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ASC1/RAS2 Suppresses the Growth Defect on Glycerol Caused by the *atp1–2* Mutation in the Yeast *Saccharomyces cerevisiae**

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To better define the regulatory role of the F_1-ATPase α-subunit in the catalytic cycle of the ATP synthase complex, we isolated suppressors of mutations occurring in *ATP1*, the gene for the α-subunit in *Saccharomyces cerevisiae*. First, two *atp1* mutations (*atp1–1* and *atp1–2*) were characterized that prevent the growth of yeast on non-fermentable carbon sources. Both mutants contained full-length $F_1\alpha$-subunit proteins in mitochondria, but in lower amounts than that in the parental strain. Both mutants exhibited barely measurable F_1-ATPase activity. The primary mutations in *atp1–1* and *atp1–2* were identified as $Thr^{383} \rightarrow Ile$ and $Gly^{291} \rightarrow Asp$, respectively. From recent structural data, position 383 lies within the catalytic site. Position 291 is located near the region affecting subunit-subunit interaction with the $F_1\beta$-subunit. An unlinked suppressor gene, *ASC1* (α-subunit complementing) of the *atp1–2* mutation ($Gly^{291} \rightarrow Asp$) restored the growth defect phenotype on glycerol, but did not suppress either *atp1–1* or the deletion mutant Δ*atp1*. Sequence analysis revealed that *ASC1* was allelic with *RAS2*, a G-protein growth regulator. The introduction of *ASC1/RAS2* into the *atp1–2* mutant increased the F_1-ATPase enzyme activity in this mutant when the transformant was grown on glycerol. The possible mechanisms of *ASC1/RAS2* suppression of *atp1–2* are discussed; we suggest that *RAS2* is part of the regulatory circuit involved in the control of F_1-ATPase subunit levels in mitochondria.

Mitochondrial ATP synthase functions as a key enzyme for ATP production in eukaryotic cells (1). The enzyme is controlled in response to the energy demands of the cell (2). Although considerable attention has been given to the central role of mitochondrial ATP production in the initiation of programmed cell death (apoptosis), little is known about the regulation of ATP synthase during its biogenesis and energy transduction or its links to growth regulatory pathways (3). The enzyme complex is composed of the F_1-ATPase (catalytic sector) and the transmembrane F_0 proton channel (embedded in inner membrane) (4–6). Both F_1 and F_0 are necessary for ATP synthase activity, whereas F_1 alone retains the ability to hydrolyze ATP (F_1-ATPase) (7). The F_1-ATPase consists of five different subunits: α, β, γ, δ, and ϵ. The minimum unit for F_1-ATPase activity resides on α-β-subunit dimer (8). The catalytic center is considered to be in the β-subunit (9, 10), and the α-subunit has been reported to play a role in the formation of the catalytic site with it (11). The α-subunit also assists the assembly of other subunits of the F_1-ATPase (12) by acting as a chaperone to assist assembly (13). In yeast, all but three F_0 subunits of the enzyme are encoded on nuclear DNA. In order to examine the control mechanism(s) of the $F_1\alpha$-subunit in the complex assembly and function, we characterized several mutants (14) and isolated extragenic suppressors of mutations in the *ATP1* gene. One extragenic suppressor for the point mutant, *atp1–2*, was *RAS2*, a well known regulator of cell proliferation and signal transduction (15–17). This work reveals for the first time that the cellular growth regulatory activity of *RAS2* is linked in some manner with the biogenesis or function of mitochondrial enzyme complexes.

EXPERIMENTAL PROCEDURES

Yeast Strains—Saccharomyces cerevisiae DC5 (*MAT*a *leu2–3 leu2–112 his3 can1–11*) (18) was used as a wild-type strain. Nuclear petite mutants XJY11 (*MAT*a *leu2–3 leu2–112 his3 atp1–2 can1–11*) and XJY12 (*MATα leu2–3 leu2–112 atp1–1 can1–11*) have been reported previously (18). The mutant allele, *atp1–2* (12), is the same as the *N9–84* mutation reported previously (14, 18). SKY4010 (*MAT*a *leu2–3 leu2–112 his3* Δ*atp1*::*HIS3 can1–11*) was constructed by disruption of the *ATP1* gene in DC5 with a 4.2-kb[1] *Eco*RI-*Sph*I fragment containing *atp1*::*HIS3* allele.

Escherichia coli Strains—MC1066 (F⁻ Δ(*lac*IPOZY) *X74 galU galK rpsL hsdR trpC9830 leuB600 pyrF74*::*Tn5*) was used for the preparation of plasmids for yeast genetics. One Shot INVαF' (Invitrogen, San Diego, CA) and XL1-BLUE MRF' KAN (Stratagene, La Jolla, CA) were used for the sequencing.

