

ATP16 Genes and Neighboring ORFs Are Duplicated on Chromosome IV in *Saccharomyces cerevisiae*

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Abstract

We present evidence that there were two closely linked copies of the *ATP16* (YDL004w) gene encoding the δ subunit of F_1F_0 -ATPase complex on chromosome IV in laboratory strains, W303-1A, W303-1B, DC5, LL20, SEY2102, YPH499, and S288C of *Saccharomyces cerevisiae*. We previously reported that there were 2-3 copies of *ATP1* (α), *ATP2* (β) and *ATP3* (γ) on chromosomes II, X, and II, respectively. Homologous recombination of the *ATP16* with *HIS3* (YOR202w), gene walking, and long-PCR analyses showed that two *ATP16* were present on the same chromosome as above genes. The gene walking estimated that the two *ATP16* were separated by approximately 8.4 kb using *ATP16* and its neighboring DNAs as probes, designated the proximal *ATP16* to the telomere as *ATP16a* and to the distal as *ATP16b*. Although the nucleotide sequences of *ATP16a* and *ATP16b* were identical including 833 bases upstream- and 937 bases downstream of *ATP16*, they might be expressed differently.

Keywords: F_1F_0 -ATPase, *ATP16*, Repetitive genes, Chromosome IV, *Saccharomyces cerevisiae*

1. Introduction

F_1 -ATPase (catalytic sector of F_1F_0 -ATPase complex, designated as F_1) was composed of five different subunits, α , β , γ , δ and ϵ in a stoichiometry of 3: 3: 1: 1: 1 in all aerobic cells (Cox et al., 1992; Boyer, 1993; Noji et al., 1997; Futai et al., 2000). In yeast *S. cerevisiae*, they were encoded for the nuclear genes *ATP1* (YBL099w), *ATP2* (YJR121w), *ATP3* (YBR039w), *ATP16* (YDL004w), and *ATP15* (YPL271w), respectively. Recently, we had revealed that multiple copies of the *ATP1* (YBL099w), *ATP2* (YJR121w), and *ATP3* (YBR039w) genes were arranged in tandem on each chromosome on which these genes are located (Takeda et al., 1995; Takeda et al., 1999; Takeda et al., 2010; Takeda et al., 2005; Ohnishi et al., 2003), not as reported by the Genome Project using S288C (Feldmann et al., 1994; Galibert et al., 1996; Mewes et al., 1997; Jacq et al., 1997). The copy numbers of these F_1F_0 subunit genes were not exactly coincident with the subunit-stoichiometry of F_1 mentioned above. In addition, these genes were repeated two or three times accompanying their several neighboring ORFs and the other tandem duplicated area on each chromosome reported by the Genome Project. Thus, the other repetitive genes such as the F_1F_0 subunit genes might exist on the chromosomes more than we have expected.

The *ATP16* (YDL004w, Giraud & Velours, 1994) gene encoding for the $F_1\delta$ subunit of *S. cerevisiae* was present on the left arm close to the centromere sequence (*CEN4*) of chromosome IV just one copy was reported by the Genome Project (Jacq et al., 1997). We showed here that two copies of the *ATP16* (YDL004w) gene were also arranged in tandem accompanying the neighboring ORFs and the other tandem duplicated area on chromosome IV.

2. Materials and Methods

Yeast strains: The yeast strains used in this study were *Saccharomyces cerevisiae* DC5 (*MATA leu2-3 leu2-112 his3 can1-11*), LL20 (*MATA leu2 his3*), W301-1A (*MATA leu2-3 leu2-112 his3-11 his3-15 trp1-1 ura3-1 ade2-1 can1-100*), W303-1B (*MATA leu2-3 leu2-112 his3-11 his3-15 trp1-1 ura3-1 ade2-1 can1-100*), YPH499 (*MATA ade2 his3 leu2 trp1 ura3 lys2*), and S288C (*MATA SUC2 mal mel gal2 CUP1*).

E. coli strains: Strains, Sure (el4⁺ (*McrA*⁺) $\Delta(mcrCB-hsdSMR-mrr)171$ *endA1 supE44 thi-1 gyrA96 relA1 lac recBrecJ sbcC umuC::Tn5* (*Kan*^r) *uvrC[F' proAB lacI^qZAM15 Tn10* (*Tet*^r)] and DH10B (F *mcrA* $\Delta(mrr-hsdRMS-mrcBC)$ $\Phi 80dlacZAM15 \Delta lacX74 deoR recA1 endA1 araD139, \Delta(ara, leu)7697 galU galK rnpG), were used in this study.$

Plasmids: The *ATP16* gene disrupted plasmid, pYEF-16/4, was constructed as follows. The *ATP16* gene was inserted into the *SphI-EcoRI* site of the pAML20 (Yep-type vector, Iha & Tsuguri, 1998) and constructed the pYEATP16. The pYEATP16 was digested with *Bam*I, and the *Bam*HI fragment of the *HIS3* gene (fill-in) was inserted into the *Bam*I site of *ATP16*.

Media: *E. coli* carrying plasmids were grown in LB (0.5% yeast extract, 1% bacto-tryptone, 1% NaCl) containing 50 µg of ampicillin per ml. Yeast strains were grown on YPG (1% yeast extract, 2% bacto-peptone, 3% glycerol), YPDM (1% yeast extract, 0.5% bacto-peptone, 0.1% ($(NH_4)_2SO_4$, 0.2% KH₂PO₄, 0.1% MgSO₄, 0.8% glucose), or SD (0.67% yeast nitrogen base without amino acids, 2% glucose, and appropriate nutrients). The solid medium contained 2% agar.

