Boban et al., http://www.jcb.org/cgi/doi/10.1083/jcb.200601011

Yeast strains

All strains used are listed in Table S1. The generation of deletion alleles stp1Δ51::Agleu2 and stp2Δ50::hphMX4 have been described previously (Andréasson and Ljungdahl, 2002). PLY127 (Kuehn et al., 1996) was transformed with a linear 3,529-bp KpnI–SacII fragment from plasmid pAZ024 (this study) containing the asi1Δ8::kanMX deletion construct, creating strain AZY68. CAY4 was constructed by transforming PLY127 with the asi3Δ1::loxP-kanMX-loxP cassette (Forsberg et al., 2001). Strain AZY111 was obtained by transforming CAY4 with a PCR-amplified asi2Δ9::URA3 cassette (Forsberg et al., 2001). The 13MYC-kanMX cassette was PCR amplified from plasmid pFA6a-13Myc-kanMX6 (Longtine et al., 1998) and was transformed into strain CAY29 (Andréasson and Ljungdahl, 2002), creating AZY262. Strains CAY60 and CAY62 are ura– derivatives of meiotic segregants obtained from the cross between CAY25 and CAY41 (Andréasson and Ljungdahl, 2002). Strain CAY47 was obtained as a meiotic segregant from the same cross. Strains CAY105 and CAY106 were obtained as segregants from a cross between CAY86 (Andréasson and Ljungdahl, 2002) and AZY68. Strain CAY120 was generated from a cross between CAY62 and CAY117 (Andréasson and Ljungdahl, 2002). Strain CAY126 was generated from the cross between CAY105 and CAY120. Strain CAY150 is a ura– derivative of a meiotic segregant obtained from a cross between CAY47 and CAY126. Strain CAY212 was generated from CAY144 (Andréasson and Ljungdahl, 2004) by replacement of the kanMX marker with hphMX4 (Goldstein and McCusker, 1999). Strains PLY1313, PLY1314, and PLY1327 were generated from a cross between CAY212 and AZY111. In all cases, the correct integration of gene deletion cassettes was confirmed by whole locus PCR analysis. Crosses and subsequent tetrad analysis were performed to verify 2:2 segregation of all deletion markers and that mutant phenotypes segregated with each respective marker gene.

Plasmids

Site-specific mutagenesis was performed according to Kunkel et al. (1987). Plasmid pPL793 contains a 3.6-kb SpeI–XbaI fragment spanning the ASI1 open reading frame, including 956-bp upstream and 729-bp downstream sequences cloned into XbaI-restricted pRS316 (Sikorski and Hieter, 1989). The XbaI site in the multicloning site of pPL793 was destroyed by digestion with XbaI, Klenow polymerase blunting, and relegation, creating pAZ001. Using single-stranded pAZ001 as a template, an XbaI site was reintroduced immediately before the translational termination codon of ASI1. An XbaI-flanked cloning cassette encoding the HA epitope reiterated three times was inserted into this unique XbaI site, creating pAZ002. pAZ013 and pAZ014 were constructed by ligating XhoI–SacII fragments obtained from pAZ001 and pAZ002, respectively, with similarly restricted pRS202 (Connelly and Hieter, 1996). Plasmid pAZ024 containing the asi1Δ8::kanMX deletion was constructed in multiple steps: pAZ001 was restricted with ClaI and was religated, and an XbaI site was introduced 207 nucleotides from the initiation codon of the ASI1 open reading frame, creating plasmid pAZ070. A 1.4-kb XbaI–XhoI fragment from plasmid pUG6 containing kanMX (Guldener et al., 1996) was ligated into XbaI–HindIII-digested pAZ070, resulting in the plasmid pAZ024. To construct the RING finger mutations in ASI1, single-stranded pAZ002 served as a template, and site-directed mutagenesis was used to replace cysteines 583 and 585 with serine (TCG and TCT, respectively), creating pAZ011, and two additional cysteines, 589 and 592, were changed to serine (TCC), creating pAZ012. The mutations were confirmed by DNA sequencing. pAZ015 and pAZ016 were constructed by ligating XhoI–SacII fragments obtained from pAZ011 and pAZ012, respectively, into similarly restricted pRS202 (Connelly and Hieter, 1996). Three N-glycosylation sites in the NH2-terminal domain of Asi1 were destroyed using site-directed mutagenesis (ser4 to ala, thr21 to ala, and asn29 to gln); single-stranded pAZ002 was used as template, creating pAZ020. Plasmids containing ASI1 gene fusion alleles with the topological reporter SUC2 cassette (Gilstring and Ljungdahl, 2000) were constructed in multiple steps. Single-stranded pAZ020 plasmid was used as template. A BglII site was individually inserted into the ASI1 coding sequence at positions corresponding to amino acids 107 (pAZ025), 144 (pAZ026), 236 (pAZ028), 313 (pAZ030), and 624 (pAZ033). In the next step, a 168-bp BamHI fragment encoding the suc2 topological reporter cassette A (pFG112; Gilstring and Ljungdahl, 2000) was ligated in frame into BglII-restricted pAZ025-pAZ033, creating pAZ034, pAZ035, pAZ037, pAZ039, and pAZ073, respectively. The functional expression of plasmid-borne ASI1, epitope-tagged proteins, fusion proteins, and RING domain mutants were tested by complementation of the asi1Δ mutation in strain YMH349 (Forsberg et al., 2001).