Media—E. coli-carrying plasmids were grown in LB (0.5% yeast extract, 1% bactotryptone, 1% NaCl) plus 50 or 125 μg of ampicillin/ml. Yeast strains were grown on YPG (1% yeast extract, 2% bactopeptone, 3% glycerol), YPGE (1% yeast extract, 2% bactopeptone, 3% glycerol, 3% ethanol), YPDM (0.5% yeast extract, 0.5% bactopeptone, 0.1% $(NH_4)_2SO_4$, 0.2% KH_2PO_4, 0.1% $MgSO_4$, 0.8% glucose), or SD (0.67% yeast nitrogen base without amino acids, 2% glucose, and appropriate nutrients). Solid medium contained 2% agar.

Gene Library—The *Sau*3AI pool of yeast genomic DNA from DC5 was cloned into the *Bam*HI site of a vector YEp13 for the construction of yeast genomic library (19).

Plasmids—A plasmid pYCL12–5 (YCp type) was constructed as follows; approximately 2.9 kb of *Eco*RI-*Sph*I fragment having *ATP1* with its 5′- and 3′-flanking regions was cloned into a derivative of YCp50 in

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The nucleotide sequence reported in this paper has been submitted to the DDBJ/GenBank™/EBI Data Bank with accession numbers D37949 (atp1-2) and D88458 (atp1-1).

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[1] The abbreviations used are: kb, kilobase pair(s); PCR, polymerase chain reaction.

which the *URA3* was disrupted by *LEU2*. pJ12–5 consists of the episomal plasmid YEp13 containing *ATP1* as described previously (18). Plasmids pOSBH20 (centromere type) and pMTYBH20 (episomal type) were constructed as follows; a 1.6-kb *Xba*I-*Nde*I fragment carrying *ASC1* with its 5′- and 3′-flanking regions was cloned into pOS31 (YCp type vector, *LEU2*, *CEN3*, *ARS1*, *TRP1*, *bla*) or into YEp13, respectively. The plasmid pYEatp1–2 (YEp type) was constructed as follows; a *Sac*I-*Hin*dIII fragment of *ATP1* region of pJD12–5 was replaced with the same fragment from the *atp1–2* mutant.

PCR—PCR was performed according to the procedure for Gene Amp DNA amplification reagent kit (Perkin-Elmer). The PCR primer pairs used are: 1 (5′-CGCAAGAACAGTAACAAAAT-3′) and 2 (5′-ATCACCAATAATCAACTCTC-3′), 3 (5′-CGGTAGAGGTCAAAGAGAGT-3′) and 4 (5′-TCAATAACAGGCAAAGCAGT-3′), and 5 (5′-TGGTGATGTCTTTTACTTGC-3′ and 6 (5′-GATTTTCAGGTTATTGTTTG-3′). The primers 1 and 6 are located just outside *ATP1* coding region. DNA was amplified in a PCR processor (Perkin-Elmer type 480) by using 30 cycles. Yeast whole DNA as templates were prepared by the previously reported method (20). PCR products were cloned into a vector pCRII (Invitrogen, San Diego, CA) or pCR-Script (Stratagene, La Jolla, CA) for sequencing.

DNA Sequencing—Nucleotide sequencing was performed by dideoxy chain termination method (21), and autoradiograms were obtained according to the method previously reported (22). In the process of sequencing, we found misreading in the *ATP1* sequence previously reported (18). We re-sequenced the wild-type *ATP1* by using a genomic clone and used corrected sequence for comparison. The nucleotide sequences described in this article are available from the DDBJ/EBI/GenBank™ nucleotide sequence data bases under accession numbers D37948 (*ATP1*), D37949 (*atp1–2*), D88458 (*atp1–1*), and D37950 (*ASC1/RAS2*).

Computer Analysis—Homology searches were calculated by BLAST (basic local alignment search tool) (23) against all data compiled in the NCBI data base (supplied October 10, 1997 by NCBI).

Complementation Tests—Growth on a non-fermentable carbon source, glycerol, was examined by incubation on YPGE medium at 30 °C for 3–4 days.

Preparation of Mitochondria—Cells were grown in 50 ml of YPDM medium. After 24 h of incubation at 30 °C, cells (~2–4 × 10^7 cells/ml) were harvested and mitochondria were prepared according to the method reported previously (24).