Polymerase chain reaction (PCR): Long-PCR analysis was performed according to the procedure recommended for the Takara Pyrobest™ polymerase PCR kit (Takara Shuzo Co., Ltd, Osaka, Japan). The primer pairs used were 5'-TATAACTGTAGCCGTGAGGA-3' (sense) and 5'-AGTTCTAGAATGCGTACCAT-3' (antisense). These primers were located just inside the *ATP16*-coding region (Takeda et al., 1999; Takeda et al., 2010; Ohnishi et al., 2003; Barnes, 1994). DNA was amplified in a PCR processor (Astec, Program TEMP Control System PC-700, Fukuoka, Japan) using 30 cycles. Yeast whole DNAs from each strain used in the experiments were made by the method previously reported (Takeda et al., 1995; Takeda et al., 1999). The PCR products (*ATP16a* and *ATP16b*, respectively) from each template were cloned into a vector pBluescript (Stratagene, La Jolla, CA) for sequencing according to the procedure recommended for the Takara Blunting Kination Ligation kit (Takara).

DNA sequencing: Nucleotide sequencing was performed by the standard dideoxy chain termination method with the ABI models 373 and 310, and LI-COR model 4200L-2 sequencers.

Pulse-field gel electrophoresis: The amplified DNAs were separated in a 1% agarose (w/v) gel on an alternating CHEF gel apparatus (Bio-Rad, CA). Electrophoresis was performed for 16 h in 0.5 x TBE buffer at 200 V (14°C) with a 2.8 to 3.4 s linear gradient, as described previously (Takeda et al., 1995; Takeda et al., 1999; Takeda et al., 2010; Takeda et al., 2005; Ohnishi et al., 2003).

Preparation of mitochondria: Cells were grown in 50 ml of YPDM medium. After incubation for 24 h at 30°C,

the cells (ca. $2 \sim 4 \times 10^6$ cells/ml) were harvested and mitochondria were prepared according to the published methods (Daum et al., 1982).

Western blotting: Immunodetection analysis of proteins was performed according to the procedures described previously (Mabuchi et al., 2000).

RT-PCR: Single colony of each yeast line was inoculated into 10 ml of liquid YPD medium and shake for 42 hours at 30°C. The yeast cells were collected by centrifugation and washed. The cell pellet was suspended in 0.3 ml of the lysis buffer (0.2M Tris-HCl pH 7.5, 0.5M NaCl, 10mM EDTA, 1% SDS) and transfer to a microtube carrying 0.6 g of 0.5 mm diameter glass beads (fujistone No. 06, Fuji Rikakogyo, Osaka) and 0.3 ml of PCI (phenol/chloroform/isoamyl alcohol). The cell was disrupted by vortex for 2.5 min. After centrifugation and alcohol precipitation, the total RNA solution was treated with Dnase (Dnase RQ, Promega) and then purified by PCI. Extraction. The total RNA (5 µg) was reverse-transcribed with Superscript III first strand cDNA synthesis system (Invitrogen) using a gene-specific reverse primer (primer c). The cDNA equivalent to 0.5 µg of the total RNA was amplified with Takara Ex Taq HS (Takara) using primers for *ATP16* (primers a & b). The PCR cycle was 30 cycles of 98°C for 10 sec., 50°C for 30 sec. and 72°C for 1min.

Miscellaneous: Southern hybridization of DIG-labeled *ATP16* (YDL004w, 1.0 kb *HincII* fragment), *HIS3* (1.7 kb *BamHI* fragment), and other *ATP16*-neighboring probes used in the experiments were performed as described previously (Takeda et al., 1995).

3. Results

3.1 Disruption of *ATP16* on the left arm of chromosome IV

The *ATP16* (YDL004w) gene encoding the $F_1\delta$ subunit was mapped on the left arm close to the centromere of chromosome IV in *S. cerevisiae* (Jacq et al., 1997; Giraud & Velours, 1994). The *ATP16* (YDL004w) gene was disrupted with the yeast *HIS3* gene (plasmid pYEF-16/4) by the homologous recombination methods as described previously (Takeda et al., 1995; Takeda et al., 1999; Takeda et al., 2010; Takeda et al., 2005; Ohnishi et al., 2003). The total DNAs (genomic DNAs) of the *ATP16*-disruptants derived from four different laboratory strains, DC5, LL20, W303-1A, and W303-1B, were digested with *HincII*, transferred on membranes, and then hybridized successively with DIG-labeled (a) *ATP16* and (b) yeast *HIS3* as probes. The DNAs of the four different strains gave the same hybridization patterns when the DNA were digested with various restriction enzymes, *EcoRI*, *BamHI*, *HindIII*, *SalI*, *PvuII*, *PstI*, *XbaI*, *NcoI*, and *SphI*. In the gene disruption experiments, the undisrupted *ATP16* (YDL004w, 1.0 kb) was detected in all laboratory strains used in this study, but some His⁺-transformants yielded undisrupted *ATP16* (YDL004w, 1.0 kb) in addition to the disrupted *ATP16* with the *HIS3* gene (*atp16::HIS3*, 2.8 kb). Lane 1 (W303-1B, WT) was detected the undisrupted *ATP16*, lanes 2-4 (transformants W30, W3, W202) were detected both undisrupted- (1.0 kb) and *HIS3*-disrupted- *ATP16* (2.8 kb). Lanes 5 and 6 (transformants W19 and W6) were only detected *HIS3*-disrupted-*ATP16* (Figure 1).

