



Figure 4. Rsc4 K25 Is Acetylated In Vivo by Gcn5

(A) Rsc4 is acetylated in vivo. Increasing amounts of purified RSC complex (87.5, 350, and 1400 ng) were analyzed by western blot with anti-Rsc4 or anti-acetyl-lysine antibodies. An asterisk denotes a proteolytic fragment of Rsc4 that is evident when RSC is overloaded.

(B) Rsc4 is acetylated in vivo by Gcn5. Western blot analysis of partially purified (via nickel, NTA-Agarose) Rsc4(1–340) TBD (p2243) following expression in WT or various HAT mutant strains. Strains were the following: WT, (YBC1895); *gcn5Δ*, (YBC1662); *sas3Δ*, (YBC1911); *hat1Δ*, (YBC2493); *esa1 L327S*, (LPY3430); and *sas2Δ*, (YBC1857).

(C) Rsc4 resident in RSC complex is acetylated at K25 by Gcn5 in vivo. Western blot analysis of partially purified RSC isolated via the Rsc2-TAP tag from strains bearing either *RSC4* or the *rsc4 K25A* mutant or *GCN5* or *gcn5Δ*. The protein A portion of the TAP tag (not cleaved in this procedure) serves as an internal control, as it is recognized by all antibodies, including anti-acetyl-lysine or anti-Rsc4. Strains included the following: *RSC4*, (YBC2814); *rsc4 K25A*, (YBC2815); *GCN5*, (YBC2825); *gcn5Δ*, (YBC2822).

(D) Alignment of the amino termini of Rsc4 orthologs from various yeast species (Clustal W). Identical amino acids are shown in green, and similar amino acid residues are shown in red, with regions of high similarity blocked together (PrettyPlot). Rsc4 K25, asterisk at top. Species include the following: *S. cerevisiae* (Scer), *S. bayanus* (Sbay), *S. mikatae* (Smik), *S. paradoxus* (Spar), *S. kudriavzevii* (Skud), *C. glabrata* (Cgla), *S. castellii* (Scas), *K. lactis* (Klac), and *A. gossypii* (Agos).

on genes of a particular inherent transcriptional frequency. Common classes of upregulated genes include those involved in cell wall integrity, those involved in the response to cell stress, and those encoding proteins that reside in

the cell membrane. Misregulation of genes involved in cell wall integrity has been observed with other *rsc* mutants, including conditional *rsc4* alleles (Angus-Hill et al., 2001; Kasten et al., 2004). Taken together, our genetic

Table 2. Competition of the *rsc4 K25A* Strain against Wild-Type

Media	s	Impact ^a	n	Number of Cultures	p
YPD 30°C	-0.018 ± 0.0036	1.8%	6	7	<0.01
SD 30°C	0.021 ± 0.0069	-2.1%	6	8	<0.01
SD 37°C	0.087 ± 0.0059	-8.7%	6	4	<0.01

Media are described in the Experimental Procedures section. Selection coefficients (s) ± the standard deviation for *rsc4 K25A* relative to the WT strain were measured as described in the Experimental Procedures section. A positive value for s indicates a greater selective advantage for the WT strain, and a negative value indicates that the mutant has a greater selective advantage. Indicated are the number of time points (n) at which genotype frequencies were measured and the number of cultures analyzed for each growth condition. The statistical significance (p) for a test of the null hypothesis that the selection coefficient is not different from zero was determined.

^aImpact indicates the approximate percent change in the mutant allele relative to the WT allele per generation in coculture.