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ATP Synthase of Yeast Mitochondria

ISOLATION OF SUBUNIT j AND DISRUPTION OF THE *ATP18* GENE*

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The subunit composition of the mitochondrial ATP synthase from *Saccharomyces cerevisiae* was analyzed using blue native gel electrophoresis and high resolution SDS-polyacrylamide gel electrophoresis. We report here the identification of a novel subunit of molecular mass of 6,687 Da, termed subunit j (Su j). An open reading frame of 127 base pairs (*ATP18*), which encodes for Su j, was identified on chromosome XIII. Su j does not display sequence similarity to ATP synthase subunits from other organisms. Data base searches, however, identified a potential homolog from *Schizosaccharomyces pombe* with 51% identity to Su j of *S. cerevisiae*. Su j, a small protein of 59 amino acid residues, has the characteristics of an integral inner membrane protein with a single transmembrane segment. Deletion of the *ATP18* gene encoding Su j led to a strain (Δ*su j*) completely deficient in oligomycin-sensitive ATPase activity and unable to grow on nonfermentable carbon sources. The presence of Su j is required for the stable expression of subunits 6 and f of the F_0 membrane sector. In the absence of Su j, spontaneously arising rho$^-$ cells were observed that lacked also ubiquinol-cytochrome *c* reductase and cytochrome *c* oxidase activities. We conclude that Su j is a novel and essential subunit of yeast ATP synthase.

Yeast mitochondrial ATP synthase (1) is similar to the corresponding bovine enzyme (2, 3) regarding its polypeptide composition, but there are also differences. All components of the bovine catalytic sector of the ATP synthase (F_1), *i.e.* subunits α, β, γ, δ, ϵ, and the inhibitor protein (IF_1), have homologous counterparts in *Saccharomyces cerevisiae* (4–9). The structural similarity extends to subunits of the membrane sector (F_0) and second stalk, *i.e.* subunit a (Su a or Su 6) (10), Su b (11), Su c (proteolipid or Su 9) (12), which is mitochondrially encoded in yeast but nuclearly encoded in mammals, Su d (13), oligomycin sensitivity conferring protein (14), Su 8 (15), and Su f (16). In addition, a gene encoding a putative homolog of subunit g of the bovine ATP synthase has been identified in the yeast genome (Su g).[1] The corresponding protein, however, has not been observed in the isolated ATP synthase yet. A yeast homolog of bovine subunit e has also been reported recently (17). Subunit e, also known as Tim11, was originally reported as being a component of the mitochondrial inner membrane import machinery (18); however, it has subsequently been shown to be a membrane-bound subunit of the ATP synthase complex (17). Although in mitochondria it is associated with the ATP synthase complex and can be co-immunoprecipitated with subunits of the F_1 sector (17), subunit e, like subunit g, has not been identified yet in the purified ATP synthase enzyme. A yeast homologue to bovine subunit F6 (19) so far has not been reported. Conversely, a novel subunit, subunit h, has been found recently in yeast ATP synthase (20), which appears not to be related to any of the known subunits of bovine or other ATP synthases.

The experiments described in this paper focus on the reassessment of the polypeptide composition of the yeast ATP synthase using a different isolation technique, namely blue native electrophoresis (BN-PAGE).[2] BN-PAGE is a microscale technique for the separation of the multiprotein complexes of oxidative phosphorylation directly from isolated mitochondrial membranes (21). Combined with SDS-polyacrylamide gel electrophoresis (SDS-PAGE) in a second dimension, an overview on the protein subunits of all oxidative phosphorylation complexes is obtained in a two-dimensional gel (22). Using this two-dimensional electrophoretic technique, we observed in the ATP synthase the presence of a previously undetected protein in the 6–7-kDa range. N-terminal protein sequencing revealed it to be a novel subunit of the ATP synthase, subunit j (Su j). Su j is encoded by a gene termed here *ATP18* and has no apparent bovine counterpart.

EXPERIMENTAL PROCEDURES

Materials—Aminocaproic acid (6-aminohexanoic acid) and imidazole were from Fluka, Tricine and Serva Blue G (Coomassie Blue G-250) were from Serva, and phenylmethylsulfonyl fluoride was from Sigma. Hydroxyapatite was prepared as described recently (23).

