100 μl bed volume of Ni-NTA agarose (QIAGEN) for 3 hr at 4°C, poured into a small column, washed three times (1.2 ml) with wash buffer (50 mM NaPhosphate [pH 8.0], 150 mM NaCl, 10% glycerol) containing increasing amounts of imidazole (from 10 mM to 40 mM). Bound protein was eluted with wash buffer containing 300 mM imidazole and protease inhibitors prior to western blot analysis.

rsc4 K25A Fitness Determination

Fitness determinations compared isogenic strains, differing only at *rsc4* K25A mutation, which were prepared by standard yeast gene transplacement methods. Comparisons used a method based on (Thatcher et al., 1998). Competition cocultures were established by inoculating 2 ml of YPD (rich media) or SD supplemented as needed for auxotrophies (minimal media) with 32 μl each from overnight cultures of YBC2898 and YBC2899 grown in YPD or SD. Equal numbers of cells (by OD reading) from each strain were inoculated, and cultures were incubated at 30°C or 37°C and were back diluted into fresh media daily. Each day, 1 ml of the competition coculture was collected and used to prepare genomic DNA using the YeaStar Genomic DNA kit (Zymo Research). Quantitative PCR (qPCR) was utilized to determine *RSC4* genotype frequencies. Primers were designed so that the 3′ ends recognize either the K25 or the K25A codon allowing these primers to differentiate between the two *RSC4* alleles. Genomic DNA harvested periodically from the competition cocultures was used in separate qPCR procedures essentially as previously described (Roberts et al., 2003) to determine the quantity of each allele individually. Primer for *RSC4* K25 detection was the following: (BC3550) GCCTAAATACTTGCCGGGAAAA. Primer for *rsc4* K25A detection was the following: (BC3551) CTAAATACTTGCCGGGAGCC. *RSC4* reverse primer used for detection of either allele was the following: (BC3539) TGTATTTGTCGATAAGAACATCCAAAGTG. The average of three PCR replicates was taken for each genomic DNA analyzed. The change in allele ratio with time is given by the equation $\ln(R_t) = \ln(R_0) - st$ (where R_0 is the initial genotype ratio, R_t is the ratio after t generations, and s is the selection coefficient) (Thatcher et al., 1998). The selection coefficient for the *rsc4* K25A mutant relative to WT in each growth condition was calculated by fitting the above equation to the qPCR frequency data.

Supplemental Data

Supplemental Data include Supplemental Experimental Procedures, four figures, four tables, and Supplemental References and can be found with this article online at http://www.molecule.org/cgi/content/full/27/5/817/DC1/.

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