The NotI site in the multicloning site of pCA029 (Andréasson and Ljungdahl, 2002) was destroyed by digestion with NotI, Klenow blunting, and relegation to create pCA031. Using single-stranded pCA031 as a template, a NotI site was reintroduced after nucleotide 879 of *STP1*, resulting in pCA083. A NotI-flanked cloning cassette encoding the HA1 epitope reiterated three times was inserted into the unique NotI site of pCA083, creating pCA088. Plasmids pCA145, pCA153, pCA181, pCA227, pMB9, pMB10, pMB12, pMB13, pMB16, pMB17, pMB27, and pMB30 were constructed using homologous recombination in yeast. pCA181 was isolated from yeast cells cotransformed with SpeI–StuI-restricted pCA088 and PstI–XhoI-restricted pCA047

Table S1. **Yeast strains**

Strain	Genotype	Reference
AZY68	*MATα ura3-52 lys2Δ201 asi1Δ8::kanMX*	This study
AZY111	*MATα ura3-52 lys2Δ201 asi2Δ9:hisG-URA3-hisG asi3Δ1::loxP-kanMX-loxP*	This study
AZY262	*MATa ura3-52 ASI3::13MYC*	This study
CAY4	*MATα ura3-52 lys2Δ201 asi3Δ1::loxP-kanMX-loxP*	This study
CAY25	*MATα ura3-52*	Andréasson and Ljungdahl, 2002
CAY29	*MATa ura3-52*	Andréasson and Ljungdahl, 2002
CAY41	*MATa ade2 leu2-3, 112 lys2Δ201 ura3-52 stp1Δ50::CaURA3MX3*	Andréasson and Ljungdahl, 2002
CAY47	*MATα ura3-52 stp1Δ50::CaURA3MX3*	This study
CAY60	*MATα ura3-52 stp1Δ51::Agleu2*	This study
CAY62	*MATα ura3-52 lys2Δ201 stp1Δ51::Agleu2*	This study
CAY86	*MATa ura3-52 grr1Δ50:: hphMX4*	Andréasson and Ljungdahl, 2002
CAY91	*MATa ura3-52 ssy1Δ13::hisG*	Andréasson and Ljungdahl, 2002
CAY105	*MATa ura3-52 asi1Δ8::kanMX*	This study
CAY106	*MATα ura3-52 asi1Δ8::kanMX*	This study
CAY117	*MATa ura3-52 stp2Δ50::hphMX4*	Andréasson and Ljungdahl, 2002
CAY120	*MATα ura3-52 lys2Δ201 stp2Δ50::hphMX4*	This study
CAY126	*MATa ura3-52 asi1Δ8::kanMX stp2Δ50::hphMX4*	This study
CAY144	*MATa ura3-52 asi1Δ8::kanMX*	Andréasson and Ljungdahl, 2004
CAY150	*MATa ura3-52 asi1Δ8::kanMX stp1Δ51::Agleu2*	This study
CAY152	*MATa ura3-52 stp1Δ51::Agleu2 stp2Δ50::hphMX4 asi1Δ8::kanMX*	Andréasson and Ljungdahl, 2004
CAY206	*MATa ura3-52 ssy1Δ13::hisG asi1Δ8::kanMX*	Andréasson and Ljungdahl, 2004
CAY212	*MATa ura3-52 asi1Δ80::hphMX4*	This study
PLY127	*MATα ura3-52 lys2Δ201*	Kuehn et al., 1996
PLY1313	*MATa ura3-52 asi1Δ80::hphMX4*	This study
PLY1314	*MATα ura3-52 asi1Δ80::hphMX4*	This study
PLY1327	*MATα ura3-52 lys2Δ201 asi1Δ80::hphMX4*	This study
