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Shrimp ATP synthase genes complement yeast null mutants for ATP hydrolysis but not synthesis activity

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Abstract

The aim of this study was to examine the feasibility of employing a yeast functional complementation assay for shrimp genes by using the shrimp mitochondrial F₁F₀-ATP synthase enzyme complex as a model. Yeast mutants defective in this complex are typically respiratory-deficient and cannot grow on non-fermentable carbon sources such as glycerol, allowing easy verification of functional complementation by yeast growth on media with them as the only carbon source. We cloned the previous published sequence of *ATP2* (coding for ATP synthase β subunit) from the Pacific white shrimp *Penaeus vannamei* (Pv) and also successfully amplified a novel *PvATP3* (coding for the ATP synthase γ subunit). Analysis of the putative amino acid sequence of *PvATP3* revealed a significant homology with the ATP synthase γ subunit of crustaceans and insects. Complementation assays were performed using full-length *ATP2* and *ATP3* as well as a chimeric form of *ATP2* containing a leader peptide sequence from yeast and a mature sequence from shrimp. However, the shrimp genes were unable to complement the growth of respective yeast mutants on glycerol medium, even though transcriptional expression of the shrimp genes from plasmid-borne constructs in the transformed yeast cells was confirmed by RT-PCR. Interestingly, both *PvATP2* and *PvATP3* suppressed the lethality of the yeast F₁ mutants after the elimination of mtDNA, which suggests the assembly of a functional F₁ complex necessary for the maintenance of membrane potential in the ρ^0 state. These data suggest an incompatibility of the shrimp/yeast chimeric F₁-ATPase with the stalk and probably also the F₀ sectors of the ATP synthase, which is essential for coupled energy transduction and ATP synthesis.

Keywords

ATPase; ATP synthase; complementation; shrimp; yeast; mitochondria

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Footnote: The GenBank accession no. for *Penaeus vannamei* ATP synthase gamma subunit (*PvATP3*) is [JN977138](#).

Introduction

F₁F₀-ATP synthases are multisubunit enzymes which are found in the plasma membranes of bacteria, thylakoid membranes of chloroplasts and the inner membranes of mitochondria. They utilize the energy of a transmembrane proton-motive force for the synthesis of ATP from ADP and inorganic phosphate [1]. The F₁F₀-ATP synthase complex is formed by two major structural domains consisting of an F₁ portion that performs the ATP synthesis and hydrolysis reactions and an F₀ portion that mediates the proton transport (for reviews, see [2],[3],[4],[5]). A peripheral stalk is also required for connecting F₁ and F₀ to facilitate energy transduction. F₁ is composed of five subunits with the stoichiometry $\alpha_3\beta_3\gamma\delta\epsilon$ while F₀ is composed of ab_2c_{10-15} subunits, where the number of c subunits varies according to species [5]. Moreover, additional protein components d, F₆ (or h) and OSCP of F₀ have been reported in eukaryotes. The yeast F₁F₀-ATP synthase complex has been well studied and is used widely as a model to study the molecular and cellular aspects of this enzyme complex in other organisms. Null mutations of either F₁ or F₀ in the ATP synthase complex impair mitochondrial oxidative phosphorylation, resulting in slow growth or lack of growth on non-fermentable carbon sources such as glycerol. Thus, this phenotype can be used in functional complementation assays with homologous proteins from other organisms. For example, bovine α -, β -, γ -, and ϵ -subunits are able to complement growth on glycerol medium of corresponding yeast null mutants [6]. In addition to reports on nucleotide sequences encoding protein subunits of the F₁F₀-ATP synthase complex in yeast (e.g., “ATP synthase” at <http://www.yeastgenome.org> for *Saccharomyces cerevisiae*), there are reports for the complex in the cow (e.g., <http://genomes.arc.georgetown.edu/drupal/bovine/> for *Bos Taurus*) and *Escherichia coli* (e.g., <http://ecogene.org>). However, there are no reports for the sequences of genes in the complete shrimp F₁F₀-ATP synthase complex. Only 4 have been reported in the Pacific white shrimp *Penaeus vannamei*. These are *ATP1* (α subunit), *ATP2* (β subunit), *ATP6* (6 or a subunit), and *ATP9* (9 or c subunit) [7],[8]. Here, we reported a novel cDNA sequence encoding an *ATP3* gene (γ subunit) from *P. vannamei* and described the first attempt to use a yeast functional complementation assay for shrimp ATPase genes.