To confirm the occurrence of gene disruption on chromosome IV, Southern blot analysis of the chromosome were performed using DIG-*ATP16* and yeast *HIS3* as probes (Figure 2). The results of the Southern analysis showed that both the undisrupted-*ATP16* and the disrupted-*ATP16* with yeast *HIS3* were mapped on chromosome IV, as described in the Genome Project. The yeast *HIS3* gene was essentially present on not only chromosome XV, but also on chromosome IV located the *ATP16*. The results indicated that both undisrupted *ATP16* and disrupted *ATP16* were present on chromosome IV, i.e., the two closely-linked copies of the *ATP16* (YDL004w) gene were arranged on chromosome IV as were those of *ATP3* in the yeast *S. cerevisiae* (YBR039w, Ohnishi et al., 2003). We designated the two copies as *ATP16a* (proximal to the telomere) and *ATP16b* (distal to the telomere).

Most of the transformants which were co-existing with the undisrupted-*ATP16* (*ATP16b*) and the disrupted-*ATP16* (*atp16a::HIS3*) were able to grow on glycerol, but the growth was not the same as those of laboratory strains (transformant W30, Figure 3-2). In contrast, few transformants were co-existed both undisrupted-*ATP16* and disrupted-*ATP16* (transformant W202, *ATP16a*, *atp16b::HIS3*, Figure 3-3) could not grow on glycerol as both *ATP16s*-disruptant (transformant W19, *atp16a::HIS3*, *atp16b::HIS3*, Figure 3-4). The *ATP16b*-disruptant (transformant W202, Figure 3-3) and the double *ATP16s*-disruptant (transformant W19, Figure 3-4) did not recover the glycerol-grown phenotype by the introduction of the wild-type *ATP16*.

3.2 Gene walking analysis of two copies of *ATP16*

Southern blot hybridization of purified genomic DNA from S288C and other laboratory strains were performed using the same probe (DIG-labeled *ATP16*). Restriction enzymes, *BamHI*, *Clal*, *KpnI*, *XhoI*, *NdeI*, *SphI*, *SphI/NcoI*, and *XbaI* were used in the experiments (Figure 4). In *SphI* digestion (Figure 4a, lane 6), two bands

(8.4 and 30.4 kb) were hybridized with the *ATP16* probe. According to the data of the Genome Project, the long DNA band (30.4 kb) was expected from S288C but not the short band (8.4 kb). This showed that one extra *Sph*I site, which had not been reported by the Genome Project, should be present between two closely linked gene copies of *ATP16s* (data not shown). *Nde*I (lane 5) and *Xba*I (lane 8) were also detected two bands used *ATP16* gene as a probe.

It also means that two closely linked copies, of *ATP16* (YDL004w) are located on the left arm of chromosome IV, as described for *ATP1* (YBL099w, Takeda et al., 1995; Takeda et al., 1999; Takeda et al., 2010), *ATP2* (YJR121w, Takeda et al., 2005), and *ATP3* (YBR039w, Ohnishi et al., 2003) in the yeast *S. cerevisiae*.

3.3 Map of two gene copies of *ATP16* on the left arm of chromosome IV

Long-PCR analysis using genomic DNA from the laboratory strains and a primer set of the starting and terminal codon just inside the *ATP16* (YDL004w) gene were performed in order to amplify the DNA region between the two *ATP16s* as described previously (Takeda et al., 1999; Takeda et al., 2005; Ohnishi et al., 2003). The results showed that only approximately 8.4-kb of PCR product were obtained (data not shown). The 8.4-kb of PCR product was hybridized with the probe of the DIG-labeled *ATP16* (YDL004w), but not hybridized with the DIG-labeled *CEN4* (localized between YDL001w and YDR001c on chromosome IV, data not shown). From the analysis by chromosome walking of the total (genomic) DNA of the laboratory strains and the PCR experiments, this 8.4-kb PCR product might contain the DNA region between the two *ATP16s*, *ATP16a*, and *ATP16b* on chromosome IV.

To confirm more detail whether this 8.4-kb PCR product was the DNA region between *ATP16a* and *ATP16b* on chromosome IV, and to identify the repeating region of the *ATP16* (YDL004w) gene. The long-PCR product was hybridized with the DIG-labeled probes neighboring genes of chromosome IV. In contrast, probes containing both *CEN4* localized between YDL007w (*RPT2*) and YDL001w to YDR001c (*NTH1*) of chromosome IV were not hybridized respectively to the PCR product, i.e., they are located outside the repeating region (data not shown).

3.4 Base sequence of *ATP16a* and *ATP16b*

To determine the nucleotide sequence of *ATP16a* and *ATP16b*, we performed the standard dideoxy chain-termination methods using the genomic DNA isolated from laboratory strains DC5, LL20, W303-1A, W303-1B, YPH499, and strain S288C, which was used in the Yeast Genome Project. As a result, no difference was detected between the two closely linked copies of *ATP16s*, *ATP16a*, and *ATP16b*, not only in the coding region, but also 5'-end 833 bases upstream of ATG and 3'-end 937 bases downstream from the terminal codon. They were completely identical with the nucleotide sequences registered both at the SGD (<http://www.yeastgenome.org/>) and at the Mips (<http://mips.gsf.de/>) of the *ATP16* (YDL004w) and the neighboring ORFs (data not shown). Nevertheless, they seemed to function differently. In other words, the *ATP16a*-disruptant could grow on the glycerol medium, but the *ATP16b*-disruptant could not.

