Supplementary methods

DNA primers used for quantitative real-time RT-PCR analysis

β-tubulin (nucleotides 634-709)
5'-TTCCGCACCCTGAAACTGA / 5'-TGACGCCGGACACAACAG

18S rRNA (nucleotides 1525-1588)
5'-CGGAATGGCACCACAAGAC / 5'-TGGTAAAGTTCCCCGTGTTGA

ND4 (nucleotides 566-654)
5'-CAATCTGACCATTCCATGTGTGA /
5'-TTTCAGCACAATACTTGCTAATAAAACA

ATP synthase subunit α (nucleotides 1330-1388)
5'-GGCCAGCAGGTACAAACGAT / 5'-TTGAACAGCGCGACGAATC

ATP synthase subunit β (nucleotides 1298-1366)
5'-CGCGTAAGTTGGTGAAGTTCCT / 5'-GGCCAGTCATTCCTGTGAAGA

DNA primers used to amplify complete coding sequences of ATP synthase subunits α, β, and γ for sequence comparisons

subunit α
5'-TATTGGTGGCACGCTT / 5'-TGCTACCGGAGGCTAA

subunit β
5'-GAAGAAGTACCCGGAG / 5'-CCACGCTTTAAACAGC

subunit γ
5'-GCTTTACTGCGTACTCTTC / 5'-CGCATACCTACCGCATTT

***K. lactis* strains use in this study**

Strain	Relevant genotype	Source
CW35	Mata, adeT-600,uraA1, atp3::kan	Clark-Walker, unpublished
CW35-ATP3	CW35 plus LEU2::pCXJ4-ATP3	This study
CW35-atp3-2	CW35 plus LEU2::pCXJ4-atp3-2(Ile281Thr)	(Clark-Walker *et al.*, 2000)
CW35-atp3-3	CW35 plus LEU2::pCXJ4-atp3-3 (Leu265Pro)	This study

Yeast strains and media

The genotypes and sources of *K. lactis* strains are listed in Table 1. GYP medium contains 2% glucose, 0.5% Bacto yeast extract and 1% Bacto peptone. EB medium is GYP with 16 mg/ml ethidium bromide, Gly YP contains 2% glycerol in place of glucose and G418 medium is GYP with 200 mg/ml of the antibiotic. For solidification, 2% Bacto agar was added.

Yeast plasmids

pTZ19-KlATP3 was constructed by insertion of a 1.8kb HinfI fragment of K. lactis genomic DNA containing the ATP3 gene (Chen and Clark-Walker, 1995) into the SmaI site of pTZ19U (Pharmacia). To create pCXJ4-KlATP3, a 1.8kb fragment was excised from the above plasmid by SacI/BamHI digestion at flanking sites in the vector and inserted into the same restriction sites in pCXJ4. pCXJ4 is an integrative vector containing the URA3 gene of *Saccharomyces cerevisiae* and the LEU2 gene of *K. lactis* (X.J. Chen, unpublished).

***In vitro* mutagenesis of *K. lactis* ATP3**

The Leu 265 Pro change in *K. lactis* corresponding to the Leu 262 Pro change in *T. brucei* was produced as described (Clark-Walker *et al.*, 2000). An oligonucleotide, KlATP3PRO1, carrying TTA(Leu) to CCA(Pro), flanked by genomic sequence for 27 nucleotides upstream and 21 nucleotides downstream, together with a 17-mer universal primer 1, 5'-GTAAAACGACGGCCATG-3', downstream of the EcoR1 site in pTZ19U, were included in a PCR reaction in the presence of pTZ19-KlATP3 cut with BglII. The PCR product was isolated from a gel, blunt ended with T4 DNA polymerase and used as a mega primer in a second PCR reaction together with SacI cut pTZ19-KlATP3 and a primer, KlMG15P7, 5'-GTGACAAGGTTAAGGGTC-3', upstream of the BglII site in ATP3. The resulting PCR product was cut with SacI/BglII and used to replace the SacI/BglII fragment in pTZ19-KlATP3 containing wild-type sequence. A plasmid containing the desired mutation was identified by sequence determination and called pTZ19Klatp3-3.

Expression of mutagenized ATP3 in *K. lactis*

To examine expression of atp3-3 containing Leu265Pro, plasmid pCXJ4-Klatp3-3 was targeted to LEU2 by cleavage at a unique HpaI site followed by transformation of *K. lactis* CW35 atp3::kan. Transformants were selected for Ura+, screened for stability, then examined for single copy integration by Southern blotting of HindIII digested DNA using 32P-labeled LEU2 as a probe. Details of these procedures have been described previously (Clark-Walker *et al.*, 2000). The phenotype of CW35 atp3::kan containing a single integrated copy of mutagenized ATP3 was examined for suppression of ρ^0/ρ^--lethality by resistance to ethidium bromide and production of petite colony mutants.

ATPase activity of *K. lactis* strains

Kinetic parameters of F_1-ATPase were determined with freshly prepared mitochondria as described (Clark-Walker, 2003). V_{max} and K_m estimates were obtained from non-linear, least-squares curve fits generated with the Kaleidagraph software using the Michelis-Menten equation.