Yeast mitochondrial F₁F₀-ATP synthase exists as a dimer: identification of three dimer-specific subunits

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Using the technique of blue native gel electrophoresis, the oligomeric state of the yeast mitochondrial F₁F₀-ATP synthase was analysed. Solubilization of mitochondrial membranes with low detergent to protein ratios led to the identification of the dimeric state of the ATP synthase. Analysis of the subunit composition of the dimer, in comparison with the monomer, revealed the presence of three additional small proteins. These dimer-specific subunits of the ATP synthase were identified as the recently described subunit e/Tim11 (Su e/Tim11), the putative subunit g homolog (Su g) and a new component termed subunit k (Su k). Although, as shown here, these three proteins are not required for the formation of enzymatically active ATP synthase, Su e/Tim11 and Su g are essential for the formation of the dimeric state. Su e/Tim11 appears to play a central role in this dimerization process. The dimer-specific subunits are associated with the membrane bound F₀-sector. The F₀-sector may thereby be involved in the dimerization of two monomeric F₁F₀-ATP synthase complexes. We speculate that the F₁F₀-ATP synthase of yeast, like the other complexes of oxidative phosphorylation, form supracomplexes to optimize transduction of energy and to enhance the stability of the complex in the membrane.

Keywords: dimer/F₁F₀-ATP synthase/mitochondria/subunits e, g and k/yeast

Introduction

F₁F₀-ATP synthase complexes play a central role in the synthesis of ATP in all living organisms. This enzyme is present in the plasma membrane of bacteria, thylakoid membranes of chloroplasts and in the inner membrane of mitochondria. In each of these locations, the F₁F₀-ATP synthase functions to synthesize ATP in a manner which is coupled to the translocation of protons across the respective membranes. Two functionally distinct parts of this complex can be distinguished, the F₁ part, which performs the ATP synthesis and hydrolysis reactions, and the membrane bound F₀-sector, which mediates the proton transport.

The mitochondrial F₁F₀-ATP synthase, complex V of the respiratory chain, is composed of both nuclear and mitochondrial encoded subunits (Law et al., 1995; Walker et al., 1991, 1995). In the yeast Saccharomyces cerevisiae, three subunits, 6, 8 and 9 (Su 6, Su 8 and Su 9), are encoded by the mitochondrial DNA and represent essential subunits of the membrane-bound F₀ sector. The remaining subunits of the F₀-sector and all subunits of the F₁ sector are nuclear encoded. Recently, several small novel subunits of the F₁F₀-ATP synthase from yeast have been reported, the subunits h (Su h), subunit e/Tim11 (Su e/Tim11) and subunit f (Su f; Arselin et al., 1996; Arnold et al., 1997; Spannagel et al., 1997). In addition, a putative homolog of the mammalian subunit g (Su g) has been reported; however, it has not yet been demonstrated as being a subunit of the yeast ATP-synthase complex (Prescott et al., 1997). Not all of these proteins appear to be essential for the activity of the F₁F₀-ATP synthase (Arnold et al., 1997; Prescott et al., 1997).

In this study we have re-analysed the subunit composition of the mitochondrial F₁F₀-ATP synthase from yeast. We used a mild detergent lysis procedure followed by blue native gel electrophoresis to isolate the ATP synthase. Using this procedure, the ATP synthase was found to exist as a dimer. Analysis of the subunit composition of the dimer by a high-resolution second dimension SDS–polyacrylamide gel electrophoresis (SDS–PAGE) lead to the identification of three novel proteins. These subunits present only in the dimeric form of the complex include Su e/Tim11, the putative Su g homolog (Prescott et al., 1997) and a new subunit, termed subunit k (Su k).

Characterization of these subunits, in particular their role in the formation of the dimeric state of the F₁F₀-ATP synthase is presented here.

Results

Dimeric structure of the yeast F₁F₀-ATPase

The subunit composition of the mitochondrial F₁F₀-ATPase from S.cerevisiae was analysed using blue native gel electrophoresis followed by resolution of protein constituents in a second dimension by SDS–PAGE. Mitochondrial membrane proteins were solubilized with a low concentration of Triton X-100 and the extract was applied to a blue native gel (Figure 1A). Two dominant complexes could be identified, one of molecular mass ñ1000 kDa and a less abundant complex of 500 kDa. Second-dimension resolution of both of these complexes by SDS–PAGE resulted in a protein subunit profile characteristic of F₁- and F₀-sector subunits of the F₁F₀-ATPase complex (Figure 1B). The estimated molecular mass of the smaller complex corresponds well to that of the monomeric F₁F₀-ATPase complex. The larger and more prominent one apparently represents a dimer of the complex. The identity of both complexes was confirmed by protein...
Dimer-specific subunits of yeast F\textsubscript{1}F\textsubscript{0}-ATP synthase

sequencing of individual subunits following SDS–PAGE (Table I) and by Western blotting using antisera specific for the subunits of the F\textsubscript{1}-sector (results not shown; see Figure 5).

