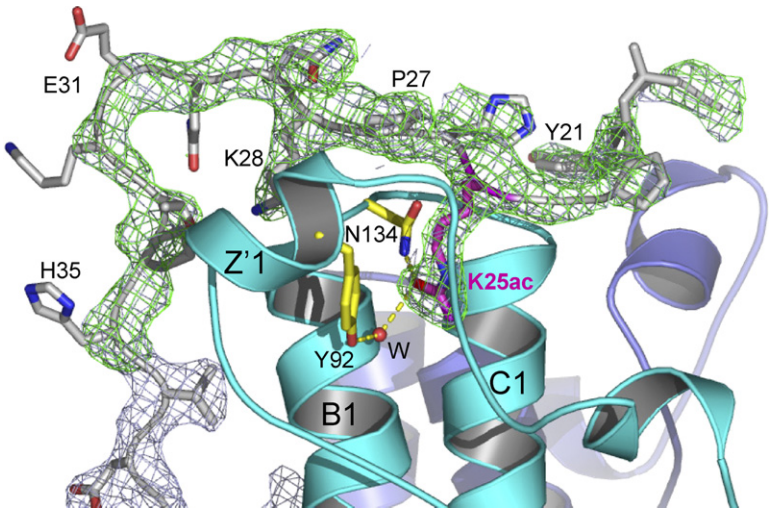

Figure 3. Binding of Rsc4 K25ac in BD1
Acetylated K25 binds BD1 and orders flanking residues. The $F_o - F_c$ map (3.0 × rmsd, green) and $2F_o - F_c$ map (1.2 × rmsd, gray) were phased from the protein model refined in the absence of residues 19–35. Residues involved in K25ac recognition are shown in yellow.

Rsc4 K25 Is Acetylated by Gcn5 In Vivo

Motivated by the surprising crystallographic observation that Rsc4 K25ac binds BD1, we sought to determine if this modification occurs in vivo. Western blot analysis of purified RSC complex using an antibody against acetyl-lysine revealed a single dominant band that comigrated with Rsc4 (Figure 4A). To identify the acetyltransferase that modifies Rsc4 in vivo, we expressed in yeast a Rsc4(1–340) construct that also included a nuclear targeting sequence and a 10 × HIS tag. We then purified this protein with nickel chelating chromatography and examined its acetylation state. Western analysis revealed clear acetylation in the WT strain and in a wide variety of strains lacking specific acetyltransferases. However, Rsc4 K25 acetylation was abolished in a strain lacking Gcn5 (Figure 4B). As a definitive test for the presence of Gcn5-dependent Rsc4 K25ac in the RSC complex in vivo, we made a Rsc4 derivative with a single amino acid replacement (K25A) as the sole source of Rsc4. This derivative or the WT Rsc4 was expressed in WT cells and purified as a component of the RSC complex using a TAP tag on the Rsc2 subunit (Figure 4C, lanes 1 and 2). Importantly, substitution of K25 abolished the acetylation of Rsc4 (Figure 4C, lanes 1 and 2). Furthermore, acetylation of WT Rsc4 present in the RSC complex was abolished when Rsc4 was isolated from a strain lacking Gcn5 (Figure 4C, lanes 3 and 4). The functional importance of K25 is supported by sequence alignment, which reveals that this lysine is highly conserved within yeast Rsc4 orthologs (Figure 4D). Moreover, G24 and P27 are also highly conserved, and the GKXP sequence matches the Gcn5 recognition motif determined from structural analysis of a Gcn5 HAT-histone H3 complex (Rojas et al., 1999).

Rsc4 K25 Is Important for Fitness and Gene Expression

To examine the in vivo consequences of Rsc4 K25 acetylation, we made a K25A mutation and assessed phenotypes. The Rsc4 K25A mutation in isolation (and also K25R and K25Q) conferred only weak plate phenotypes, such as slightly slower growth on minimal medium under moderate heat stress (Figure S4). However, combining K25A with a conditional *rsc4* allele (*rsc4-2*, which has a point mutation in each bromodomain [Kasten et al., 2004]) greatly enhanced temperature sensitivity (Figure S4). This supports the proposal that acetylation of Rsc4 K25 contributes to RSC function in vivo. Subtle phenotypic effects can confer a fitness advantage that might be more clearly revealed in a growth competition assay. We therefore measured the fitness of the *rsc4* K25A mutant in direct competition with an isogenic WT strain and quantified gene fitness by the selection coefficient (*s*), which was obtained by comparing growth of the *rsc4* K25A mutant and the WT *RSC4* strain in coculture (Table 2). In this analysis, positive values of *s* indicate a selective disadvantage for the K25A mutant, while negative values of *s* indicate a greater selective advantage compared to WT. Surprisingly, under rich growth conditions (YPD, 30°C) the K25A mutant has a greater selective advantage ($s = -0.018$), indicating a 1.8% increase in the mutant K25A allele per generation in the coculture population under these conditions. In contrast, growth in minimal medium conditions (SD, 30°C) provides a selective advantage for the WT ($s = 0.021$), and this advantage is further increased ($s = 0.087$) when grown in minimal medium at elevated temperature (SD, 37°C). The loss of fitness for the K25A mutation when grown in minimal media conditions is highly significant ($p < 0.01$) and corresponds to an approximate 2.1% (30°C) or 8.7% (37°C) decrease in the K25A allele every generation.

We also examined the *rsc4* K25A mutant for changes in gene expression by performing transcriptional profiling from cells grown in minimal medium at slightly elevated temperature (33°C). In the K25A mutant, 80 genes were upregulated 2-fold or greater, and 61 genes were downregulated 2-fold or greater (Tables S1 and S2). Among the upregulated class, we did not observe a greater affect