Determination of F_1-ATPase Activity—F_1-ATPase activity was measured by minor modification of the method of Pullman *et al.* (25) by following the decrease of the absorbency at 340 nm. The assay mixture consisted of 50 μmol of Tris acetate (pH 7.4), 1 μmol of $MgCl_2$, 0.02 μmol of NADH, 2 μmol of phosphoenolpyruvate, 2 μmol of ATP, 13 μg of lactate dehydrogenase, and 16 μg of pyruvate kinase in 1 ml.

Western Blotting—Immunodetection of protein was carried out essentially according to the published procedure (26). Isolated mitochondria (0.3 μg of protein) were suspended in solubilization buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8, 1% SDS, 10% sucrose, 1% β-mercaptoethanol), heated at 100 °C for 5 min, and then subjected to SDS-polyacrylamide gel electrophoresis (10% polyacrylamide gel, Tefco Corp., Tokyo, Japan). After electrophoretic transfer of gel-resolved F_1α-subunit, bound anti-F_1α-subunit antibody on the polyvinylidene difluoride membranes were assayed mainly by using a Western-Light rabbit kit (Tropix, Inc., Bedford, MA).

RESULTS

Biochemical Properties of atp1–2 or atp1–1 Mutants—Yeast strains carrying *atp1–2* (XJY11) and *atp1–1* (XJY12) mutations fail to grow on the non-fermentable carbon source (Fig. 1) and lack ATPase activity (Table I) (14, 18). Both strains, however, were able to synthesize their full length α-subunits, although in reduced amounts (Fig. 2). The amount of mutant α-subunit was approximately 47% of the parental wild type strain DC5 (Table I) in *atp1–2* and 52% in *atp1–1* (14, 18). The F_1-ATPase activity in the *atp1–2* mutant was barely measurable above that of *ATP1* deletion, SKY4010. Thus, the *atp1–2* mutation essentially led to complete loss of ATPase activity (Table I). The enzyme activity of Δ*atp1* strain, SKY4010, could be restored to 54% of wild-type enzyme activity by introduction of *ATP1* on pYCL12–5. The recovery of enzyme activities in strains *atp1–2* and *atp1–1* transformed with the same plasmid was 43% and 37%, respectively (Table I). The lower ATPase specific activity

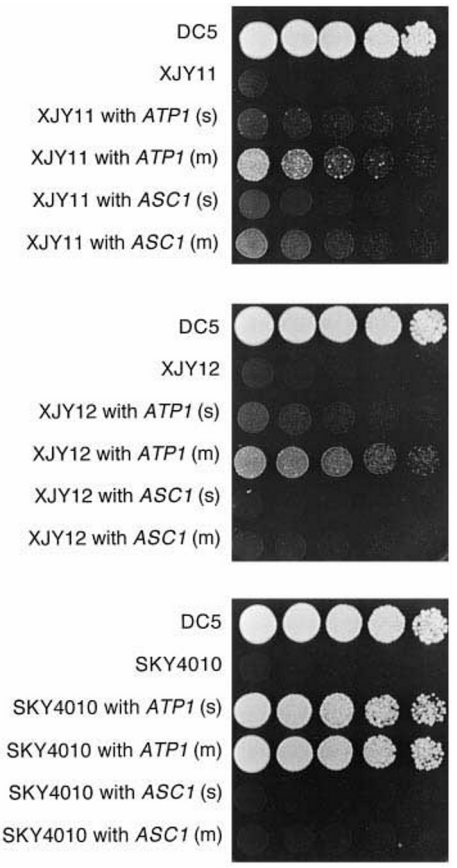

FIG. 1. **Growth phenotype on glycerol.** Strains were diluted (5-fold) and were spotted on YPGE media and cultured for 4 days at 30 °C. The most concentrated spot contains ~100,000 cells. Here, *s* and *m* indicate single-copy and multicopy plasmids, respectively.

of the point mutants transformed with the centromeric plasmid pYCL12–5 suggests that the mutant α-subunit protein probably competes with the plasmid-encoded wild type subunits for assembly into the functional enzyme complex.

Characterization of Mutations in atp1–1 and atp1–2—The nucleotide sequence of *atp1–2* (Table II) revealed a guanine to adenine change at the second position in codon 291, is strictly conserved in *ATP1* genes of other organisms (Table III), and results in an amino acid substitution from Gly^{291} to Asp. Sequence analysis of *atp1–1* also revealed a cytosine to thymine at the second position in codon 383 (Table II) generated an amino acid substitution from Thr^{383} to Ile. The residue at Thr^{383} is also a strictly conserved amino acid residue among all of the ATP synthase α-subunit genes reported so far. Therefore, these data indicate that both Gly^{291} and Thr^{383} of the F_1α-subunit are each essential for function of the ATP synthase.