Yeast Strains and Growth Conditions—For construction of the Δ*su j::HIS3* yeast strain, introduction of the *HIS3* gene resulting in a partial deletion and disruption of the *ATP18*/*Su j* gene was performed as follows. The *HIS3* gene was amplified from the plasmid pFA6a-HIS3MX6 (24) using the following primers: S1, 5′-GTTTAACATACGACGACAGATTAATTGATTGGATTGTACTGCCATGCGTACGCTGCAGGTCGAC-3′ (corresponding to nucleotides −43 to +3 of the *ATP18*/*Su j* gene locus and 18 nucleotides of the multiple cloning site of pFA6a-HIS3MX6 from the 5′ flanking region of the *HIS3* gene); and S2, 5′-TGGATCATTGATAAATTCCTTCGTGTTAGAAGAAAGGTCAGCAGCATCGATGAATTCGAGCTCG-3′ (corresponding to nucleotides +90 to +132 of the *ATP18*/*Su j* gene and 19 nucleotides of the multiple cloning site of pFA6a-HIS3MX6 from the 3′ flanking region of the *HIS3* gene). The resulting polymerase chain reaction product was transformed into the haploid yeast strain W303-1A using the lithium acetate method (25), and *HIS3* positive clones were selected. Correct integration of the *HIS3* marker into the *ATP18*/*Su j* locus was

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The protein sequence data have been submitted to the SWISS-PROT data base with accession number P81450 (ATPase j chain).

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[1] EMBL accession number Z71255, SWISS-PROT accession number Q12233.

[2] The abbreviations used are: BN, blue native; PAGE, polyacrylamide gel electrophoresis; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