The protein subunit composition of the monomeric and dimeric forms of the F\textsubscript{1}F\textsubscript{0}-ATPase was very similar, with the exception of three small proteins, present exclusively in the dimeric form (Figure 1B; Table I). N-terminal sequencing of these proteins revealed them to be the recently described subunit e/Tim11 (Su e/Tim11) (Tokatlidis et al., 1996; Arnold et al., 1997), the putative Su g homolog (Prescott et al., 1997) and a novel protein which we term Su k. Although they are not entirely reliable indices of stoichiometry, the Coomassie staining intensities suggest a copy number of two per dimer for Su g and Su e/Tim11. Su k seems to be poorly stained by Coomassie, thus making it technically difficult to estimate its precise stoichiometry.

On the basis of these data we conclude that mild solubilization of yeast mitochondria yields a dimeric form of the F\textsubscript{1}F\textsubscript{0}-ATPase complex. This dimer contains three small proteins which are not found in the monomer.

**Topology of dimer-specific components and function in the catalytic activity of the F\textsubscript{1}F\textsubscript{0}-ATPase**

Su e/Tim11 is known to span the inner membrane once with an N\textsubscript{in}–C\textsubscript{out} orientation and requires the presence of the mitochondrially encoded F\textsubscript{0}-subunits for stable expression (Tokatlidis et al., 1996; Arnold et al., 1997). We cloned the corresponding genes for the other two dimer-specific subunits and raised specific polyclonal antisera against them (Figure 2). The hydropathy plots for Su g and Su k suggests that they are membrane proteins with a single transmembrane domain. Su g- and Su k-specific antibodies were raised against peptides corresponding to the C-terminal regions of both subunits. With these antibodies it could be shown that both Su g and Su k, like Su e/Tim11 (Arnold et al., 1997), were inaccessible to exogenously added protease in intact mitochondria (Figure 2). Opening of the outer membrane by hypotonic swelling rendered the subunits accessible to the added protease. Furthermore, Su g appears to be an integral inner-membrane protein, as it was largely retained with the mitochondrial membranes upon alkaline extraction. In contrast, Su k was solubilized by this procedure, indicating that it was probably peripherally associated with the inner membrane. The submitochondrial localization and association of these subunits with the inner membrane would suggest they are probably part of the F\textsubscript{0}-sector.

These three subunits are not essential for the catalytic activity of the F\textsubscript{1}F\textsubscript{0}-ATPase complex, as yeast strains harbouring deletions of the individual genes remained respiratory competent (Figure 3A). A weak petite phenotype, however, was observed, in particular for the ΔTIM11 and ΔSU g strains. Growth of these strains on non-
fermentable carbon sources was inhibited in the presence of oligomycin, demonstrating that none of these subunits played a role in conferring oligomycin sensitivity on the F₁F₀-ATPase complex (results not shown). This observation was supported by the enzymatic measurement of the oligomycin-sensitive ATP hydrolysis activity of the F₁F₀-ATPase complex in mitochondria from the various deletion strains (Figure 3B). The protein amounts of the F₁F₀-ATPase complex in mitochondria from the various deletion strains were resolved. Both dimers and monomers of the complex could be shown to contain the Δsuα-subunit of the F₁-sector, whereas the Δsuα and Δsuγ subunits were located exclusively in the dimeric form of the F₁F₀-ATPase complex. The levels of the Δsuα-subunit were influenced by the presence of Suγ. Deletion of Suγ, however, had no appreciable effect on the steady state levels of either Suα or Suγ. Furthermore, the levels of another bona fide F₀-sector subunit, Suγ, was reduced significantly in the absence of Suγ. Deletion of Suγ, although not required for the accumulation of Suα/Tim11 or Suγ, had no appreciable effect on the steady state levels of either Suα or Suγ. Furthermore, the levels of another bona fide F₀-sector subunit, Suγ, was reduced significantly in the absence of Suα/Tim11 or Suγ. In conclusion, Suα, Suγ and Suκ are non-catalytic mitochondrial proteins, including other known mitochondrial proteins, such as Tim11 and Suγ, but appeared to be unaffected by the deletion of Suκ. The levels of the α-subunit of the F₁-sector and an unrelated control protein, cytochrome b₂, were unaffected in the various deletion mutants.