Materials and methods

Primer design

Primers were designed to amplify mitochondrial F₁-ATPase genes (*ATP2* and *ATP3*) from yeast *S. cerevisiae* (Sc) and the Pacific white shrimp *P. vannamei* (Pv). For *ScATP2*, *ScATP3*, and *PvATP2* genes, primers were designed based on GenBank database accession no. **U46215**, **U09305**, and **EU401720**, respectively. For *PvATP3* gene, primers were designed via a BLASTN search using a reported sequence of *Drosophila melanogaster ATP3* (**NM_079826**) as a query against NCBI in the EST-others database and Marine Genomics database (<http://www.marinegenomics.org/>). Table 1 summarizes primers used in this study.

Yeast strains and media

Saccharomyces cerevisiae strains shown in Table 2 were derived from the same isogenic background. All strains were grown at 30°C. Complete medium called YPD contained 0.5% Bacto yeast extract, 1% Bacto peptone and 2% glucose while YPGly medium contained 2%

glycerol in place of glucose. EB medium is YPD plus ethidium bromide at 25 µg/ml. Yeast synthetic drop-out medium supplement without uracil (-Ura) was purchased from BD Biosciences.

DNA isolation and cloning of yeast ATPase genes

Total DNA was extracted from *S. cerevisiae* strain M2915-6A using salting out/phenol/chloroform method [9]. The resulting DNA was resuspended in 1X TE buffer, pH 7.4. DNA concentration and quality were measured by spectrophotometric analysis at 260 and 280 nm. PCR reactions were carried out in a 50 µl reaction solution containing 100 ng of DNA template, 0.25 µM of each forward and reverse primer, 5 units of *Taq* polymerase (Invitrogen), 1X reaction buffer, 0.5 mM of MgCl₂ and 0.25 mM of dNTP. PCR was performed using the following protocol: 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min with a final extension at 72°C for 5 min. PCR products were analyzed on 1% agarose gels.

RNA isolation and cloning of shrimp ATPase genes

Hemocytes from the Pacific white shrimp *P. vannamei* were collected and subjected to total RNA extraction using Trizol reagent (Invitrogen). The quantity and quality of RNA samples were measured by spectrophotometry. RT-PCR reactions are carried out in a 25 µl reaction solution containing 100 ng of total RNA, 0.2 µM of each forward and reverse primer, 0.5 µl of SuperScript One-Step RT/Platinum *Taq* mix (Invitrogen) and 1X reaction buffer. The RT-PCR protocol comprised incubation at 50°C for 30 min and 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min with a final extension at 72°C for 5 min.

Construction of recombinant plasmids

PCR products of yeast genes (*ScATP2* and *ScATP3*) and RT-PCR products of shrimp genes (*PvATP2* and *PvATP3*) were gel purified and cloned into pDrive using a cloning kit (Qiagen). After sequence verification, the inserted amplicon which is flanked by *EcoRI* restriction sites was released from the cloning vector by *EcoRI* digestion. The high copy number plasmid pCXJ27 containing the promoter and terminator of the yeast gene encoding the glycolytic enzyme phosphoglycerate kinase (PGK) was employed for cloning (Table 2). The plasmid was digested with *EcoRI* and treated with alkaline phosphatase to prevent self-ligation prior to ligation. Correct orientation of inserted fragments of each recombinant plasmid was verified by colony PCR using combinations of a vector primer and a gene specific primer (Table 1) prior to DNA sequencing. A chimeric *ATP2* gene was constructed to carry a putative leader peptide sequence of *ScATP2* attached to a mature sequence of *PvATP2*. This was done by homologous recombination in yeast cells. Firstly two individual PCR reactions were performed using *ScATP2* and *PvATP2* (constructed in pCXJ27 as mentioned above) as templates. The former reaction employed primers pCXJ27_seq_F (specific to PGK promoter of the plasmid) and *ScATP2*-signal-R (target to 3' end of the leader peptide sequence of *ScATP2*) (see primer list in Table 1). The latter PCR reaction used primers *PvATP2*-mature-F (specific to mature sequence of *PvATP2* and containing an extended 5'-overhang for homologous recombination cloning), and pCXJ27_seq_R (targeted to PGK terminator of the plasmid). Respective amplified products of 196 bp and