Isolation of an atp1–2 Suppressor Gene—Suppressor genes to *atp1–2* (Gly^{291} → Asp) that restored growth of the mutants on a non-fermentable carbon source were selected as described (see "Experimental Procedures"). From 10,000 Leu^+ transformants, five were able to grow on glycerol and were further characterized. Two of the five plasmids exhibited restriction maps different to that of *ATP1* but shared a common region. We denoted the gene contained in these fragments as *ASC* (for α-subunit complementing gene). The *ASC1* suppressor of *atp1–2* did not restore the growth defect on glycerol of *atp1–1* (Fig. 1). Thus, these results support that mutations in *atp1–2* and *atp1–1* may affect different functions or cellular activities of the F_1-ATPase α-subunit. *ASC1* failed to restore the growth on glycerol of other ATPase subunit mutants including the *ATP1* deletion mutant SKY4010 (data not shown). When the

TABLE I
F_1-ATPase activity and quantitation of α subunit in mitochondria of transformants with ATP1 or ASC1 on glucose

Strain	Allele	Introduced gene (plasmid[a])	F_1-ATPase activity	Quantitation of α subunit	Growth on glycerol
			μmol/min/mg mt protein		
DC5	ATP1		1.69 ± 0.36	1.00	+
XJY11	*atp1–2*		0.07 ± 0.00	0.47	−
XJY11	*atp1–2*	*ATP1* (pYCL12–5)	0.72 ± 0.05	0.67	+
XJY11	*atp1–2*	*ASC1* (pOSBH20)	0.08 ± 0.02	1.10	+
XJY12	*atp1–1*		0.40 ± 0.00	0.52	−
XJY12	*atp1–1*	*ATP1* (pYCL12–5)	0.62 ± 0.02	0.84	+
XJY12	*atp1–1*	*ASC1* (pOSBH20)	0.13 ± 0.02	0.93	−
SKY4010	Δ*atp1*		0.07 ± 0.00	0	−
SKY4010	Δ*atp1*	*ATP1* (pYCL12–5)	0.92 ± 0.07	1.00	+
SKY4010	Δ*atp1*	*ASC1* (pOSBH20)	0.07 ± 0.00	0	−

[a] Each gene was introduced into each strain using centromeric plasmids.

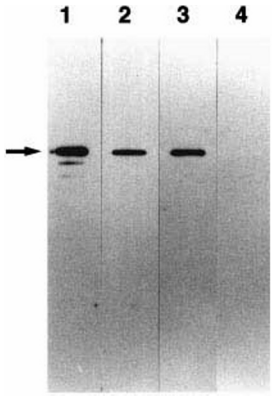

FIG. 2. **F_1-ATPase α-subunit in strains carrying different *ATP1* alleles.** Wild-type strain DC5 (*lane 1*), XJY11 carrying *atp1–2* (*lane 2*), XJY12 carrying *atp1–1* (*lane 3*), and SKY4010 carrying Δ*atp1* (*lane 4*) are shown. Fifty nanograms of protein from mitochondria of each strain was subjected to Western blotting. The *arrow* indicates the location of the α-subunit.

TABLE II
Nucleotide and amino acid changes in atp1 mutants

The nucleotide positions of *ATP1* gene were counted from the first nucleotide of start codon.

Strain	Allele	Nucleotide changes	Codon changes	Amino acid change
XJY11	*atp1–2*	402C → T	GTC → GTT	None
		576C → T	GCC → GCT	None
		872G → A	GGT → GAT	G291D
XJY12	*atp1–1*	402C → T	GTC → GTT	None
		576C → T	GCC → GCT	None
		1148G → T	ACC → ATC	T383I

ASC1 gene was placed on a centromeric plasmic, the *atp1–2* transformant grew on glycerol but at one third the rate of the wild type (180 min doubling, Fig. 3). Thus, low copy gene dosage of *ASC1* suppressed the *atp1–2* mutation. When the *atp1–1* (Thr^{383} → Ile) was transformed with the same genomic library, only *ATP1* genes and no other were isolated on different plasmids using this selection.