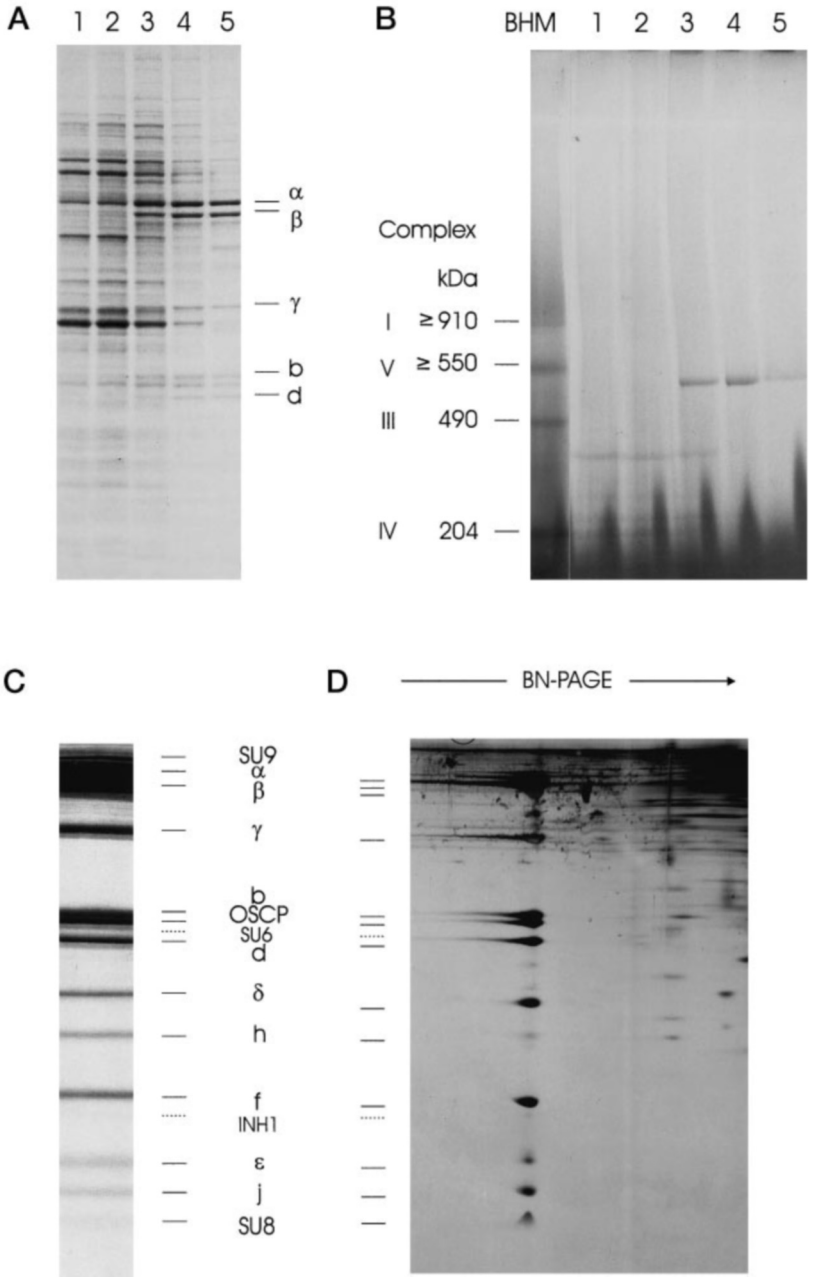

FIG. 1. **Isolation and polypeptide composition of ATP synthase from *S. cerevisiae*.** *A,* enrichment of ATP synthase by hydroxyapatite chromatography is shown. *Lane 1,* flow-through; *lane 2,* wash with buffer 3; *lanes 3–5,* fractions eluted with buffer 4. Equal volumes of the fractions (10 μl each) were analyzed by Tricine-SDS-PAGE using a 10% acrylamide gel. *B,* resolution of the same fractions as in *A* by BN-PAGE. The oxidative phosphorylation complexes from solubilized bovine heart mitochondria (*BHM*) were used for molecular mass calibration in a linear 4–13% acrylamide gradient gel. *C*, polypeptide composition of ATP synthase purified by preparative BN-PAGE of fraction 4 and electroelution of the ATP synthase band. The polypeptides were resolved by Tricine-SDS-PAGE (14% acrylamide, 6 M urea gel) (see "Experimental Procedures"). Proteins were stained with Coomassie Blue G-250. *D*, resolution of fraction 4 by BN-PAGE (*B*) followed by Tricine-SDS-PAGE in the second dimension. Silver stain, gel type as in *C*. *OSCP*, oligomycin sensitivity conferring protein; *INH1,* inhibitor protein.

confirmed by polymerase chain reaction using oligonucleotides that primed upstream and downstream of the disrupted *ATP18* gene.

The resulting yeast strain Δ*su j*, the corresponding wild-type W303-1A, and Δ*su f* were grown in YPGal medium supplemented with 0.5% lactate at 30 °C (26). Cells were harvested by centrifugation at 1,800 × *g*, washed three times with sucrose buffer (250 mM sucrose, 5 mM 6-aminohexanoic acid, 10 mM Tris/HCl, pH 7.0), and used directly for preparation of mitochondrial membranes.

Isolation of Mitochondrial Membranes—About 5 g (wet weight) of sedimented cells, 5 ml of glass beads (0.25–0.5 mm), and 5 ml of sucrose buffer were vortexed for 10 min in a 50-ml tube. After dilution with sucrose buffer, the sedimented glass beads were removed, and the supernatant was centrifuged for 20 min at 1,250 × *g*. Mitochondrial membranes were then collected by centrifugation for 30 min at 18,000 × *g*, taken up with sucrose buffer at a protein concentration of 10–30 mg/ml, and stored at −80 °C.

For the analysis of the submitochondrial localization of Su j, intact mitochondria were isolated according to previously published methods (26).

Electrophoretic Techniques—BN-PAGE was performed as described previously (22) with the following modifications. Mitochondrial membranes (400 μg of protein) were sedimented by centrifugation for 10 min at 100,000 × *g*. The pellet was suspended with 40 μl of 50 mM NaCl, 2 mM 6-aminohexanoic acid, 1 mM EDTA, 50 mM imidazole HCl, pH 7.0, and 1.0 μl of 0.5 M phenylmethylsulfonyl fluoride in Me_2SO was added. Membrane protein complexes were solubilized by the addition of Triton X-100 (9.6 μl from a 10% (w/v) stock solution, 2.4 g of Triton X-100/g of protein). After centrifugation for 20 min at 100,000 × *g*, the supernatant was supplemented with 5 μl of a Coomassie Blue G-250 dye suspension (5% Serva Blue G (w/v) in 750 mM 6-aminohexanoic acid) and immediately applied to a 1.6-mm acrylamide gradient gel for analytical BN-PAGE (1-cm gel well, linear 4–13% acrylamide gradient gel overlaid with a 4% sample gel).

For SDS electrophoresis, the Tricine-SDS-PAGE (27) or the Laemmli system (28) was used. Two-dimensional electrophoresis (BN-PAGE/Tricine-SDS-PAGE), staining, and densitometric quantification were performed as described previously (29, 30).

Isolation of ATP Synthase, Separation of Subunits, and N-terminal Sequencing—All steps were performed at 4 °C, and the pH values of all buffers were adjusted to 4 °C unless otherwise indicated. Mitochondrial membranes from the W303-1A strain (50 mg of protein) were washed with a 4-fold volume of buffer 1 (50 mM NaCl, 2 mM 6-aminohexanoic acid, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 50 mM imidazole HCl, pH 7.0) and collected by centrifugation for 60 min at 100,000 × *g*. The pellet was homogenized in 2.35 ml of buffer 1, and 0.6 ml of Triton X-100 (20% w/v) was added (2.4 g of Triton X-100/g of protein).

TABLE I
Proteins identified in isolated ATP synthase of S. cerevisiae

Proteins were identified by direct Edman degradation or after deformylation (*), except Su 6, which was identified by Western blotting. The sequence of Su d was obtained without deacylation, although the protein was reported to be acetylated (‡). Small letters in the sequences indicate amino acids that were not identified. The masses of the mature proteins do not include N-terminal modifications. AA, number of amino acids.