1576 bp were transformed together with *Eco*RI-digested pCXJ27 into *S.cerevisiae* YK9 strain [10] and transformant colonies were selected on -Ura plates. Recombinant plasmids were rescued into *E.coli* XL-1Blue and correct chimeric sequence and orientation were confirmed by sequencing.

Functional assay by complementation test

Competent yeast cells were prepared from *atp2* (CY1433) and *atp3* (CY1434) mutant strains (Table 2). Recombinant pCXJ27-based plasmids were transformed into respective yeast strains *i.e.* *ScATP2*, *PvATP2*, and chimeric *ATP2* recombinant plasmids into *atp2*, while *ScATP3* and *PvATP3* were transformed into *atp3*. Yeast transformation was performed using the lithium acetate-dimethyl sulfoxide method [11], and transformed cells were spread on -Ura plates incubated at 30°C for 3–5 days. The resulting transformants, namely *atp2*[*ScATP2*], *atp2*[*PvATP2*], *atp2*[Chimeric *ATP2*], *atp3*[*ScATP3*], and *atp3*[*PvATP3*] were subjected to complementation assays. Cultures of yeast transformants were 10-fold serially diluted and spotted onto YPD, YPGly, EB, and -Ura plates and incubated at 30°C for 4–7 days. Wild-type (WT) and mutant strains were also included as controls. Functional complementation was indicated by the growth on YPGly and EB media while the presence of the pCXJ27-based plasmids was verified by growth on plates containing -Ura medium.

Verification of gene expression in yeast cells

To verify whether a plasmid-borne shrimp *ATP2* could be expressed in yeast cells, transcriptional expression was monitored by RT-PCR. RNA was extracted from *atp2*[*PvATP2*] and *atp2*[Chimeric *ATP2*] transformed yeast cells as well as WT and *atp2* mutant strains as controls. Cell pellets from 2×10^6 cells were subjected to RNA extraction using Trizol reagent (Invitrogen) and the RNA obtained was treated with DNase I to eliminate contaminating DNA. RT-PCR reactions were performed as described above except that ATP2-express-F and ATP2-express-R primers (Table 1) were used.

DNA sequence analysis

Recombinant plasmids were sequenced by 1st BASE Malaysia. DNA and protein analysis were carried out using the EXPASY web server (<http://au.expasy.org/>). To identify related sequences, a protein-protein basic local alignment search tool (blastp) search was carried out using the NCBI protein database (<http://www.ncbi.nlm.nih.gov/BLAST/>). Alignments of protein sequences were performed using ClustalW (<http://www.ebi.ac.uk/clustalw/>) and a phylogenetic tree was generated using MEGA version 4 [12]. An analysis for protein domains was carried out using Conserved Domain Database software [13].

Results and discussion

Isolation of a novel *ATP3* cDNA sequence in shrimp

We chose *ATP2* and *ATP3* genes that encode mitochondrial ATPase β and γ subunits respectively. In shrimp the complete sequence of *ATP2* has been published [14], [8] but not that of *ATP3*. We therefore employed a BLAST search tool using *ATP3* gene from *Drosophila melanogaster* (accession number **NM_079826**) against the NCBI and Marine