3.5 Transcripts of *ATP16a* and *ATP16b*

To verify the expression of both the *ATP16a* and the *ATP16b*, RT-PCR experiments were performed using sense (primer a) and anti-sense primers (primers b and c) as the lower panel of Figure 6. The results of the RT-PCR experiments were shown in the upper panel of Figure 6. Clearly both the *ATP16a* and the *ATP16b* were expressed. The disruptant of both the *ATP16a* and the *ATP16b* (lane 4, transformant W19) could not observe any bands (products). The transcript of the *ATP16a*-disruptant (lane 2, transformant W30) deeper than that of the *ATP16b*-disruptant (lane 3, transformant W202), i.e., the *ATP16b* might be expressed more than the *ATP16a* (Figure 6). In the preliminary experiments, the *ATP16b*-disruptant was easy to mutate the mtDNA. The difference of the copy-specific disruptant might be come from the difference of the conformation of the *ATP16a* or the *ATP16b* on chromosome. The detailed experiments were under the progress.

4. Discussion

The F₁δ subunit gene, *ATP16* was repetitive on the left arm of chromosome IV in *S. cerevisiae*. Two closely linked copies of the *ATP16s*, the *ATP16a*, and the *ATP16b* were present near the *CEN4* localized between YDL001w and YDR001c of chromosome IV. The nucleotide sequences and the deduced amino acid sequences of the *ATP16a* and the *ATP16b* were the same as those of two copies of *ATP3* (YBR039w, ref. 9). Neither *ATP16*-disruptant (*Δatp16a*, *Δatp16b*) could grow on glycerol. It was easy to eliminate the mtDNA that both the Southern blot analyses of the two *ATP16*-disruptants using DIG-labeled wild-type-mtDNA and the glycerol-grown phenotype of the diploid strain with that of the p⁰-tester strain confirmed (data not shown). Co-existing transformants of the disrupted-*ATP16* and the undisrupted-*ATP16* gene showed two different

phenotypes. That is, the *ATP16a*-disruptant ($\Delta atp16a$, *ATP16b*, transformant W30) could grow on glycerol, although the level of its growth was less than that of the laboratory strain (*ATP16a*, *ATP16b*, WT). When the wild-type *ATP16* gene was introduced into the *ATP16a*-disruptant, the growth on glycerol recovered to the level of that of the laboratory strain. In contrast, the *ATP16b*-disruptant (*ATP16a*, $\Delta atp16b$, transformant W202) could not grow on glycerol and easily mutated mtDNA as did all *ATP16*-disruptants ($\Delta atp16a$, $\Delta atp16b$, transformant W19) (data not shown). Nonetheless, both the nucleotide and the deduced amino acid sequences were apparently the same, containing 5'-, and 3'-non-coding regions of the *ATP16a* and the *ATP16b*. From these observations, the two *ATP16s* might be expressed differentially as in the cases of the two *ATP3s* (Ohnishi et al., 2003).

Although no difference in base sequence between the two *ATP3s* or two *ATP16s* was observed, most of the *ATP3*-, or *ATP16*-disruption occurred in the *ATP3a* or the *ATP16a*, which is located proximal to the left telomere in comparison with that of the *ATP3b* or the *ATP16b*, which was located distal to the left telomere in all strains used in the experiments.

The reasons for the presence of two closely linked copies such as the *ATP3s* and the *ATP16s*, which had different functions, were not yet known. However, some possibilities could be considered. First, the regulatory region (promoter) might be located farther than 833 bases upstream of the ATG-starting codon of *ATP16*. Second, the expression time or conditions of *ATP16a* and *ATP16b* might be different; there might be some difference in the higher-ordered structure of chromosome, such as the nucleosome structure of two *ATP16s* on the chromosome between these two *ATP16s* (Kawasaki et al., 2004; Krogan et al., 2004). Third, some *trans*-regulated elements of the two *ATP3s* or *ATP16s* gene copies might be expressed in cells such as in the *GAL* genes (Kodama et al., 2003) and the lipogenic enzyme genes (Stoekman et al., 2004). The regulatory regions of most yeast genes are usually located within several hundred bases upstream of the ATG (Basehoar et al., 2004; Ferrer-Martinez et al., 2004), so the probability of the first explanation ought to be low. The second or third explanations could be more feasible.

Of the five gene subunits (α , β , γ , δ , ϵ) in F_1 , four subunits were repetitive accompanying their neighboring ORFs, although the repeating unit on each chromosome was different for each ATP gene. $F_1\epsilon$ subunit gene, *ATP15* (YPL271w), was also repetitive on chromosome XVI (manuscript in preparation). In addition, unpublished results showed that of the F_0 -sector in the F_1F_0 , the nuclear-coded *ATP4* (YPL078c), *ATP5* (YDR298c) and *ATP7* (YKL016c) genes were also repetitive as other F_1F_0 subunit genes, *ATP1*, *ATP2*, and *ATP3*, in *S. cerevisiae* (manuscript in preparation). Thus, all F_1 subunit genes and 3 nuclear-coded- F_0 subunit genes were repetitive, and other genes neighboring these F_1F_0 subunit genes were also present in duplicate or triplicate in *S. cerevisiae*. These repetitive genes expressed and functioned, so they probably had some biological meaning(s) and played some biological role(s). The F_1F_0 was one of essential enzymes in eukaryotic cells to maintain the life, so the subunit genes of the complex might be necessarily maintained even if some of subunit genes become disrupted. Also, the expression of the genes might be sophisticatedly regulated using multiple-copies of the genes to produce ATP (Takeda & Nakahara, 2009; Nakahara & Takeda, 2010a; Nakahara & Takeda, 2010b). In other words, the nuclear-coded subunit genes in F_1F_0 should participate in ATP synthesis. We did not yet know precisely whether the gene repetition was confined to the area of F_1F_0 subunit genes on each chromosome. The physiological meaning of these multiple genes and other regions containing repeated units was still unknown. However, we needed continue to consider them in order to understand why living cells harbor gene repetition on chromosomes.