nup133⁻	*MATα ura3 leu2 his3 trp1 ade2 nup133::HIS3*	Doye et al., 1994
YMH349	*MATa ade2 leu2-3, 112 lys2Δ201 ura3-52 ssy1Δ13 asi1Δ8::kanMX*	Forsberg et al., 2001

(Andréasson and Ljungdahl, 2002). pMB9 was obtained by cotransforming a PCR product amplified using plasmid pFA6a-13myc-kanMX6 (Longtine et al., 1998) as a template and NotI-restricted pCA042 (Andréasson and Ljungdahl, 2002). Plasmid pMB10 was isolated from yeast cotransformed with NcoI–PvuII-restricted pMB9 and EcoRI–MluI-restricted pCA181. pCA145 was obtained by cotransforming MluI–XhoI-restricted pRS202 and a PCR-amplified *lexA* fragment from pEG202 (Gyuris et al., 1993) as a template. pCA153 was created by cotransforming BssHII-linearized pCA145 and PCR-amplified *LYS2* fragment from pRS317 (Sikorski and Hieter, 1989). pMB16 was generated by cotransforming SalI-restricted pCA153 and a PCR-amplified *STP1* fragment encoding amino acids 2–69 from plasmid pCA029 (Andréasson and Ljungdahl, 2002). pMB17 was constructed by cotransforming SalI-restricted pCA153 and a PCR-amplified *STP2* fragment encoding amino acids 2–77 from genomic DNA of an *STP2* wild-type yeast strain. pMB18, a centromeric version of pJK101 (Brent and Ptashne, 1984), was constructed by cotransforming EcoRV-restricted pRS316, BamHI-restricted pCA030 (Andréasson and Ljungdahl, 2004), and PstI–SacI-restricted pJK101. Plasmid pMB12 was isolated from yeast cotransformed with SacI–KpnI-restricted pRS202 (Connelly and Hieter, 1996) and PmlI–NcoI-restricted pCA111 (Andréasson and Ljungdahl, 2002). pMB13 was obtained by cotransforming a PCR product encompassing the 6HA cassette amplified from plasmid pYM3 (Knop et al., 1999) and EagI-restricted pMB12. pCA106 was generated from pCA103 (Andréasson and Ljungdahl, 2002) by introducing an EagI site in *STP2* just after the start codon by single-strand mutagenesis. pMB27 was obtained by cotransforming a PCR product amplified using plasmid pFA6a-13myc-kanMX6 (Longtine et al., 1998) as a template and EagI-restricted pCA106. Plasmid pMB30 was isolated from yeast cotransformed with SphI–KpnI-restricted pMB13 and PvuII–XcmI-restricted pMB27. pCA227 was obtained by cotransforming a PCR-amplified P*GNP1* fragment from genomic DNA of a *GNP1* wild-type yeast strain and BamHI–HindIII-restricted YCpAGP1-lacZ (Iraqui et al., 1999).