Genomics databases. Several *P. vannamei* EST sequences gave hits for the query but none contained the complete coding sequences. Thus, we selected two overlapping sequences (GeneBank accession numbers **FE153680** and **FE191871**, corresponding to accession numbers **MGID432560** and **MGID426357**, respectively, in the Marine Genomics database). The assembled sequence appeared to cover the complete *ATP3* ORF of *P. vannamei*. Primers were then designed (Table 1) and used to amplify the full-length sequence of *PvATP3* from shrimp hemocyte RNA. The sequence obtained was deposited in GenBank (accession number **JN977138**). The entire 870 bp-open reading frame encoded a novel deduced protein of 289 amino acids with a predicted molecular weight of 31.24 kDa and an isoelectric point of 9.04. A BLASTP homology search [15] revealed high similarity to putative or annotated ATP synthase γ subunit sequences of various animals such as insects, crustaceans, and vertebrates. In addition, a conserved domain characteristic of the ATP synthase γ subunit was detected by a Conserved Domain Database [16]. For sequence comparison to *PvATP3* by ClustalW alignment, we selected full-length ATP3 protein sequences from the first 27 distinct species (Supplemental Table 1) giving high similarities (E-values between 7e-132 and 1e-119) in the BLAST search. ATP synthase γ subunit sequences from budding (*Saccharomyces cerevisiae*) and fission (*Schizosaccharomyces pombe*) yeasts were also included in the comparison. A phylogenetic tree was constructed by Molecular Evolutionary Genetics Analysis (MEGA 4) software. The automatically generated tree revealed that *PvATP3* grouped in a clade that included ATP3 of other crustaceans and was distinct from the clades including vertebrates and yeasts (Fig. 1). Since we planned to perform the functional complementation test in yeast, we made detailed comparison of the published *PvATP2* and our newly identified *PvATP3* sequences to those of the homologues in the yeast *S. cerevisiae*. This revealed that homology between the shrimp and yeast proteins was 73% for ATP2 and 36% for ATP3, in terms of amino acid sequence identity (Table 3, Supplemental Figs. S1 and S2).

Construction of chimeric ATP2 gene

For functional complementation assays, we used the native full-length sequences of ATPase genes together with a constructed chimeric sequence containing a leader peptide of yeast *ATP2* gene followed by the mature sequence of shrimp *ATP2*. This was to exclude the possibility of mitochondrial mislocation of shrimp ATPase genes in yeast cells. Fig. 2a illustrates the N-terminal sequences of native yeast and shrimp ATP2 and the chimeric form of ATP2. Although leader peptide cleavage sites were not detected from yeast or shrimp ATP2 by InterPro [17] or SMART [18], a cleavage position for yeast ATP2 had previously been published [6]. That report also constructed a chimeric form carrying a leader peptide from yeast and a mature sequence of bovine ATP2. Therefore, a cleavage site of ⁴³Y↓AAKA⁴⁷ in *PvATP2* was determined similar to ⁴⁶Y↓AAQA⁵⁰ in bovine ATP2 [6]. Fig. 2b summarizes the procedure for constructing the chimeric *ATP2* sequence by homologous recombination. Two overlapping amplified amplicons were correctly assembled and joined to *EcoRI*-linearized pCXJ27 plasmid and verified by DNA sequencing (data not shown).

Functional assay by complementation test

Shrimp *PvATP2*, *PvATP3*, and chimeric *ATP2* constructed in pCXJ27 plasmid were transformed into respective yeast mutants. In addition, plasmid constructs containing wild-type yeast *ScATP2* and *ScATP3* were used as controls. The obtained transformants together with wild-type (WT) and mutant strains were subjected to complementation assays. The results (Fig. 3) revealed that all of the tested yeast cells grew relatively well on YPD medium while respiratory-deficient *atp2* and *atp3* mutants did not grow on YPGly containing glycerol as the only carbon source (non-fermentable). Only WT and *atp2*[*ScATP2*] transformants could grow on YPGly plates. The growth on YPGly of *atp2*[*ScATP2*] transformants was a typical result for functional complementation of respiratory-deficiency. However, the transformants *atp2*[*PvATP2*], *atp3*[*PvATP3*], and *atp2*[Chimeric *ATP2*] were not able to grow on YPGly, indicating that shrimp *PvATP2* and *PvATP3* were not able to functionally complement the *atp2* and *atp3* mutations, regardless of the presence of a yeast leader peptide sequence in the chimeric construct. The presence of plasmids in transformed yeast cells was indicated by growth on -Ura plates (Fig. 3). The complementation phenotype of growth on YPGly was not observed for *atp3*[*ScATP3*] transformants. The failure of complementation by *ScATP3* confirmed previous observations that the *atp3* mutants have unstable mtDNA [19], [6]. The lack of a conditional *atp3* mutant precluded the possibility of a direct complementation test in this regard. However, the shrimp *ATP3* clearly complemented the ATP hydrolysis as described below.