The δ subunit played an important role of F_1 and cell death in eukaryotic cells (Matsuyama et al., 1998). In bacteria and bovine F_1 -ATPase analyses, the δ subunit located and interacted with the α , β and γ subunits (Duvezin-Caubet, 2003). However, details of the role of the δ subunit of yeast F_1 were not known, although the δ subunit was a member of the F_1F_0 and plays an important role in energy-transduction machinery. Certainly α , β and γ were important in the structure and function of the complex, but other subunits δ and ϵ might be necessary for the control of the complex, and interact with other proteins and factors. Unfortunately, little was known about the small subunits of the F_1 .

As mentioned above, in aerobic cells, ATP was the energy source of biological phenomena, and the F_1F_0 was an important enzyme in the production of ATP. The multiple subunit genes on each chromosome might regulate in some way gene expression and assembly for functions based on the combination and organization of these multiple F_1F_0 subunit genes on each chromosome by e.g., aging, the cell division-cycle, and environmental interactions. It might not be so unusual that a protein plays different roles in living cells. We did not know still what different roles or the minute regulation to the biological phenomena were played by the two closely linked copies of the *ATP16* genes in *S. cerevisiae*, but understanding their roles would contribute to a better understanding of living cells (Dujon et al., 2004).

References

- Barnes, W. M. (1994). PCR amplification of up to 35 kb DNA with high fidelity and high yield from 1 bacteriophage templates. *Proc. Natl. Acad. Sci. USA*, 91, 2216-2220. <http://dx.doi.org/10.1073/pnas.91.6.2216>
- Basehoar, A. D. Zanton, S. J., & Pugh, B.F . (2004). Identification and distinct regulation of yeast TATA box-containing genes. *Cell*, 116, 699-709. [http://dx.doi.org/10.1016/S0092-8674\(04\)00205-3](http://dx.doi.org/10.1016/S0092-8674(04)00205-3)
- Boyer, P. D. (1993). The binding change mechanism for ATP synthase- Some probabilities and possibilities. *Biochim. Biophys. Acta*, 1140, 215-250. [http://dx.doi.org/10.1016/0005-2728\(93\)90063-L](http://dx.doi.org/10.1016/0005-2728(93)90063-L)
- Cox, G. B. et al. (1992). The structure and assembly of ATP synthase. In Ernster L Ed. In *Molecular Mechanism in Bioenergetics*, Ernster L (ed.,). Elsevier, 283-315.
- Daum, G., Böhni P. C., & Schatz G. (1982). Import of proteins into mitochondria. *J. Biol. Chem*, 257, 13028-13033.
- Dujon, B. et al. (2004). Genome evolution in yeasts. *Nature*, 430, 35-44. <http://dx.doi.org/10.1038/nature02579>
- Duvezin-Caubet, S. et al. (2003). The two rotor components of yeast mitochondrial ATP synthase are mechanically coupled by subunit δ . *Proc. Natl. Acad. Sci. U.S.A.*, 100, 13235-13240. <http://dx.doi.org/10.1073/pnas.2135169100>
- Feldmann, H. et al. (1994). Complete DNA sequence of yeast chromosome II. *EMBO J.*, 13, 5795-5809.
- Ferrer-Martinez, A. et al. (2004). A glucose response element from the *S. cerevisiae* hexose transporter HXT1 gene is sensitive to glucose in human fibroblast. *J. Mol. Biol*, 338, 657-667. <http://dx.doi.org/10.1016/j.jmb.2004.03.036>
- Futai, M. et al. (2000). Synthase (H^+ -ATPase) coupling between catalysis, mechanical work, and proton translocation. *Biochim. Biophys. Acta*, 1458, 276-288. [http://dx.doi.org/10.1016/S0005-2728\(00\)00080-3](http://dx.doi.org/10.1016/S0005-2728(00)00080-3)
- Galibert, F. et al. (1996). Complete nucleotide sequence of *Saccharomyces cerevisiae* chromosome X. *EMBO J*, 15, 2031-2049.
- Giraud, M-F. & Velours, J. (1994). ATP synthase of yeast mitochondria: isolation of the $F_1 \delta$ subunit, sequence and disruption of the structural gene. *Eur. J. Biochem*, 22, 851-859. <http://dx.doi.org/10.1111/j.1432-1033.1994.tb18932.x>
- Iha, H. & Tsurugi, K. (1998). Shuttle-Vector System for *Saccharomyces cerevisiae* designed to produce C-terminal-myc-tagged fusion proteins. *Biotechniques*, 25, 936-938.
- Jacq, C. et al. (1997). The nucleotide sequence of *Saccharomyces cerevisiae* chromosome IV. *Nature*, 387, 75-78.
- Kawasaki, H., Wadhwa, R., & Taira, K. (2004). World of small RNAs: from ribozymes to siRNA and miRNA. *Differentiation*, 72, 58-64. <http://dx.doi.org/10.1111/j.1432-0436.2004.07202006.x>
- Kodama, T. et al. (2003). Unique distribution of GAL genes on chromosome XI in the yeast *Saccharomyces naganishii*. *Curr. Microbiol*, 47, 497-500. <http://dx.doi.org/10.1007/s00284-003-4083-5>
- Krogan, N. J. et al. (2004). High-definition macromolecular composition of yeast RNA-processing complexes. *Mol. Cell*, 13, 225-239. [http://dx.doi.org/10.1016/S1097-2765\(04\)00003-6](http://dx.doi.org/10.1016/S1097-2765(04)00003-6)
- Mabuchi, T. et al. (2000). *ASCI/RAS2* suppresses the growth defect on glycerol caused by the *atp1-2* mutation in the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem*, 275, 10492-10497. <http://dx.doi.org/10.1074/jbc.275.14.10492>
- Matsuyama, S. et al. (1998). The mitochondrial F_0F_1 -ATPase proton pump is required for function of the proapoptotic protein Bax in yeast and mammalian cells. *Mol. Cell*, 1, 327-336. [http://dx.doi.org/10.1016/S1097-2765\(00\)80033-7](http://dx.doi.org/10.1016/S1097-2765(00)80033-7)
- Mewes, H. W. et al. (1997). Overview of the yeast genome. *Nature*, 387, 7-65. <http://dx.doi.org/10.1038/42755>
- Nakahara, M. & Takeda, M. (2010a). Characterization of the sequence spectrum of DNA based on the appearance frequency of the nucleotide sequences of the genome -A new method for analysis of genome structure-. *J. Biomed. Sci. Eng*, 3, 340-350. <http://dx.doi.org/10.4236/jbise.2010.34047>
- Nakahara, M. & Takeda, M. (2010b). Identification of the Interactive Region by the Homology of the Sequence Spectrum. *J. Biomed. Sci. Eng*, 3, 868-883. <http://dx.doi.org/10.4236/jbise.2010.39117>
- Noji, H. et al. (1997). Direct observation of the rotation of F_1 -ATPase. *Nature* 1997; 386: 299-302. 1997. Direct