Band in SDS gel	Assignment	Gene	N-terminal sequence	Mature protein AA	Mass	SWISS-PROT accession
					Da	
1	Su 9 oligomer	*ATP9*	MQLVLA(*)	76	7,759	P00841
2	Su α	*ATP1*	ASTKAQPTEV	510	54,952	P07251
3	Su β	*ATP2*	ASAAQSTPIT	478	51,254	P00830
4	Su γ	*ATP3*	ATLKEVEMRL	278	30,614	P38077
5	Su 4 or Su b	*ATP4*	MSSTPEKQTD	209	23,249	P05626
6	Su 5 or OSCP	*ATP5*	ASKAAAPPPV	195	20,870	P09457
7	Su 6 or Su a	*ATP6*	No sequence	249	27,956	P00854
8	Su d	*ATP7*	sLAKsAANKL(‡)	173	19,677	P30902
9	Su δ	*ATP16*	AEAAAASSGL	138	14,553	Q12165
10	Su h	*ATP14*	DVIQDLYLRE	92	10,408	Q12349
11	Su f	*ATP17*	VSTLIPPKVV	95	10,565	Q06405
12	Inhibitor protein	*INH1*	sEGsTGtPRG	63	7,383	P01097
13	Su ϵ	*ATP15*	sAwRKAGI	61	6,611	P21306
14	Su j	*ATP18*	MLKRFPTPILKVY	59	6,687	P81450
15	Su 8	*ATP8*	MPQLVPFYF(*)	48	5,822	P00856

After centrifugation for 60 min at 100,000 × *g*, the supernatant (2.6 ml) was adjusted to 150 mM Na^+ phosphate and loaded onto a 3-ml hydroxyapatite column equilibrated with buffer 2 (0.05% Triton X-100, 2 mM 6-aminohexanoic acid, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 150 mM Na^+ phosphate, pH 7.7). Hydroxyapatite-bound ATP synthase was washed at room temperature with 1 column volume of buffer 3 (0.1% Triton X-100, 333 μM egg yolk phospholipid, 200 mM Na^+ phosphate, pH 7.3) and eluted with buffer 4 (0.1% Triton X-100, 333 μM egg yolk phospholipid, 300 mM Na^+ phosphate, pH 7.3). One ml of the fraction with highest ATP hydrolysis activity (about 30% of the total yield, 0.5 mg of total protein) was supplemented with 200 mM 6-aminohexanoic acid and loaded onto a 3-mm-thick preparative gel for BN-PAGE. The major blue band comprising ATP synthase, which was visible during BN-PAGE, was excised and cut into 4 pieces. A stack of these 4 pieces was processed by Tricine-SDS-PAGE in a second dimension and electroblotted onto Immobilon P membranes (30). The transferred proteins were sequenced directly using a 473A protein sequencer (Applied Biosystems) or after incubation in a 1:1 (v/v) mixture of trifluoroacetic acid and methanol (24 h at 37 °C) for deformylation (31).

Catalytic Activities—Oligomycin-sensitive ATP hydrolysis was measured at 25 °C using an assay coupled to the oxidation of NADH. Shortly before the test, 0.25 mM NADH, 1 mM phosphoenolpyruvate, 2.5 units/ml lactate dehydrogenase, and 2 units/ml pyruvate kinase were added to the test buffer (250 mM sucrose, 50 mM KCl, 5 mM $MgCl_2$, 2 mM NaCN, 20 mM Tris/HCl, pH 7.5). The reaction was started with protein without detergent and stopped by the addition of 25 μg of oligomycin from a 5 mg/ml stock solution in Me_2SO.

Antibody Production—Antisera against the C-terminal region of Su j were raised in rabbits against a chemically synthesized peptide CRFAKGGKFVEVD that had been coupled to activated ovalbumin (Pierce).

Miscellaneous—Hypotonic swelling and carbonate extraction of mitochondria were performed as described previously (32, 33). Protein determination was performed according to Bradford (34) and a Lowry protocol in the presence of SDS (35).