DNA-targeting agents such as ethidium bromide (EB) can deplete mitochondrial DNA (mtDNA) from living cells (see review in [20]). *S.cerevisiae* forms petite colonies when mtDNA is lost and cells without mtDNA are referred to as ρ^0 . Viability of ρ^0 strains on glucose medium has been proposed to be mainly due to sufficient mitochondrial membrane potential (Ψ) maintained by electrogenic exchange of cytosolic ATP^{4-} against matrix ADP^{3-} generated by the $\text{F}_1\text{-ATPase}$ [21], [22]. Thus, we examined the growth phenotype of yeast strains on EB glucose medium (i.e., in the absence of mtDNA) to determine whether or not shrimp proteins generated by tested plasmids resulted in a functional F_1 as distinct from the whole functional F_1F_0 -ATP synthase complex.

The growth results on EB shown in Fig. 3 suggested that a shrimp/yeast chimeric F_1 -complex is formed in the transformed cells. EB-treated *S. cerevisiae* WT (i.e. EB-converted to ρ^0) is petite-positive while *atp2* mutant cells (no F_1 β subunit) are petite-negative and cannot grow upon loss of mtDNA, as previously reported [23]. However, when transformed with the plasmid expressing *ScATP2*, growth of the *atp2* mutant on EB was restored and it became petite-positive indicating a functional F_1 complex but not a functional F_1F_0 complex (Fig. 3). Interestingly, growth of *atp2* mutant cells on EB was also improved and partially improved with plasmids containing shrimp *ATP2* (*PvATP2*) and chimeric *ATP2* (yeast leader peptide + mature *PvATP2*), respectively (Fig. 3). *atp3* mutant strain that grew slowly on EB medium also gave better growth when transformed with *ScATP3* and *PvATP3* plasmid constructs. EB plates photographed on day 4 (not shown) showed similar growth phenotypes to plates photographed on day 7 (Fig. 3), although the latter showed somewhat more pronounced growth. It has been proposed that in the absence of mtDNA, the essential mitochondrial membrane potential Ψ is maintained by electrogenic exchange between

cytoplasmic ATP⁴⁻ (generated by glycolysis) and mitochondrial ADP³⁻ produced by F₁-ATPase via ATP hydrolysis in the matrix [21], [22]. Thus, our results indicated that *PvATP2*, *PvATP3*, and chimeric ATP2 were implicated in the function of ATP hydrolysis by F₁-ATPase in generating sufficient Ψ for viability of the corresponding yeast null mutants. The findings also implied that the shrimp genes in the plasmid constructs could be expressed and correctly targeted to mitochondria and that the tested genes and especially the newly identified *PvATP3* gene are truly functional genes.