observation of the rotation of F₁-ATPase. *Nature*, 386, 299-302. <http://dx.doi.org/10.1038/386299a0>

Ohnishi, K. et al. (2003). Studies on the *ATP3* gene of *Saccharomyces cerevisiae*: presence of two closely linked copies, *ATP3a* and *ATP3b*, on the right arm of chromosome II. *Yeast*, 20, 943-954. <http://dx.doi.org/10.1002/yea.1009>

Stoekman, A. K. et al. (2004). Mix is the functional heteromeric partner of the carbohydrate response element-binding protein in glucose regulation of lipogenic enzyme gene. *J. Biol. Chem.*, 279, 15662-15669. <http://dx.doi.org/10.1074/jbc.M311301200>

Takeda, M. & Nakahara, M. (2009). Structural Features of the Nucleotide Sequences of Genomes. *J. Comput. Aided Chem.*, 10, 38-52. <http://dx.doi.org/10.2751/jcac.10.38>

Takeda, M. et al. (1995). Three *ATP1* genes are present on chromosome II in *Saccharomyces cerevisiae*. *J. Biochem (Tokyo)*, 118, 607-613.

Takeda, M. et al. (1999). The three copies of *ATP1* gene are arranged in tandem on chromosome II of the yeast *Saccharomyces cerevisiae* S288C. *Yeast*, 15, 873-878. [http://dx.doi.org/10.1002/\(SICI\)1097-0061\(199907\)15:10A<873::AID-YEA427>3.0.CO;2-A](http://dx.doi.org/10.1002/(SICI)1097-0061(199907)15:10A<873::AID-YEA427>3.0.CO;2-A)

Takeda, M. et al. (2005). The three copies of *ATP2* gene are arranged in tandem on chromosome X of yeast *Saccharomyces cerevisiae*. *Curr Genet*, 47, 265-272. <http://dx.doi.org/10.1007/s00294-005-0565-5>

Takeda, M., Nakamura, S., & Matsushita, S. (2010). Genome organization of the three identical *ATP1* genes on the left arm of chromosome II of *Saccharomyces cerevisiae*. Sequence analysis of the 35-kb region containing three *ATP1* genes. *The Open Genomics Journal*, 3, 9-17.

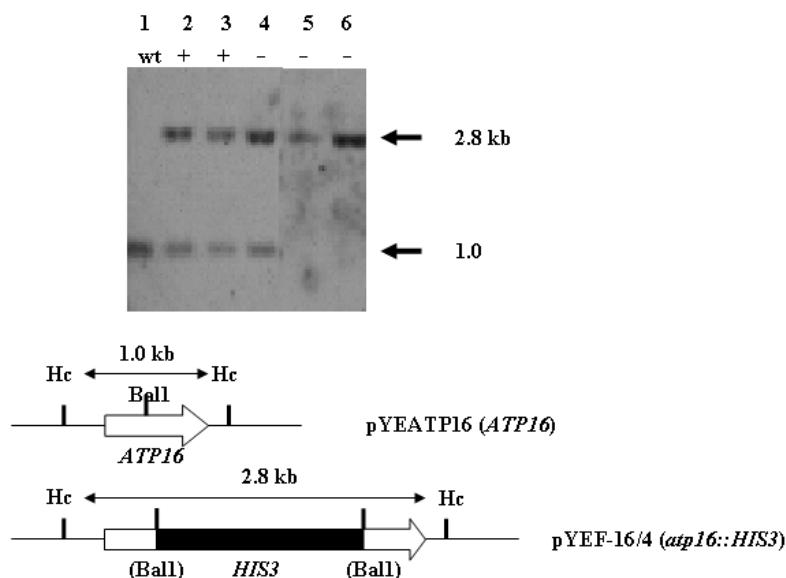


Figure 1. Southern hybridization of genomic DNA isolated from *ATP16*-disruptants with *HIS3*

Various yeast strains, DC5, LL20, W303-1A and W303-1B were used in the experiments. The total DNA (genomic DNA) was isolated from the yeast strains and digested with *Hinc*II. After the digestion the genomic DNA was applied to a 0.8% agarose gel electrophoresis, blotted on to a nylon membrane and then hybridized to DIG-labeled *ATP16*. Lower panel showed the plasmids containing *ATP16* (pYEATP16), and *ATP16* disrupted with *HIS3* (pYEF-16/4) using the homologous recombination described in Materials and Methods.