RESULTS

Isolation of ATP Synthase and Analysis Protein Subunit Composition—The ATP synthase binds stronger to hydroxyapatite than most other mitochondrial proteins of yeast. Therefore hydroxyapatite chromatography is an efficient technique for its isolation. After this first purification step, some of the known ATP synthase subunits can already be recognized in SDS-PAGE (Fig. 1*A*, *lanes 3–5*). The same fractions of the hydroxyapatite column were also applied to a gel for BN-PAGE. The *S. cerevisiae* ATP synthase (Fig. 1*B*, *lanes 3–5*) is slightly smaller than the bovine ATP synthase that was loaded in parallel. BN-PAGE was then repeated on the preparative scale using the hydroxyapatite fraction with the highest amount of ATP synthase complex (Fig. 1*B*, *lane 4*). The band of ATP synthase was excised, and the subunit composition of this

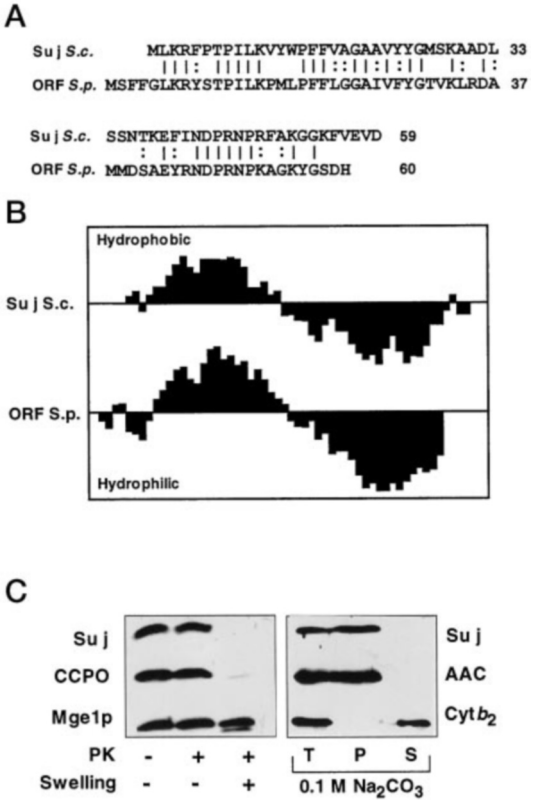

FIG. 2. **Sequence and submitochondrial localization of Su j from *S. cerevisiae* and comparison to a *S. pombe* homolog.** *A,* alignment of the sequences of Su j from *S. cerevisiae* (*S.c.*) and potential homolog from *S. pombe* (*ORF S.p.*) (O13931, EMBL). Identical (*connecting line*) and similar residues (*colons*) between sequences (36) are indicated. *B*, hydropathy profiles of Su j from *S. cerevisiae* and homologous open reading frame (*ORF*) from *S. pombe*. *C,* submitochondrial localization of Su j is shown. *Left panel,* mitochondria and mitoplasts were generated by hypotonic swelling and incubated for 30 min on ice in the presence or absence of proteinase K (*PK*) (200 μg/ml). *Right panel,* mitochondria were subjected to alkaline extraction (0.1 M Na_2CO_3, pH 11.5) for 30 min on ice. The sample was divided; one-half was directly trichloroacetic acid-precipitated (*T,* total), and the other was separated by centrifugation (60 min at 226,000 × *g*) into pellet (*P*) and supernatant (*S*) fractions, prior to trichloroacetic acid precipitation. All samples were analyzed by SDS-PAGE and Western blot analysis. Specific antisera were used against cytochrome *c* peroxidase (*CCPO*) and cytochrome b_2 (*Cytb*$_2$), both soluble proteins of the intermembrane space; *Mge1p*, a matrix localized soluble protein; *AAC*, ADP/ATP carrier protein, an integral inner membrane protein; and *Su j*.

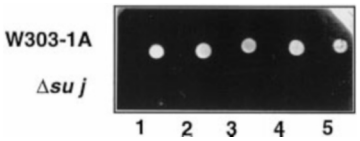

FIG. 3. **Su j is essential for growth on nonfermentable carbon sources.** Yeast strains Δ*su j* and corresponding isogenic wild-type W303-1A grown on YPD (glucose-containing) medium were resuspended in sterile water at a concentration of 10 A_{578}/ml. A dilution series was generated by serially diluting this suspension 10-fold each time. 2 μl of each of the resulting dilutions were spotted onto a YPG (glycerol-containing) plate (*spots 1–5*) and were incubated at 30 °C for 2 days.

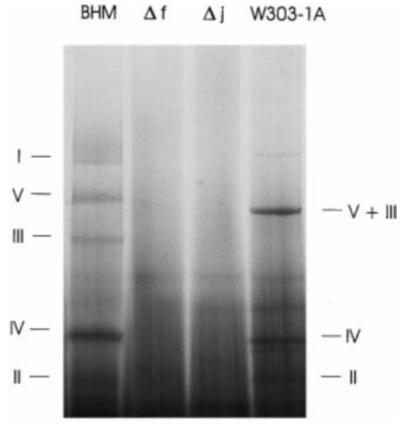

FIG. 4. **BN-PAGE of Triton X-100-solubilized mitochondria from *S. cerevisiae* wild-type strain (W303-1A) and the deletion strains Δ*su j* and Δ*su f*.** ATP synthase (complex *V*) and cytochrome oxidase (complex *IV*) are missing in both deletion strains. An additional loss of ubiquinol-cytochrome *c* reductase (complex *III*), which gives rise to a broad band not detectable in BN-PAGE, was revealed after resolution by SDS-PAGE in a second dimension (not shown). *BHM,* bovine heart mitochondria.