References

Andréasson, C., and P.O. Ljungdahl. 2002. Receptor-mediated endoproteolytic activation of two transcription factors in yeast. *Genes Dev.* 16:3158–3172.

Andréasson, C., and P.O. Ljungdahl. 2004. The N-terminal regulatory domain of Stp1p is modular and fused to an artificial transcription factor confers full SPS-sensor control. *Mol. Cell. Biol.* 24:7503–7513.

Brent, R., and M. Ptashne. 1984. A bacterial repressor protein or a yeast transcriptional terminator can block upstream activation of a yeast gene. *Nature.* 312:612–615.

Connelly, C., and P. Hieter. 1996. Budding yeast SKP1 encodes an evolutionarily conserved kinetochore protein required for cell cycle progression. *Cell.* 86:275–285.

Doye, V., R. Wepf, and E.C. Hurt. 1994. A novel nuclear pore protein Nup133p with distinct roles in poly(A)+ RNA transport and nuclear pore distribution. *EMBO J.* 13:6062–6075.

Forsberg, H., M. Hammar, C. Andréasson, A. Moliner, and P.O. Ljungdahl. 2001. Suppressors of ssy1 and ptr3 null mutations define novel amino acid sensor-independent genes in *Saccharomyces cerevisiae*. *Genetics.* 158:973–988.

Gilstring, C.F., and P.O. Ljungdahl. 2000. A method for determining the in vivo topology of yeast polytopic membrane proteins demonstrates that Gap1p fully integrates into the membrane independently of Shr3p. *J. Biol. Chem.* 275:31488–31495.

Goldstein, A.L., and J.H. McCusker. 1999. Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. *Yeast.* 15:1541–1553.

Guldener, U., S. Heck, T. Fielder, J. Beinhauer, and J.H. Hegemann. 1996. A new efficient gene disruption cassette for repeated use in budding yeast. *Nucleic Acids Res.* 24:2519–2524.

Gyuris, J., E. Golemis, H. Chertkov, and R. Brent. 1993. Cdi1, a human G1 and S phase protein phosphatase that associates with Cdk2. *Cell.* 75:791–803.

Iraqui, I., S. Vissers, F. Bernard, J.O. de Craene, E. Boles, A. Urrestarazu, and B. André. 1999. Amino acid signaling in *Saccharomyces cerevisiae*: a permease-like sensor of external amino acids and F-Box protein Grr1p are required for transcriptional induction of the AGP1 gene, which encodes a broad-specificity amino acid permease. *Mol. Cell. Biol.* 19:989–1001.

Knop, M., K. Siegers, G. Pereira, W. Zachariae, B. Winsor, K. Nasmyth, and E. Schiebel. 1999. Epitope tagging of yeast genes using a PCR-based strategy: more tags and improved practical routines. *Yeast.* 15:963–972.

Kuehn, M.J., R. Schekman, and P.O. Ljungdahl. 1996. Amino acid permeases require COPII components and the ER resident membrane protein Shr3p for packaging into transport vesicles in vitro. *J. Cell Biol.* 135:585–595.

Kunkel, T.A., J.D. Roberts, and R.A. Zakour. 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Methods Enzymol.* 154:367–382.

Longtine, M.S., A. McKenzie III, D.J. Demarini, N.G. Shah, A. Wach, A. Brachat, P. Philippsen, and J.R. Pringle. 1998. Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*. *Yeast.* 14:953–961.

Sikorski, R.S., and P. Hieter. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics.* 122:19–27.