We conclude that Suα/Tim11 plays a central role in the assembly and/or stability of both Suγ and Suκ. The accumulation of Suα/Tim11 does not depend on the presence of Suγ and Suκ. On the other hand, the presence of Suγ appears to enhance the stability of Suα/Tim11. Suκ, although not required for the accumulation of Suα/Tim11 and Suγ, appears to need these two subunits for its own assembly and/or stability.

Expression of dimer-specific subunits

We next addressed whether these subunits were interdependent on each other for stable expression and if their levels were influenced by the presence of the mitochondrial encoded subunits of the F₁-sector. Mitochondria were isolated from the three deletion yeast strains and a rho⁰ strain, which lacks a mitochondrial genome, and were analysed by Western blotting together with mitochondria from the corresponding wild-type strain. The levels of Suα/Tim11, Suγ and Suκ were analysed and compared with other mitochondrial proteins, including other known subunits of the F₁F₀-ATPase complex (Figure 4). The presence of Suα/Tim11 influences the stable expression of both Suγ and Suκ, as they were not detected in mitochondria isolated from the Δtim11 strain. On the other hand, Suα/Tim11 was present in the Δsuγ mitochondria, albeit at reduced levels, whereas stable expression of Suκ clearly was affected in the absence of Suγ. Deletion of Suκ, however, had no appreciable effect on the steady state levels of either Suα/Tim11 or Suγ. Furthermore, the levels of another bona fide F₀-sector subunit, Suγ, was reduced significantly in the absence of both Suα/Tim11 and Suγ, but appeared to be unaffected by the absence of Suκ. The levels of the α-subunit of the F₁-sector and an unrelated control protein, cytochrome b₂, were unaffected in the various deletion mutants.

Table 1. Proteins associated with dimeric ATP synthase of S.cerevisiae

<table>
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<tr>
<th>Band in SDS gel</th>
<th>Assignment</th>
<th>Gene</th>
<th>Mature protein</th>
<th>N-terminal sequence</th>
<th>AA</th>
<th>Mass (Da)</th>
<th>Staining ratio</th>
<th>SWISS-PROT accession No.</th>
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aProteins were identified by direct Edman degradation, or after deamidation (f), or deacylation (g), except Su 6 which was identified by Western blotting. Lower case letters indicate amino acids which were not identified.

bAA, number of amino acids.
cThe masses of the mature proteins do not include N-terminal modifications.
dThe staining ratios were obtained by densitometric quantification of Coomassie-stained SDS gels. The stain intensities (arbitrary units) were divided by the molecular masses of the individual subunits, and normalized to Suγ.

Dimerization of the F₁F₀-ATPase

The involvement of the novel subunits in the dimerization of the F₁F₀-ATPase complex was addressed. Wild-type mitochondria were solubilized with the detergent digitonin and the oligomeric state of the F₁F₀-ATPase was analysed by blue native gel electrophoresis followed by Western blotting (Figure 5A). Similar to the solubilization procedure in Triton X-100, both dimeric and monomeric forms of the F₁F₀-ATPase complex were resolved. Both forms of the complex could be shown to contain the α-subunit of the F₁-sector, whereas the Suα/Tim11 and Suγ were located exclusively in the dimeric form of the complex.

The role of the novel subunits in the formation of...
Fig. 2. Amino acid sequence of Su g and Su k of the mitochondrial F1F0-ATPase from *S. cerevisiae*; hydropathy profiles and submitochondrial localization of both proteins. (A) The amino acid sequences of Su g and Su k from *S. cerevisiae* together with their hydropathy profiles, are shown. (B) Submitochondrial localization of Su g and Su k. Mitochondria and mitoplasts generated from hypotonic swelling were incubated for 30 min on ice in the presence or absence of proteinase K (PK, 200 μg/ml), as indicated. Mitochondria were subjected to alkaline extraction (0.1 M Na₂CO₃, pH 11.5) for 30 min on ice. The sample was divided, one half was directly TCA-precipitated (total, T) and the other was separated by centrifugation (60 min at 226,000 g) into pellet (P) and supernatant (S) fractions, and then all samples were subjected to TCA precipitation. Samples were analysed by SDS–PAGE and Western blot analysis, using specific antisera for cytochrome c peroxidase (CCPO) and cytochrome b₃ (Cytb₃), both soluble proteins of the intermembrane space; Mge1p, a matrix-localized soluble protein; the ADP/ATP carrier protein (AAC), an integral inner membrane protein; and Su g and Su k.