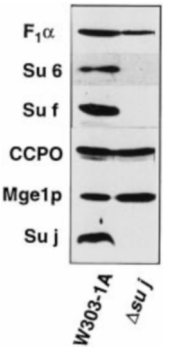

FIG. 5. **The presence of Su j is essential for the stable expression of F_0 sector subunits.** Mitochondria (50 μg of protein) isolated from the Δ*su j* strain and corresponding isogenic wild-type strain W303-1A were subjected to SDS-PAGE and analyzed by Western blotting for the presence of marker proteins, as indicated. *CCPO,* cytochrome *c* peroxidase; *Mge1p*, a matrix-localized soluble protein.

complex was analyzed further by SDS-PAGE. N-terminal protein sequencing of the resolved proteins (Fig. 1*C*) showed the presence of known subunits of the ATPase and one additional protein that was termed subunit j, Su j. To exclude the possibility that this protein may represent a contamination of the ATP synthase, a two-dimensional resolution of the sample from Fig. 1, *lane 4*, was performed. The two-dimensional gel clearly shows that Su j fits the pattern of established subunits of the complex (Fig. 1*D*). Because no streaking of this protein was observed, we conclude that Su j is a true constituent of the ATP synthase complex.

Subunit j could not be removed from the ATP synthase isolated by hydroxyapatite chromatography by adding 7 g of Triton X-100/g of protein and application to BN-PAGE. After the addition of Triton X-100 and 2 M urea and application to BN-PAGE, most of the ATP synthase was dissociated into the individual subunits. The residual fraction of holo-ATP synthase still contained Su j (data not shown).

Polypeptide Composition of ATP Synthase—Direct Edman degradation of the proteins transferred to Immobilon P or after deformylation (Su 8 and Su 9) confirmed the presence of known subunits of ATP synthase. The mature subunits α and β were found to be 4 and 14 amino acids, respectively, shorter than described in protein data bases (Table I). The presence of subunit 6 was confirmed by Western blotting and subsequent immunodecoration using a specific antiserum. The novel Su j was directly accessible to Edman degradation, and a sequence of 13 N-terminal amino acids could be obtained (Table I).

Su j Is an Integral Mitochondrial Inner Membrane Protein—Using the obtained N-terminal sequence of Su j, a search for an open reading frame corresponding to a 6.5-kDa protein in the yeast genome data base was performed. A 177-base pair sequence on chromosome XIII was identified with the potential to encode for a protein of 59 amino acid residues (Fig. 2*A*). This gene encoding for Su j was termed *ATP18*. We identified a potential homolog in *Schizosaccharomyces pombe*, a hypothetical protein of 6.8 kDa. This potential Su j homolog in *S. pombe* was 51% identical to the Su j of *S. cerevisiae*. The hydropathy plots for both proteins were very similar and suggested them to be membrane proteins with a single transmembrane domain (Fig. 2*B*).

A peptide corresponding to the C-terminal region of the protein was used to raise antibodies against Su j. Su j was localized to mitochondria by immunostaining. It was inaccessible to added protease in intact mitochondria (Fig. 2*C*). Disruption of the outer membrane by hypotonic swelling rendered Su j sensitive to the added protease. In addition, Su j was resistant to alkaline extraction and therefore most likely is an integral membrane protein (Fig. 2*C*).

In summary, Su j is a protein anchored to the inner membrane by a single transmembrane segment at its N terminus and has an N_{in}-C_{out} orientation.

Deletion of the ATP18 Gene Leads to Spontaneously Arising Rho$^-$ Cells—To test whether the presence of Su j is essential for the activity of the F_1F_0-ATP synthase, the gene encoding Su j was deleted. The resulting yeast strain Δ*su j* was respiratory incompetent, as it could no longer grow on the nonfermentable carbon source glycerol, in contrast to its isogenic wild-type strain (Fig. 3). Enzymatic measurement of the F_1F_0-ATP synthase activity confirmed the loss of oligomycin-sensitive ATPase activity in isolated Δ*su j* in contrast to the wild-type control (results not shown); therefore Su j seemed to be an essential subunit of the yeast F_1F_0-ATP synthase. However, comparison of the mitochondrial proteins of the Δ*su j* and Δ*su f* strains by BN-PAGE (Fig. 4) and two-dimensional resolution (not shown) revealed that not only the ATP synthase but also cytochrome oxidase (complex *IV*) and ubiquinol-cytochrome *c* reductase (complex *III*) were below the limit of detection (<10% as compared with wild-type W303-1A). The reduced levels of complex III and IV can be explained by the fact that a spontaneous transition of the Δ*su j* cells to the rho$^-$ state was observed.[3] Interestingly a similar formation of rho$^-$ cells was observed in the Δ*su f* strain, as reported by Spannagel *et al.* (16).