Many bovine subunit (α , β , γ , ϵ , δ , h) and a rat OSCP (oligomycin sensitivity conferring protein) components of F₁F₀-ATPase complex have been functionally tested using the yeast complementation assay [24], [6], [25], [26]. It was shown that α , β , γ , ϵ , F₆ and OSCP complemented the respective corresponding mutations in yeast for genes *ATP1*, *ATP2*, *ATP3*, *ATP15*, *ATP14*, and *ATP5*. However, the bovine δ subunit was unable to complement the corresponding yeast *ATP16* mutation. Among the protein subunits of mitochondrial F₁F₀-ATPase, α and β subunits are highly conserved. For example, yeast and bovine α and β subunits share 70 and 74% sequence identity, respectively. Nevertheless, functional complementation was not necessarily successful simply due to high sequence homology. For example, bovine F₆ which shared a very low sequence identity of only 14.5% to its yeast counterpart (ATP14 protein) was still able to functionally replace the yeast subunit [25], while expression of the bovine δ subunit was unable to complement the corresponding yeast mutation of ATP16, even though they shared a higher identity of 30% [6]. This suggested that position-specific amino acid composition was more important in accounting for functionally folded protein structure and topology and was more likely to determine the success of specific interactions in the enzyme complex. Two genes (*PvATP2* and *PvATP3*) chosen in this study encode putative β and γ subunits that are protein components of the F₁ sector. A hexamer of alternating α and β subunits serves as catalytic reaction center for ATP hydrolysis and synthesis [27],[28] while the γ subunit is a central component inside the $\alpha_3\beta_3$ hexamer, essential for enzyme complex cooperativity [2], [29]. In addition to subunit interactions in the F₁ sector, there is also a physical connection between F₁ and the membrane embedded F₀ unit. The α and β subunits are also in contact with the stalk sector that links F₁ with F₀. Since there are many contacts and interactions among the protein components, incompatibility of heterologous proteins with the native components might lead to defects in integrity of the enzyme complex. In summary, our results indicated that shrimp ATP synthases were more divergent from yeast than those of the cow. Although a functionally active chimeric F₁ complex could be assembled between subunits from yeast and shrimp, it did not result in normal energy transduction and ATP synthesis probably due to its failure in essential interaction with the stalk and/or F₀ sectors.

Expression of shrimp ATP2 in yeast cells

Transcriptional expression of a plasmid-borne shrimp *ATP2* in transformed yeast cells was verified by RT-PCR. Since the nucleotide sequences encoding *ATP2* in yeast and shrimp share relatively high identity (69%)(Table 3, Supplemental Fig. S1), a pair of degenerate primers (Table 1) could be designed to target *ATP2* sequences from both species. The expected amplified band from yeast *ATP2* was 593 bp while that of shrimp was 596 bp. Fig. 4 shows that the expected product was obtained from yeast WT RNA but that no product

was obtained from the *atp2* mutant lacking the endogenous *ATP2* gene. However, the expected amplified products of 596 bp was obtained from *atp2* mutant cells transformed with *PvATP2* and the chimeric *ATP2* plasmid construct, confirming transcriptional expression of shrimp *ATP2* in yeast cells. Taken together, the results indicated that expression of shrimp genes led to the assembly of a functionally active F_1 -ATPase but at least in the case of *PvATP2* it did not support respiratory growth, probably due to a defective interaction with the stalk or F_0 sectors. Interestingly, a recent publication has revealed an increase in the ATP hydrolyzing activity in muscle mitochondria of shrimp under hypoxia condition [30]. These data raise an intriguing question as to whether native shrimp F_1 has evolved a robust ATP hydrolyzing activity independent of F_0 , capable of maintaining membrane potential and providing some capacity for shrimp to survive under low oxygen conditions (partial hypoxia), as opposed to survival under complete hypoxia that has evolved in the petite-positive yeast. Future studies would be required to address this question

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Phylogenetic tree showing the relationship among ATP3 from various organisms. See Supplemental Table 1 for keys to protein sequences from the GenBank database.

Fig. 2.

Construction of chimeric ATP2. **(a)** N-terminal sequences of yeast *ScATP2*, shrimp *PvATP2*, and a chimeric form containing a leader peptide sequence from yeast and a mature sequence from shrimp ATP2. Putative leader peptide sequences are marked by dashed boxes. The sequence derived from yeast is shown by gray shading while that of shrimp is indicated by white. **(b)** Chimeric ATP2 was constructed by homologous recombination. A fragment containing a potential leader peptide of *ScATP2* was amplified by primers 1 and 2 which are pCXJ27_seq_F and *ScATP2*-signal-R, respectively. A mature *PvATP2* sequence was amplified by primers 3 and 4 which are *PvATP2*-mature-F and pCXJ27_seq_R, respectively. Two amplified fragments were then transformed together with *Eco*RI-digested pCXJ27 vector into yeasts as detailed in the materials and methods.