Fig. 3. Su g, Su k and Su e/Tim11 of the F₁F₀-ATPase are not essential for growth on non-fermentable carbon sources. (A) Yeast cells of strains Δtim11, Δsu g and Δsu k and corresponding isogenic wild-type W303-1A, grown on YPD (glucose) medium were resuspended in sterile water at a concentration of 10 OD₅78/ml. A dilution series was generated by serially diluting this suspension 10-fold each time. Two microlitres of each of the resulting dilutions were spotted onto a YPG (glycerol) plate (spots 1–5) and were incubated at 30°C for 2 days. (B) Oligomycin-sensitive ATP hydrolysis measured with isolated mitochondria (full bars) and correlated to the amount of ATP synthase (determined following BN–PAGE and second-dimension electrophoresis) (shaded bars) from deletion and wild-type strains were compared (determinations with three different mitochondrial preparations each; n = 3). Wild-type levels were set to 100%.

We conclude that Su e/Tim11 and Su g play an essential role in the formation of the dimer of the F₁F₀-ATPase complex. In contrast, the presence of Su k appears not to be necessary.
The dimeric F₁F₀-ATPase complex can be dissociated into intact monomers. Isolated wild-type mitochondria were solubilized with increasing Triton X-100 concentrations and the F₁F₀-ATPase complex was analysed by blue native gel electrophoresis. Increasing the detergent concentration resulted in a decrease of the dimer and a corresponding increase in the monomeric form (Figure 6A). Western blotting and decoration with F₁α-specific antisera confirmed the presence of dimeric F₁F₀-ATPase at low Triton X-100 and its conversion to monomer with increasing amounts of the detergent (Figure 6B, upper panel). Dissociation of the dimer into the monomeric form resulted in the loss of Su e/Tim11 and Su g from the dimer (Figure 6B, lower panel). Loss of Su k from the dimer was also observed as confirmed in silver-stained two-dimensional gels and by microsequencing (results not shown; the titer of the antibody was not sufficiently high for use in blots of blue native gels). The three subunits were not recovered with the resulting monomer F₁F₀-ATPase complexes. Whether Su e/Tim11, Su g and Su k are released together as a subcomplex from the dimer was subsequently analysed. Gel-filtration analysis of Triton X-100 solubilized mitochondria was performed, using conditions where only the monomeric form of the ATP synthase was present. The three dimer-specific subunits did not co-elute from the column (results not shown). We therefore conclude these three subunits are not released in a subcomplex together upon dissociation of the dimer.

**Discussion**

In the present study we report the observation that the F₁F₀-ATP synthase of yeast mitochondria exists as a dimer. The dimeric form is observed under conditions when mitochondrial membrane proteins are solubilized by low detergent to protein ratios. Analysis of the protein composition of the dimer of the F₁F₀-ATP synthase by high-resolution second dimension electrophoresis revealed the presence of three additional small proteins, which are not observed in the monomeric form. Sequencing of these proteins indicated them to be the recently identified Su e/Tim11, the putative homolog of mammalian Su g proteins and a third novel protein, Su k.

Disruption of the respective genes for these proteins demonstrated them to be non-essential subunits for an enzymatically active F₁F₀-ATP synthase. Although the mitochondrial content of the ATP synthase complex was reduced in the absence of these proteins, in particular in the Su e and Su g deletion strains, the specific oligomycin-sensitive ATP hydrolysis activity remained unaltered. The functional relevance of these proteins was revealed following the electrophoretic analysis of the composition of the F₁F₀-ATP synthase in their absence. Mitochondria isolated from the Δtim11 and Δsu g yeast strains contained the monomeric form of the F₁F₀-ATP synthase, whereas the dimer was absent. We conclude therefore that Su e/Tim11 and Su g are essential for the formation of the dimeric form of the complex.