Su j Is Required for the Stable Expression of Other Subunits of the F_0 Sector—The nature of the association of Su j with the inner membrane and its predicted orientation in the membrane would suggest Su j to be a subunit of the F_0 sector of the

[3] I. Arnold, K. Pfeiffer, W. Neupert, R. A. Stuart, and H. Schägger, unpublished observations.

F_1F_0-ATP synthase. We therefore asked whether the expression of Su j was required for the stable expression of other F_0 sector subunits. Mitochondria from the Δ*su j* strain were analyzed by Western blotting for the presence of various subunits of the ATP synthase (Fig. 5). In the absence of Su j, subunits 6 and f of the F_0 sector were not detectable. The α-subunit of the F_1 sector was reduced in the Δ*su j* strain. Levels of other mitochondrial marker proteins, such as cytochrome *c* peroxidase and Mge1p, were not altered in the absence of Su j.

DISCUSSION

The subunit composition of the yeast mitochondrial F_1F_0-ATP synthase was analyzed using the combined techniques of BN-PAGE and high resolution Tricine-SDS-PAGE. We present evidence here for the existence of a novel ATP synthase subunit, Su j. A homologue in the purified bovine ATP synthase complex has so far not been reported. The presence of an open reading frame in *S. pombe* with 51% amino acid sequence identity to the *S. cerevisiae* Su j suggests that Su j represents a general component of eukaryotic ATP synthases.

The novel Su j protein appears to represent a bona fide subunit of the ATP synthase. Su j purified with the ATP synthase after BN-PAGE. As this technique resolves proteins by their native molecular mass (22), Su j is unlikely to be a contaminant of the ATP synthase; this would require it to have the same native size. No other polypeptides, which could not be assigned to the ATP synthase complex according to the amino acid sequence, were present in the purified ATP synthase fractions. Su j was observed to be tightly bound to the ATP synthase. Treatment of the isolated complex under conditions that led to its almost complete dissociation resulted in a residual fraction of holo-ATP synthase, which still contained Su j. We therefore suggest that Su j is required for the structural integrity of the ATP synthase complex. Consistent with this view, Su j appears to be an essential subunit of the yeast ATP synthase complex. Deletion of the gene encoding Su j (*ATP18*) gave rise to a respiratory-deficient phenotype and loss of measurable oligomycin-sensitive ATP synthase activity.

The tight binding of subunit j to the isolated yeast ATP synthase raises the question as to why subunit j was not previously identified in other ATP synthase preparations. The previous use of SDS gels probably did not yield sufficient resolution of the smaller subunits of the complex. As shown here, the use of high resolution Tricine-SDS-PAGE has optimized the separation of the yeast ATP synthase subunits in the molecular mass range of Su j. Furthermore, although we used mild solubilization with Triton X-100 and BN-PAGE as a one-step procedure, subunits g and e (Tim11) or a potential homologue of bovine subunit F6 were not found in association with the ATP synthase complex. Notably, recent variation of the conditions for protein solubilization and BN-PAGE led to the isolation of an ATP synthase with three more bound proteins, including subunits e (Tim11) and subunit g. The analysis of the role of these proteins for the structure and function of ATP synthase will be discussed separately (37).

In conclusion, we demonstrate here that Su j is an integral inner membrane protein, spanning the membrane once in an N_{in}-C_{out} orientation. The membrane association of Su j is compatible with it being a subunit of the F_0 sector of the ATP synthase. We are currently investigating the association of Su j with other known F_0 sector subunits.