Lane 1, W303-1B (WT, *ATP16a*, *ATP16b*, Gly+); lanes 2, W30 (*atp16a::HIS3*, *ATP16b*, Gly+); lane 3, W3 (*atp16a::HIS3*, *ATP16b*, Gly+) lane 4, W202 (*ATP16a*, *atp16b::HIS3*, Gly-); lane 5, W19 (*atp16a::HIS3*, *Atp16b::HIS3*, Gly-); lane 6, W6 (*atp16a::HIS3*, *atp16b::HIS3*, Gly-). The disrupted *ATP16* with *HIS3* was determined by the Southern hybridization of the *Sph*1-digested genomic DNA used *HIS3* as a probe.

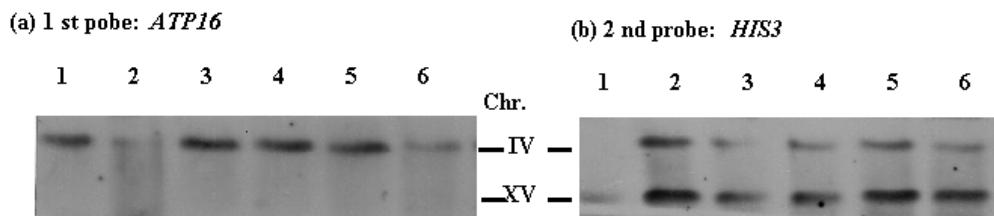


Figure 2. Chromosome Southern hybridization of *ATP16* disruptants

Chromosomes were isolated and electrophoresed in 1% agarose gel on a CHEF apparatus, blotted onto a nylon membrane and then hybridized successively to DIG-labeled *ATP16* (a) and *HIS3* (b). Electrophoresis was performed for 34 h with a switching interval of 55 s for 17 h and 90 s for 17 h (1-4). Lane 1, W303-1B (WT, *ATP16a*, *ATP16B*); lane 2, W30 (*atp16a::HIS3*, *ATP16b*, Gly+); lane 3, W3 (*atp16a::HIS3*, *ATP16b*, Gly+); lane 4, W202 (*ATP16a*, *atp16b::HIS3*, Gly-); lane 5, W19 (*atp16a::HIS3*, *ATP16b::HIS3*, Gly-); lane 6, W6 (*atp16a::HIS3*, *atp16b::HIS3*, Gly-).

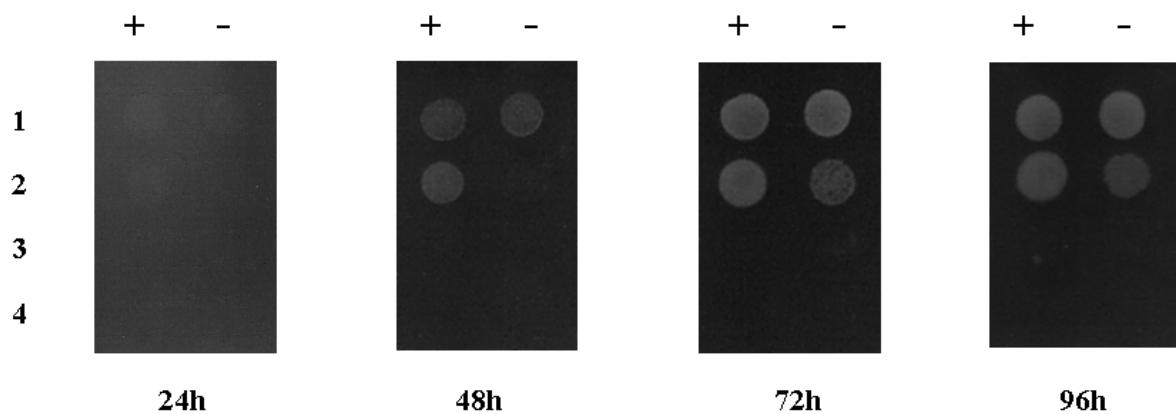


Figure 3. Glycerol phenotype of *ATP16* disruptants

Various *ATP16*-disruptants were counted ($10^6/\text{ml}$) and grown on a glycerol medium (YPG) for 24, 48, 72, and 96 h at 30 °C. Top (1), W0303-1B (WT, *ATP16a*, *ATP16b*); second (2), W30 (*atp16a::HIS3*, *ATP16b*); third (3), W202 (*ATP16a*, *atp16b::HIS3*); bottom (4), W19 (*atp16a::HIS3*, *atp16b::HIS3*).

In each dish at left (+) are the wild-type *ATP16* (YDL004w) gene with multi-copy plasmid (pYEATP16) added to the laboratory strain and the *ATP16* disruptants. The dishes at right (-) are without the added wild-type *ATP16* (YDL004w) gene (only the control vector, pAML20 was added).