Fig. 3. Functional assay of shrimp *ATP* genes by complementation test in yeast cells. Cultures of wild type (WT), mutants (*atp2* and *atp3*), and transformed yeast cells (*atp2*[*PvATP2*], *atp3*[*PvATP3*], *atp2*[Chimeric *ATP2*], *atp2*[*ScATP2*], and *atp3*[*ScATP3*]) were serially diluted and dropped onto indicated plates. Plates were incubated at 30°C for 4 days with YPD, YPGly and -Ura and 7 days with EB before the photograph was taken.

Fig. 4. RT-PCR assay of *ATP2* transcripts from yeast cells. Total RNAs extracted from wild-type (WT), mutant (*atp2*), and transformed yeast cells (*atp2*[*PvATP2*], and *atp2*[Chimeric *ATP2*]) were subjected to RT-PCR using degenerate primers specific to both yeast and shrimp *ATP2*. Arrow marks expected band products. M, 2-log DNA ladder (New England Biolabs).

Table 1

Primers used in this study.

Names	Sequences (5' to 3')	Remarks
Pv2_F	GGGGTACCATGTTGGGAGCTGCACAGCG	Full-length amplification & Cloning purpose
Pv2_R	CCGCTCGAGTTAGCTGGCCTGGGCAGCCA	
Pv3_F	GGGGTACCATGTTCAAGCCGGGCGACTCT	
Pv3_R	CCGCTCGAGTTAGACAGCAGCAGCACCAG	
Sc2_F	CCATCGATATGGTTTTGCCAAGACTATA	
Sc2_R	GGAATTCCCTAGTTGGCTTCAGCGGCTAA	
Sc3_F	CCATCGATATGTTGTCAAGAATTGTATC	
Sc3_R	GGAATTCTCATCCCAAAGAGGAAGCACC	
ScATP2-signal-R	CATACATCTTTTCCACGAAGT	Chimeric ATP2 construction
PvATP2-mature-F	<i>GCTCCGCTTCTATCCACTTCGTGGAAAAG ATGTATGGCTGCCAAGGCTGAGGCTG</i>	
pCXJ27_seq_F	CAAGTTCTTAGATGCTTTC	Vector primers
pCXJ27_seq_R	TCGGATAAGAAAGCAACAC	
ATP2-express-F	AAGATTGGTCTKTTTCGGTGGTG, K= G or T	Transcription expression
ATP2-express-R	TCCACWGCAGGGTAAATACCTAA, W=A or T	

Underlined sequences indicate restriction sites.

Italic represents extended 5'-overhang sequence.

Table 2

S.cerevisiae strains and plasmids used in this study.

Yeast	Description	Source
M2915-6A	<i>MATa, ade2, ura3, leu2</i>	[23]
CY1433	<i>MATa, leu2, ade2, ura3, atp2::KAN</i>	Chen, unpublished
CY1434	<i>MATa, leu2, ade2, ura3, atp3::KAN</i>	Chen, unpublished
Plasmid		
pCXJ27	Expression vector containing PGK promoter and terminator, and a URA3 auxotrophic marker	Chen, unpublished

Table 3

Sequence comparison of ATPase sequences of yeast and shrimp.

Sequence	Organism	Accession no.	Size of coding sequence (bp/aa)	% identity	
				Nucleotide sequence	Amino acid sequence
ATP2	<i>S. cerevisiae</i>	U46215	1536/511	69%	73%
	<i>P. vannamei</i>	EU401720	1578/525		
ATP3	<i>S. cerevisiae</i>	U09305	936/311	56%	36%
	<i>P. vannamei</i>	JN977138	870/289		

bp, basepairs

aa, amino acids