What mediates this dimerization? The dimer-specific subunits were found to be associated with the membrane and exposed to the intermembrane space. They appear to be associated with the F₀-sector of the ATPase complex. As Su e/Tim11 and Su g are required for the formation of the dimer, we predict that the dimerization is mediated by the F₀-sector and probably involves two monomeric F₁F₀-ATP synthase complexes (Figure 7). Su e/Tim11 apparently plays a central role in this dimerization process for the following reasons: (i) the sequence of Su e/Tim11, like its mammalian counterparts, predicts the ability to form a coiled-coil structure, often the basis for homodimerization events; and (ii) the presence of Su e/Tim11 was essential for the stable expression of the other two dimer-specific subunits, whereas low level expression or normal expression of Su e/Tim11 was observed in the absence of Su g and Su k, respectively. The prediction underlying this model of F₁F₀-ATP synthase dimerization is that Su e/Tim11 can form a homodimer. Preliminary evidence supporting a dimeric state of the bovine Su e has been reported recently (Belogrudov et al., 1996). Investigations are currently in progress to verify a dimeric form of the yeast Su e/Tim11 protein.

What are the functions of Su g and Su k? Su g is essential for the dimerization process. In contrast to Su e/Tim11, but like all other known subunits of the F₀-sector, Su g does not contain a predicted coiled-coil structure. Su g thus appears to play an accessory role to Su e/Tim11 in the dimerization event. The role of Su k remains unclear. Although it is exclusively located in the dimeric form, its presence is not required for stable expression of Su e/Tim11 or Su g, and does not appear to be essential for dimer formation either. However, it is unlikely that Su k represents a contaminant of the ATP synthase. First, its steady-state levels are influenced by those of other ATP synthase subunits, Su k is clearly downregulated in Δtim11, Δsu g and rho⁰ mitochondria. Secondly, the technique of blue native (BN)–PAGE resolves proteins by their molecular mass. To be a contaminant, Su k would need to have the same native size as the ATP synthase. No other polypeptides which could not be assigned to the ATP synthase were present in the dimer. This result, together with the finding that the abundance of Su k was comparable to that of other F₀-subunits, argues that Su k represents a bona fide subunit of the ATP synthase complex. Su k appears to be peripherally associated with the intermembrane space side of the inner membrane. There, it may interact with the intermembrane space...
Fig. 5. Expression of Su e/Tim11 and Su g are essential for the formation of dimeric F₀F₁-ATPase. (A) Wild-type mitochondria were solubilized with digitonin, as described in Materials and methods and analysed by BN–PAGE followed by Western blotting. Antisera specific for the α-subunit of the F₁-ATPase, Su e/Tim11 (α-Tim11) and Su g (α-Su g) were used for immunodecoration. The positions of the dimeric and monomeric forms of the F₀F₁-ATPase, are indicated by V Dim. and V Mon., respectively, as well as those of the molecular mass standards thyroglobulin (669 kDa) and apoferritin (443 kDa). (B) Mitochondria isolated from wild-type yeast (W303-1A) or from yeast mutants deficient in either Su e/Tim11 (Δtim11), Su g (Δsu g) or Su k (Δsu k) were solubilized in digitonin, as described above. The dimeric state of the F₀F₁-ATPase was analysed by BN–PAGE followed by Western blotting and decoration with antiserum specific for the α-subunit of the F₁-sector. The positions of dimeric (V Dim.) and monomeric (V Mon.) ATP synthase and free F₁-sector (F₁), are indicated. (C and D) Dimeric and monomeric forms of the ATP synthase in wild-type, Δtim11, Δsu g and Δsu k strains. Mitochondria were solubilized at a low Triton X-100/protein ratio (0.6 g/g) and BN–PAGE was performed (C). BN–PAGE samples were processed in a second dimension by Tricine-SDS–PAGE (D).

exposed C-termini of Su e/Tim11 and/or Su g. An investigation into the possible interactions of Su e, Su g and Su k with each other is currently being undertaken.

Why does the mitochondrial F₀F₁-ATP synthase from yeast form a dimer? The specific activity of the ATP synthase did not appear to be adversely affected in the Δtim11 or Δsu g mutant mitochondria, indicating that the monomer is as active as the dimer found in wild type.