ASC1 Is Identical to RAS2—To identify *ASC1*, the common region of the plasmid, inserts were sequenced (Fig. 4). This indicated that *ASC1* was identical to a previously sequenced gene *RAS2*. This gene is a homologue to human oncogene involved in signal transduction (30, 31). Further, Southern hybridization of chromosomes separated by contour-clamped homogenous electric field gel electrophoresis confirmed that digoxigenin-labeled *ASC1* hybridized with chromosome XIV on which *RAS2* is localized (data not shown) (32). Primary clone analysis also showed that *ASC1* hybridized with ATCC clone 70762 alone, which contains *RAS2* (data not shown). Finally,

TABLE III
Conservation of the amino acid residues 291 and 383 in α subunit of ATP synthase

	Amino acid position					
	Gly^{291}		Ala^{291}		Thr^{383}	
Eukaryote						
Mitochondria	38/40	95%	2[a]/40	5%	39[b]/40	98%
Chloroplast	0/17	0%	17/17	100%	17/17	100%
Prokaryote						
Cyanobacteria	0/4	0%	4/4	100%	4/4	100%
Others	16/24	67%	8[c]/24	33%	24/24	100%

[a] *Chlamydomonas reinhardtii* and *Physarum polycephalum*.
[b] An exceptional organism is evening primrose.
[c] *Bacillus subtilis*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Mycoplasma preumoniae*, *Mycoplasma gentalium*, *Mycoplasma sp.*, *Streptococcus mutans*, and *Rhodobacter capsulatus*.

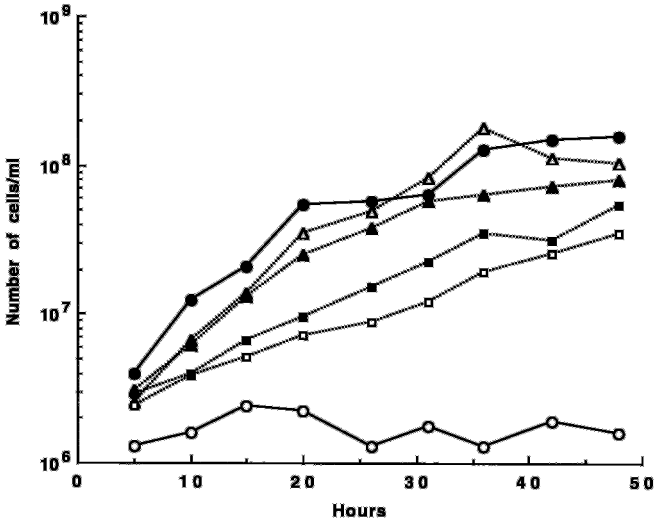

FIG. 3. **Growth curves of DC5 and XJY11 transformants with *ATP1* or *ASC1* in glucose.** Symbols are as follows: ●, DC5 (wild-type); ○, XJY11 (*atp1–2*); ▲, XJY11 with *ATP1* (*s*); △, XJY11 with *ATP1* (*m*); ■, XJY11 with *ASC1* (*s*); □, XJY11 with *ASC1* (*m*). Here, *s* and *m* indicate single-copy and multicopy plasmid, respectively. Each strain was cultured in 50 ml of YPG with shaking at 30 °C. Cell numbers were counted every 5 h using a counting cell.

the yeast *RAS2* gene obtained from another source suppressed *atp1–2* in the same manner as *ASC1* (data not shown).

Biochemical Properties of the atp1–2 Mutant Transformed with ASC1/RAS2—The presence of the plasmid encoded *ASC1/RAS2* in the *atp1–2* mutant caused no change in mitochondrial ATPase activity as long as the cells were grown on glucose (Table I). On glucose in this medium, there is no selective pressure for the mutant to retain the plasmid encoded *ASC1/RAS2*. However, when the transformant was grown on

