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’-CGGAATGGCACACCACAAGAC / 5’-TGGTAAAGTTCCCCGTGTGA

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

ATP synthase subunit α (nucleotides 1330-1388)
5’-GGCCAGCAGGTACAAACGAT / 5’-TTGAACACGCGACGAATC

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’-TATTGGTGCGCAGCCTT / 5’-TGCTACCGGAGGCTAA

subunit β
5’-GAAGAAGTACCCGGAG / 5’-CCACGCTTTAAACAGC

subunit γ
5’-GCTTTACTGCCTACTCCCTT / 5’-CGCATAACCTACCCGAC

K. lactis strains use in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>CW35</td>
<td>Mata, adeT-600,uraA1, atp3::kan</td>
<td>Clark-Walker, unpublished</td>
</tr>
<tr>
<td>CW35-ATP3</td>
<td>CW35 plus LEU2::pCXJ4-ATP3</td>
<td>This study</td>
</tr>
<tr>
<td>CW35-atp3-2</td>
<td>CW35 plus LEU2::pCXJ4-atp3-2(Ile281Thr)</td>
<td>(Clark-Walker et al., 2000)</td>
</tr>
<tr>
<td>CW35-atp3-3</td>
<td>CW35 plus LEU2::pCXJ4-atp3-3 (Leu265Pro)</td>
<td>This study</td>
</tr>
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Yeast strains and media
The genotypes and sources of \textit{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 Hinfl fragment of \textit{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containing the URA3 gene of \textit{Saccharomyces cerevisiae} and the LEU2 gene of \textit{K. lactis} (X.J. Chen, unpublished).

**In vitro mutagenesis of \textit{K. lactis} ATP3**

The Leu 265 Pro change in \textit{K. lactis} corresponding to the Leu 262 Pro change in \textit{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/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 \textit{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 \textit{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 \(\rho^0/\rho^{-}\)-lethality by resistance to ethidium bromide and production of petite colony mutants.

**ATPase activity of \textit{K. lactis} strains**
Kinetic parameters of F₁-ATPase were determined with freshly prepared mitochondria as described (Clark-Walker, 2003). $V_{\text{max}}$ and $K_m$ estimates were obtained from non-linear, least-squares curve fits generated with the Kaleidagraph software using the Michaelis-Menten equation.