The dimerization, however, appears to confer a stability advantage on the ATP synthase. During preparations of the ATP synthase, we observed that the monomer form is more labile and susceptible to proteolysis than the dimeric form. The decreased levels of the ATP synthase complex in the Δtim11 or Δsu g mutant mitochondria probably reflects this increased lability. Could the dimeric ATP synthase complex display a dynamic relationship with the
Fig. 6. Release of Su e/Tim11 and Su g from the dimeric form of the F₁F₀-ATPase upon its dissociation into the monomeric form. (A) BN–PAGE of mitochondria solubilized at varying Triton X-100:protein ratios. The positions of the dimeric and monomeric forms of the F₁F₀-ATPase, are indicated by V Dim. and V Mon., respectively. Complexes I–V from bovine heart mitochondria were used as molecular mass standards (Std.; see Figure 1B). (B) Isolated wild-type mitochondria were solubilized with increasing amounts of Triton X-100. The mitochondrial lysates were applied to three gels in parallel, and the dimeric and monomeric forms of the F₁F₀-ATPase were analysed by BN–PAGE and Western blotting. The three blots were decorated with antisera specific for either the F₁α-subunit, Su e/Tim11 or Su g. Upper panel: decoration with F₁α-antiserum. Lower panel: the levels of F₁α, Su e/Tim11 and Su g associated with the dimer and monomer were quantified by densitometry. The levels of F₁α associated with the dimer (open squares) and monomer (filled squares) is expressed as a percentage of the sum dimer and monomer signals at each Triton X-100 concentration. No Su e/Tim11 or Su g was observed associated with the monomeric form, therefore only the dimer-associated species could be quantified for each point; Su e/Tim11 (open circles), Su g (open triangles).

![Diagram of F₁F₀-ATPase dimer]

Fig. 7. Schematic representation of the F₁F₀-ATPase dimer. Dimerization of two monomeric F₁F₀-ATPase complexes occurs through their F₀-sectors and involves three subunits, Su e/Tim11, Su g and Su k. Abbreviations: e, Su e/Tim11; g, Su g; k, Su k; IM, inner membrane; IMS, intermembrane space.

The enzymatic activity of the F₁F₀-ATP synthase remains an open question. Furthermore, the dimeric form of the ATP synthase may confer the ability, in vitro, to modulate the activity or levels of the ATP synthase under different growth conditions, such as aerobic and anaerobic conditions. Finally, the mitochondrial inner membrane is a protein-rich membrane. Formation of dimers of respiratory chain complexes could serve to accommodate more protein in the membrane, as less lipid to surround the protein complex would be required. Interestingly, not only the ATP synthase, but also other complexes of the respiratory chain, such as the cytochrome b₆c₁ complex and cytochrome oxidase complex are known to form stable dimers.

Materials and methods

Yeast strains and growth conditions

Construction of the Δtim11::HIS3 (Δtim11), Δsu g::HIS3 (Δsu g) and Δsu k::HIS3 (Δsu k) yeast strains is described below. The introduction of the HIS3 gene resulting in a partial deletion and disruption of the Su e, Su g and Su k genes was performed as follows: the HIS3 gene was amplified from the plasmid pFA6a-HIS3MX6 (Wach et al., 1994) using the following primers:

For Su e, Δtim11::HIS3 (Δtim11), S1: 5'-CGGAACATACGTTA-3' [corresponding to nucleotides –42 to +3 of the TIM11 gene (ATP21) locus and 18 nucleotides of the multiple cloning site (MCS) of
Dimer-specific subunits of yeast F_{1}F_{0}-ATP synthase

The blue bands of the dimeric and monomeric F_{1}F_{0}-ATPase visible following BN–PAGE were excised from the preparative gel and cut into four pieces. These pieces were loaded as a stack of four slices onto a Tricine-SDS gel. Following electrophoresis, the proteins were blotted onto Immobilon P (Schägger, 1994) and 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 deamidation; 57 h at 37°C for partial deacylation) (Gheorghe et al., 1997).

**Antibody production**

Antisera against the C-terminal region of both Su g and Su k were raised in rabbits against chemically synthesized peptides (CIGRRKLVGYKHH) (Su g) and (CENYLLKHSKQDQA) (Su k) which had been coupled to ovalbumin (Pierce). 

**Miscellaneous**

Hypotonic swelling and carbonate extraction of mitochondria were performed as described previously (Pfanner et al., 1988; Fölsch et al., 1996). Protein determination and SDS–PAGE were performed according to the published methods of Bradford (1976) and Laemmli (1970), respectively.

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I. Arnold et al.


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