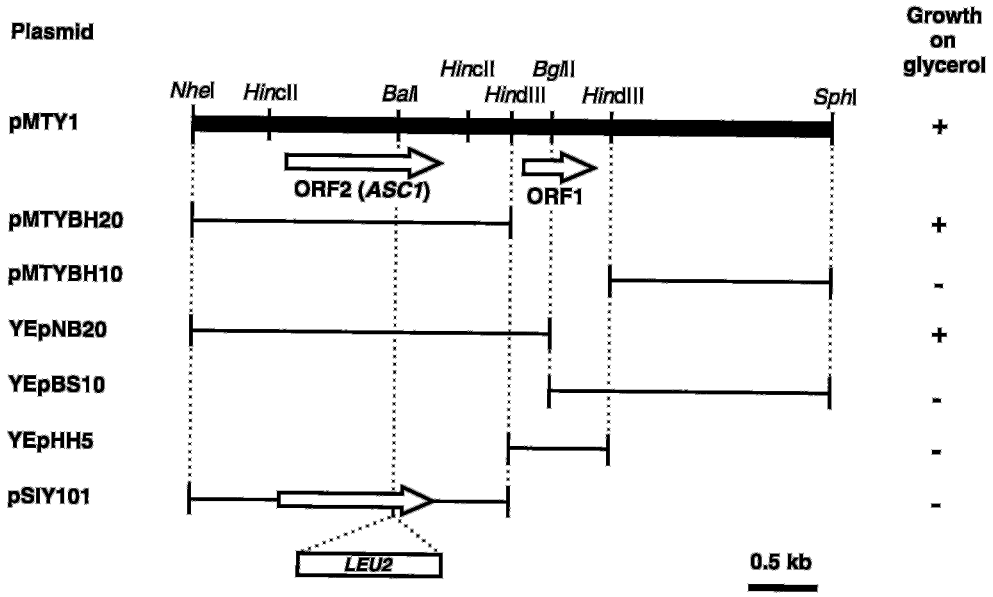

FIG. 4. **Identification of *ASC1* region.** *Arrows* indicate open reading frames. The fragment (*Nhe*I-*Sph*I, 3.7 kb) from pMTY1 was digested using enzymes and generated DNA fragments shown were cloned into YEp13, resulting in pMTYBH20, pMTYBH10, YEpNB20, YEpBS10, and YEpHH5, respectively. pSIY101 contains the disrupted open reading frame 2 by the insertion of *LEU2*. Yeast strain XJY11 was transformed with modified plasmids to Leu$^+$, and their glycerol phenotypes were subsequently analyzed as described under "Experimental Procedures."

the non-fermentable substrate like glycerol, the F_1-ATPase activities increased in *atp1–2* almost 6-fold. The level of enzymatic activity in *atp1–2* transformed with *ASC1* reached 30% that of the parental strain grown under the same conditions (Table IV). These results suggest that *ASC1/RAS2* functions as part of the regulatory circuit linked to the control of F_1-ATPase subunit synthesis on a non-fermentable carbon source.

The amount of F_1-ATPase α-subunit protein was measured under different growth conditions to determine if increases in activity reflected enzyme content. The amount of Atp1–2p in XJY11 grown on glycerol only increased 1.3-fold following the introduction of *ASC1/RAS2* (Table IV) on a centromeric plasmid. The amount of α-subunit increased about 2-fold in both the XJY11 and XJY12 mutants transformed with *ASC1/RAS2* on glucose (Table I). This increase in the F_1-ATPase α-subunit protein occurred when additional copies of *ASC1/RAS2* were present. To further confirm the positive regulatory effect of *RAS2* on Atp1p, we determined the level of $F_1\alpha$-subunit in a *RAS2* deletion mutant. Disruption of *ASC1/RAS2* in DC5 decreased the level of Atp1p compared with that of the parental strain (data not shown).

The combination of protein and enzymatic analysis (Tables I and IV) was consistent with a model that additional copies of *RAS2* increased the amount of the F_1-ATPase subunit protein in some fashion. In the case of the mutant containing a partially functional *atp1–2* subunit, the increase in protein likely provides a threshold level of mutant subunit and activity sufficient to support growth on glycerol. If additional *RAS2* gene product suppressed *atp1–2* by causing increased levels of the partially active Atp1–2p, then we should restore growth on glycerol of the *atp1–2* mutant by introducing and expressing additional copies of the mutant gene. To test this, additional copies of *atp1–2* were placed into the XJY11 mutant on the multicopy plasmid (pYEatp1–2). Following transformation, the level of Atp1–2p and F_1-ATPase activity increased and growth was restored on glycerol (Table IV). These data support the model that *ASC1/RAS2* controls the amount of α-subunit and ATP synthase activity in mitochondria in response to a non-fermentable carbon source.

TABLE IV
F_1-ATPase activity and quantitation of α subunit in mitochondria of transformants with ASC1 or atp1–2

Strain	Allele	Medium	Introduced gene (plasmid)	F_1-ATPase activity	Quantitation of α subunit
				μmol/min/mg mt protein	
DC5	*ATP1*	YPG		1.29 ± 0.24	1.00
XJY11	*atp1–2*	YPG	*ASC1* (pOSBH20[a])	0.39 ± 0.14	0.60
XJY11	*atp1–2*	YPG	*atp1–2* (pYEatp1–2[b])	0.36 ± 0.08	1.12
DC5	*ATP1*	YPDM		1.69 ± 0.36	1.00
XJY11	*atp1–2*	YPDM		0.07 ± 0.00	0.47

[a] A centromeric plasmid.
[b] A multi-copy plasmid.

