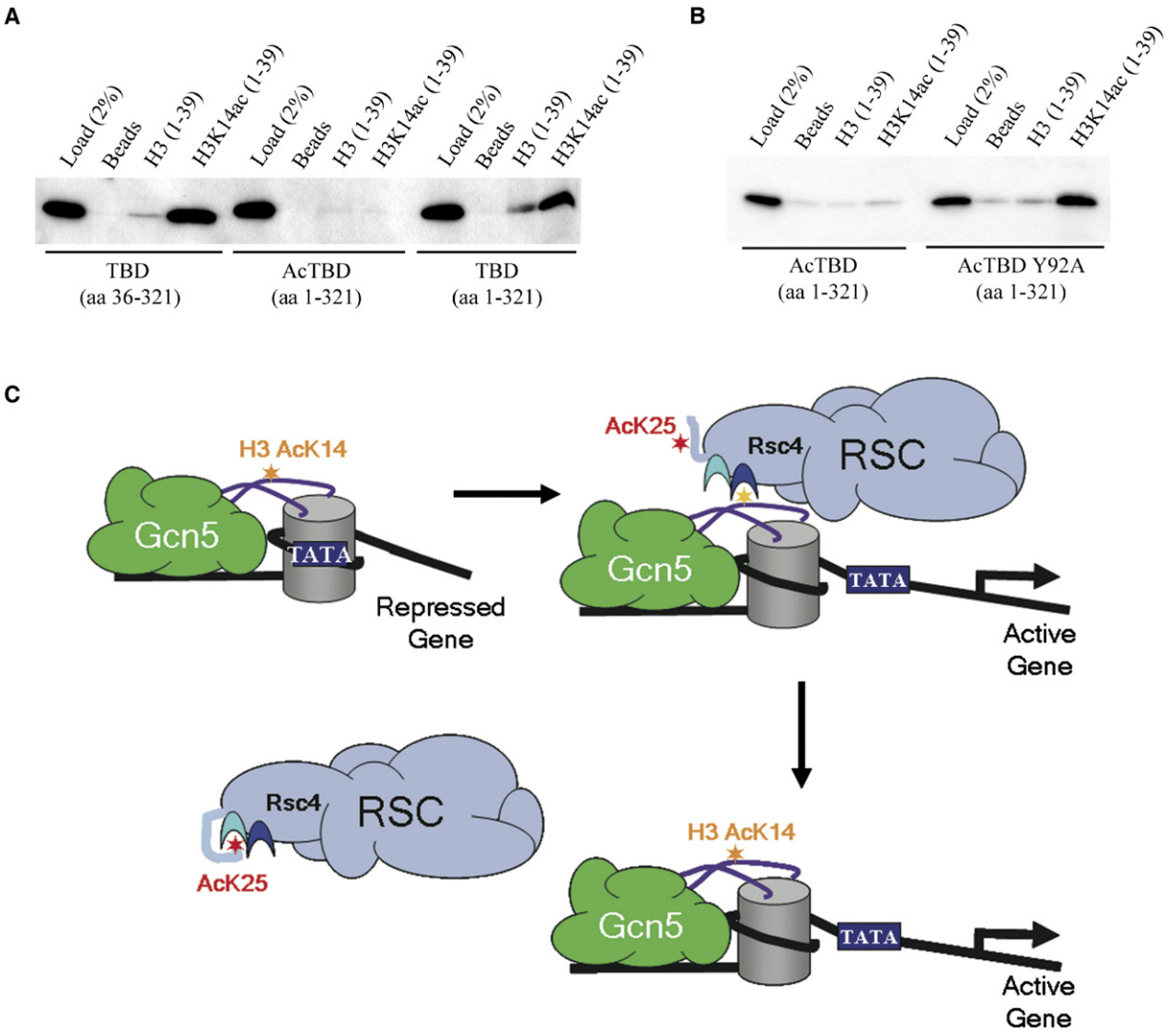

Figure 5. Inhibition of H3K14ac Binding Involves Rsc4 K25ac Binding to BD1

(A) Rsc4 K25 acetylation inhibits H3K14ac binding. Binding of purified nonacetylated and acetylated TBD to biotinylated histone H3 tail peptides conjugated to streptavidin beads was examined by western analysis. Rsc4(36–321) TBD was expressed from p1617 and Rsc4(1–321) TBD was expressed from p1616. Rsc4(1–321) TBD was acetylated by Gcn5 where indicated, and acetylation at K25 was confirmed by mass spectrometry analysis (data not shown). Binding and wash buffers contained 100 mM NaCl.

(B) Western blot analysis of purified WT and mutant acetylated Rsc4(1–321) TBD bound to H3 peptides. Bead bindings and washes were conducted at 150 mM NaCl. Rsc4 K25ac(1–321) TBD was expressed from p1617 and Rsc4 K25Ac(1–321) Y92A TBD was expressed from p2296. Proteins were acetylated by Gcn5, and acetylation was confirmed by mass spectrometry analysis (data not shown).

(C) Model for Rsc4 autoregulation. Gcn5 acetylation of H3K14 facilitates Rsc4 interaction with H3K14ac and also the subsequent release of Rsc4 from chromatin by acetylating Rsc4 K25, which binds in BD1 and antagonizes Rsc4 binding to H3K14ac.

analysis, fitness determination, and gene profiling analysis demonstrate a significant role for Rsc4 K25 in RSC function in vivo.

Rsc4 K25ac Binding to BD1 Inhibits H3K14ac Binding to BD2

Having demonstrated that Rsc4 K25 is acetylated by Gcn5 both in vitro and in vivo, we investigated possible mechanistic roles for this modification. In principle, the binding of K25ac in BD1 could have a positive, neutral, or negative impact on H3K14ac binding to BD2. To test this, we applied the histone tail peptide binding assay utilized above and prepared both K25-acetylated and nonacetylated versions of Rsc4(1–321). The results show that K25 acetylation of Rsc4(1–321) significantly inhibited binding of H3K14ac peptides to BD2 (Figure 5A). The importance of K25ac binding to BD1 for this inhibition was tested more precisely by using Rsc4(1–321) Y92A protein, which is crippled for binding to BD1. Importantly, binding to H3K14ac peptides is largely restored in the Y92A