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REFERENCES

1. Law, R. H. P., Manon, S., Devenish, R. J., and Nagley, P. (1995) *Methods Enzymol.* **260,** 133–163
2. Collinson, I. R., Runswick, M. J., Buchanan, S. K., Fearnley, I. M., Skehel, J. M., Van Raaij, M. J., Griffith, D. E., and Walker, J. E. (1994) *Biochemistry* **33,** 7971–7978
3. Walker, J. E., Collinson, I. R., Van Raaij, M. J., and Runswick, M. J. (1995) *Methods Enzymol.* **260,** 163–190
4. Takeda, M., Chen, W. J., Saltzgaber, J., and Douglas, M. G. (1986) *J. Biol. Chem.* **261,** 15126–15133
5. Takeda, M., Vassarotti, A., and Douglas, M. G. (1985) *J. Biol. Chem.* **260,** 15458–15465
6. Paul, M. F., Ackermann, S., Yue, J., Arselin, G., Velours, J., and Tzagoloff, A. (1994) *J. Biol. Chem.* **269,** 26158–26164
7. Giraud, M. F., and Velours, J. (1994) *Eur. J. Biochem.* **222,** 851–859
8. Guelin, E., Chevallier, J., Rigoulet, M., Guerin, B., and Velours, J. (1993) *J. Biol. Chem.* **268,** 161–167
9. Ichikawa, N., Yoshida, Y., Hashimoto, T., Ogasawara, N., Yoshikawa, H., Imamoto, F., and Tagawa, K. (1990) *J. Biol. Chem.* **265,** 6274–6278
10. Macino, G., and Tzagoloff, A. (1980) *Cell* **20,** 507–517
11. Velours, J., Durrens, P., Aigle, M., and Guerin, B. (1988) *Eur. J. Biochem.* **170**, 637–642
12. Macino, G., and Tzagoloff, A. (1979) *J. Biol. Chem.* **254,** 4617–4623
13. Norais, N., Prome, D., and Velours, J. (1991) *J. Biol. Chem.* **266,** 16541–16549
14. Uh, M., Jones, D., and Mueller, D. M. (1990) *J. Biol. Chem.* **265,** 19047–19052
15. Macreadie, I. G., Novitzki, C. E., Maxwell, R. J., John, U., Ooi, B. G., McMullen, G. L., Lukins, H. B., Linnane, A. W., and Nagley, P. (1983) *Nucleic Acids Res.* **11,** 4435–4451
16. Spannagel, C., Vaillier, J., Arselin, G., Graves, P. V., and Velours, J. (1997) *Eur. J. Biochem.* **247,** 1111–1117
17. Arnold, I., Bauer, M. F., Brunner, M., Neupert, W., and Stuart, R. A. (1997) *FEBS Lett.* **411,** 195–200
18. Tokatlidis, K., Junne, T., Moes, S., Schatz, G., Glick, B. S., and Kronidou, N. (1996) *Nature* **384,** 585–588
19. Fang, J.-K., Jacobs, J. W., Kanner, B. I., Racker, E., and Bradshaw, R. A. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81,** 6603–6607
20. Arselin, G., Vaillier, J., Graves, P. V., and Velours, J. (1996) *J. Biol. Chem.* **271,** 20284–20290
21. Schägger, H., and von Jagow, G. (1991) *Anal. Biochem.* **199,** 223–231
22. Schägger, H., Cramer, W. A., and von Jagow, G. (1994) *Anal. Biochem.* **217,** 220–230
23. Schägger, H. (1994) in *A Practical Guide to Membrane Protein Purification* (von Jagow, G., and Schägger, H., eds) pp. 107–124, Academic Press, Orlando, FL
24. Wach, A., Brachat, A., Poehlmann, R., and Philippsen, P. (1994) *Yeast* **10,** 1793–1808
25. Gietz, D., St. Jean, A., Woods, R. A., and Schiestl, R. H. (1992) *Nucleic Acids Res.* **20,** 1425
26. Herrmann, J. M., Fölsch, H., Neupert, W., and Stuart, R. A. (1994) in *Cell Biology: A Laboratory Handbook* (Celis, D. E., ed) Vol. 1, pp. 538–544, Academic Press, San Diego, CA
27. Schägger, H., and von Jagow, G. (1987) *Anal. Biochem.* **166,** 368–379
28. Laemmli, U. K. (1970) *Nature* **227,** 680–685
29. Schägger, H. (1995) *Electrophoresis* **16,** 763–770
30. Schägger, H. (1994) in *A Practical Guide to Membrane Protein Purification* (von Jagow, G., and Schägger, H., eds) pp. 59–79, Academic Press, Orlando, FL
31. Gheorghe, M. T., Jörnvall, H., and Bergman, T. (1997) *Anal. Biochem.* **254,** 119–125
32. Fölsch, H., Guiard, B., Neupert, W., and Stuart, R. A. (1996) *EMBO J.* **15,** 479–487
33. Pfanner, N., Hartl, F.-U., and Neupert, W. (1988) *Eur. J. Biochem.* **175,** 205–212
34. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254
35. Helenius, A., and Simons, K. (1972) *J. Biol. Chem.* **247,** 3656–3661
36. Dayhoff, M. O., Schwartz, R. M., and Orcutt, B. C. (1981) in *Atlas of Protein Sequence and Structure* (Dayhoff, M. O., ed) Vol. 5, pp. 342–352, National Biomedical Research Foundation, Silver Spring, MD
37. Arnold, I., Pfeiffer, K., Neupert, W., Stuart, R. A., and Schägger, H. (1998) *EMBO J.* **17,** in press