DISCUSSION

In this paper we show that the specific mutant of *ATP1*, *atp1–2,* but not *atp1–1*, can be partially suppressed by *RAS2*, a mediator of signal transduction. Additionally, this is the first report of mutation sites in *ATP1* in *S. cerevisiae* and will allow further examination of the role of this subunit in cell growth and its control. These studies identify an essential residue in α-subunit function at Thr^{383} in the active site and another at Gly^{291}, which retains partial enzymatic function and can be suppressed by *RAS2* in multiple copies (Fig. 5). There are three copies of *ATP1* in yeast (39, 40); however, the results of sequence analysis of an extensive number of cloned PCR products indicate that only one mutation site was present in each mutant described here. In the mitochondria from *atp1–2* as well as *atp1–1*, the F_1-ATPase activity was barely measurable, although Western blotting revealed that there were 47% or 52% level of α-subunits, respectively, compared with the level in the wild-type strain. Expression of *ATP1* in the same cell with either Atp1–2p or Atp1–1p yielded a lower specific activity than that for *ATP1* expression in the absence of the mutants alleles. This indicated competition for assembly between the defective α-subunits, Atp1–2p or Atp1–1p, and the wild type α-subunit expressed from *ATP1*.

The unusual observation that *RAS2* could specifically suppress the *atp1–2* mutation indicates a relationship between a