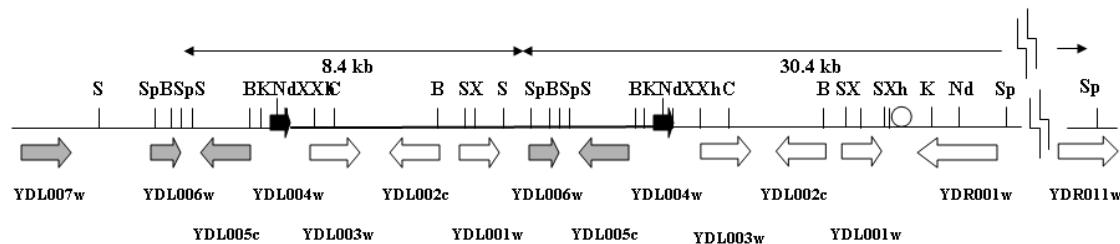
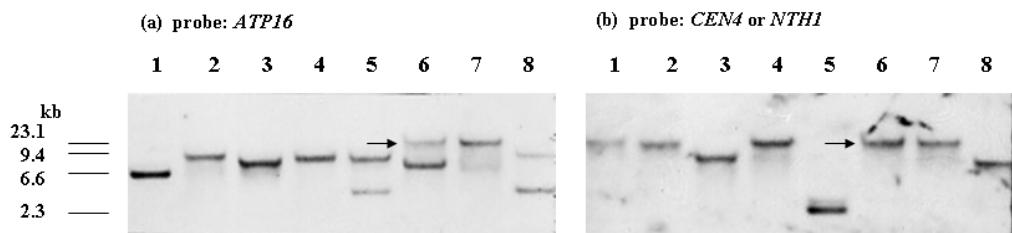


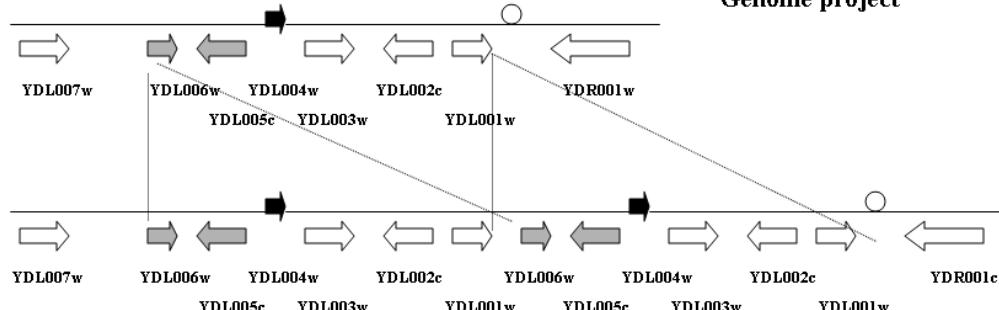
Figure 4. Analyses of DNA region between *ATP16a* and *ATP16b*

The total (genomic) DNAs from laboratory strains W303-1A, W303-1B, or S288C were isolated and digested with different restriction enzymes and applied to a 0.8% agarose gel. After electrophoresis, the digestive DNA fragments were blotted onto a nylon membrane and then hybridized with DIG-labeled *ATP16* (a) and *CEN4* and *NTH1*. Lane 1, *BamHI*; lane 2, *Clal*; lane 3, *KpnI*; lane 4, *XhoI*; lane 5, *NdeI*; lane 6, *SphI*; lane 7, *SphI/NcoI*; lane 8, *XbaI*. Arrow indicated the 30.4 kb fragment.

B, *BamHI*; C, *Clal*; K, *KpnI*; N, *NcoI*; Nd, *NdeI*; S, *Scal*; Sp, *SphI*; X, *Xba*; Xh *XhoI*.

Chromosome IV, left arm.

Genome project



This study

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○ :*CEN4*

Figure 5. Map of two *ATP16s* and neighboring ORFs

ORFs on chromosome IV were taken as systematic designations in the *S.cerevisiae* genome.

Black arrow, *ATP16* (YDL004w); gray arrow, upstream ORFs (YDL006w and YDL005w); white arrow, downstream ORFs (YDL003w, YDL002c, and YDL001w) of *ATP16* (YDL004w, mips, <http://mips.gsf.de/>).

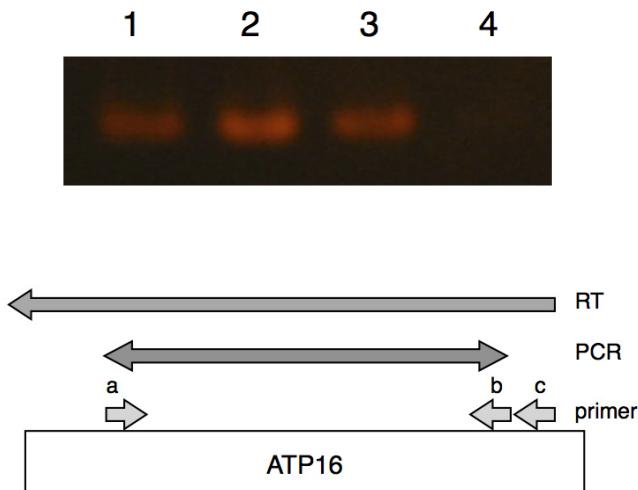


Figure 6. Transcripts of *ATP16a* and *ATP16b*

The RT-PCR experiments were carried out to see the transcripts of both *ATP16a* and *ATP16b* using the sense (primer a) and anti-sense primers (primers b or c) in the lower panel. The procedures of the RT-PCR experiments were described in Materials and Methods. The upper panel was shown the products of the RT-PCR. Lane 1, W303-1B (WT, *ATP16a*, *ATP16b*); lane 2, W30 (*atp16a::HIS3*, *ATP16b*); lane 3, W202 (*ATP16a*, *atp16b::HIS3*); lane 4, W19 (*atp16a::HIS3*, *atp16b::HIS3*).