

## MATERIALS AND METHODS

**Construction of *CYC1-lacZ* plasmids.** Plasmids pLG $\Delta$ 312S $\Delta$ SS, pKB144, pKB148, pKB150, and pKB160 were a gift from A. Mitchell (Columbia University, New York, NY). Plasmids pKBmtURS1 and pLG(URS1)-52 were constructed by digesting plasmid pLG $\Delta$ 312S $\Delta$ SS with *Xho*I and ligation to phosphorylated, annealed oligonucleotides mtURS1-A (5'-TCGAGGTTACGTACGCTATTTTC-3') and mtURS1-B (5'-TCGAGAAATAGCGTACGTAACC-3'), or URS1-A (5'-TCG-AGGTTACGGCGGCTATTTTC-3') and URS1-B (5'-TCGAG-AAATAGCCGCCGTAACC-3') (Bowdish *et al*, 1995). All insertions were confirmed by sequencing.

**Construction of *HAC1<sup>i</sup>* expression plasmids.** The 3.7 kB *Sac*I-*Cl*I insert from pRS316-*HAC1<sup>i</sup>* (Schröder *et al*, 2000) was cloned into pRS314 (Sikorski and Hieter, 1989) to create pRS314-*HAC1<sup>i</sup>*. For inducible expression of HA-Hac1<sup>i</sup>p the *HAC1* ORF was amplified from pRS316-*HAC1<sup>i</sup>* by PCR and cloned into *Bam*HI and *Sac*I digested p2UG (Schena *et al*, 1991) to generate p2UG-HA-*HAC1<sup>i</sup>*. The *HAC1* sequence in p2UG-HA-*HAC1<sup>i</sup>* was verified by sequencing.

**Yeast strain constructions.** Yeast strains are summarized in Table II. The alleles *arg6*, *his3 $\Delta$ SK*, *ho::LYS2*, *IME2-20-lacZ::LEU2* (Vidan and Mitchell, 1997), *leu2::hisG*, *lys2*, *rme1 $\Delta$ 5::LEU2* (Covitz *et al*, 1991), *rpd3 $\Delta$ ::URA3* (Lamb and Mitchell, 2001), *sds3 $\Delta$ ::URA3* (Dorland *et al*, 2000), *sin3 $\Delta$ ::LEU3* (Lamb and Mitchell, 2001), *trp1::hisG*, *ume6-5::LEU2* (Strich *et al*, 1994), and *ura3* were provided by A. Mitchell (Columbia University, New York, NY). The *RPD3* alleles *RPD3-FLAG*, <sup>H150A</sup>*rpd3-FLAG*, <sup>H151A</sup>*rpd3-FLAG*, and <sup>H188A</sup>*rpd3-FLAG* (Kadosh and Struhl, 1998) were provided by K.

Struhl (Harvard Medical School, Boston, MA). The mutations *hac1Δ::TRP1* (Schröder *et al*, 2003) and *hac1Δ::URA3* (Schröder *et al*, 2000) were described previously.

Mutated URS1 elements were introduced into the genomic *DMC1* and *REC104* loci as follows. Each gene, including its URS1, was deleted by PCR based gene disruption using the *Candida albicans URA3* marker in pAG60 (Goldstein *et al*, 1999). The WT loci, including additional 400 - 500 bp of 5' and 3' sequence for homologous recombination, were amplified from genomic DNA and the mutation in the URS1 element introduced by PCR mediated mutagenesis. PCR products were digested with *Sall* and cloned into *Sall* digested, dephosphorylated pBC SK- (Stratagene, La Jolla, CA). The sequence of at least two independent clones was confirmed by sequencing the whole insert. Next, the inserts in pBC SK- were released with *Sall*, purified by gel electrophoresis, transformed into *dmc1Δ::URA3(C.a.)* and *rec104Δ::URA3(C.a.)* strains, and transformants selected with 1 g/l 5-fluoroorotic acid.

Replacement of the WT *RPD3* allele with FLAG tagged WT, H150A, H151A, and H188A *RPD3* alleles was done in a similar way. The source of the FLAG tagged *RPD3* alleles was YEplac112-*RPD3*-F (Kadosh and Struhl, 1998). About 500 bp of immediate 3' region of *RPD3* were amplified by PCR from genomic DNA, the sequence confirmed by sequencing, and cloned into the *SacI* site of YEplac112-*RPD3*-F. At this step a *ClaI* site at the 3' end of the insert was also introduced. The 2.2 kbp *ClaI* piece from YEplac112-*RPD3*-F and similar plasmids carrying the H150A, H151A, and H188A point mutation in *RPD3* were transformed into *rdp3Δ::URA3* strains and transformants selected with 1 g/l 5-fluoroorotic acid.

The *isw2Δ::hphMX4*, *itc1Δ::kanMX2*, *rpd3Δ::kanMX2*, and *rpd3Δ::natMX4* alleles were generated by PCR mediated gene disruption using plasmids pFA6a-kanMX2 (Wach *et al*, 1994), pAG32 (Goldstein and McCusker, 1999), and pAG25 (Goldstein and McCusker, 1999) as templates. The *ISW2* and *RPD3* deletions remove nearly the complete ORFs (bp 2 to 3341 for *ISW2* and 1 to 1300 for *RPD3*). The *ITC1* deletion removes bp 191 to 1946. Transformants were selected by replica plating to 400 mg/l G-418 (Invitrogen, Carlsbad, CA), 600 mg/l hygromycin B (Invitrogen), or 50 mg/l clonNAT (Werner Bioagents, Jena, Germany) after growth for one day on YPD plates.

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