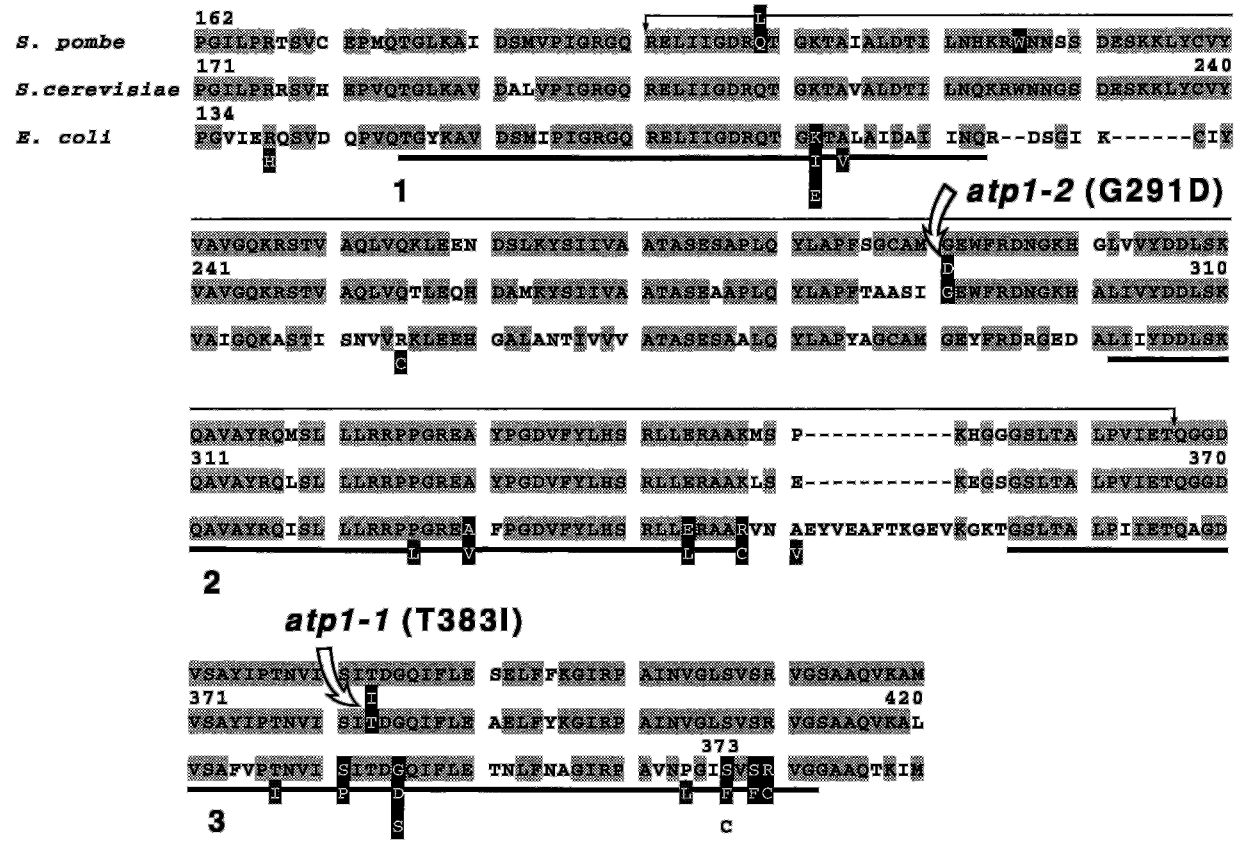

FIG. 5. **Comparison of amino acid sequences between the main central segment of α-subunit in *S. cerevisiae*, *Schizosaccharomyces pombe*, and *E. coli*.** The *numbered underlines* indicate the putative regions from *E. coli* research as follows: *1*, nucleotide-binding domain; *2*, important region for subunit-subunit interaction; *3*, catalytic domain. The *lines* over the sequence indicate putative nucleotide-binding domain from *S. pombe*. Identical amino acid residues are depicted on a *shaded background*. *Dark shaded* amino acids indicate the substitutions that can affect enzyme activity. Amino acids in *black boxes* show the substitutions that lead to loss of function.

growth regulatory pathways involving *RAS* and mitochondrial energy transduction. Analysis of the *atp1–2* mutation site revealed that it is not located in the catalytic domain like *atp1–1* but at the boundary where subunit-subunit interaction is noted (Fig. 5). All of the information presented in this study is consistent with the α-subunit of *atp1–2* exhibiting residual activity that in the presence of multiple copies of *RAS2* reaches a threshold sufficient for growth on a mitochondrial-dependent substrate. On the other hand, the presence of additional copies of *RAS2* in the *atp1–1* mutant, XJY12, does not partially restore F_1-ATPase activity or growth on glycerol. This is because the *atp1–1* mutation is in the catalytic domain (Fig. 5).

Previous studies have shown that threshold levels of the energy transducing ATPase complex containing as little as 15% of the oligomycin-sensitive ATPase activity of wild type strains is sufficient to support growth on glycerol (41). This is consistent with the model that increasing the level of Atp1–2p and/or enhancing its assembly will yield sufficient energy transducing complex for growth on glycerol. This model for suppression of *atp1–2* was confirmed in the experiment in which the gene dosage of *atp1–2* was increased using a multicopy plasmid containing *atp1–2*. The resulting transformants exhibited an increase in F_1-ATPase activity and were able to grow on glycerol (Table IV). Thus, increased Atp1–2p due to gene dosage was a necessary condition to support growth on a non-fermentable carbon source. In the XJY12 transformant, the amount of Atp1–1p also increased (Table I) due to *ASC1/RAS2*. Thus, *ASC1/RAS2* appears to be involved in the regulation of α-subunit content in mitochondria. On glucose, the lack of *ASC1/RAS2* stimulation of ATPase protein and activity likely reflects the loss of *ASC1/RAS2* plasmid due to non-selective growth conditions.

RAS2 has been previously characterized in yeast in earlier studies (16). One phenotype of different alleles of *ras2* is the failure to grow on a non-fermentable carbon source, although a firm characterization of the mechanism of this remains open. *RAS2* activates adenylyl cyclase, followed by activation of cAMP-dependent protein kinases (42–44). The presence of a cAMP-dependent protein kinase on the inner membrane of mitochondria has been reported (45, 46). More recent work indicates a role for protein kinase activity in the regulation of mitochondrial transcription (47, 48). Dupont *et al.* also reported that *ccs1/ira2*, an attenuator gene of *RAS1* and *RAS2*, confers the resistance to inhibitors of the F_0 part of ATP synthase and increased in the ATP synthesis rate in mitochondria (49, 50). This could suggest a role for *RAS* in the assembly of mitochondrial and nuclear encoded subunits of the complex.

The present study directly links a growth mediator to mitochondrial energy coupling. We demonstrate here that the *ASC1/RAS2* stimulates the F_1-ATPase in the *atp1–2* mutant. This activation allowed growth on glycerol. Studies of the relationship between mitochondrial function and growth regulatory pathways have yet to be convincingly established in detail. However, considerable activity has recently focused on the pathways that link the function of mitochondria and mitochondrial components to the growth regulatory activities with which they are now firmly established. Most recently, Akt, a protein kinase B, which has been shown to act as an anti apoptotic regulator at many points in the pathway, is itself regulated by phosphorylation (3). So far, *RAS2* functions have

been investigated with glucose as a carbon source. It is well established that the *RAS2*-cAMP pathway is activated by glucose and that *RAS2* is necessary to grow on a non-fermentable carbon source. Studies are currently in progress to better understand if these regulatory pathways share common control points and why gene dosage of *RAS2* can influence the amount and function of ATPase subunits in mitochondria.

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