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Structural organization of mitochondrial ATP synthase

Ilka Wittig *, Hermann Schägger

Molecular Bioenergetics Group, Cluster of Excellence Frankfurt "Macromolecular Complexes", Medical School, Johann Wolfgang Goethe-Universität, Theodor-Stern-Kai 7, D-60590 Frankfurt am Main, Germany

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ABSTRACT

Specific modules and subcomplexes like F_1 and F_0-parts, F_1-c subcomplexes, peripheral and central stalks, and the rotor part comprising a ring of c-subunits with attached subunits γ, δ, and ε can be identified in yeast and mammalian ATP synthase. Four subunits, $\alpha_3\beta_3$, OSCP, and h, seem to form a structural entity at the extramembranous rotor/stator interface ($\gamma/\alpha_3\beta_3$) to hold and stabilize the rotor in the holo-enzyme. The intramembranous rotor/stator interface (c-ring/a-subunit) must be dynamic to guarantee unhindered rotation. Unexpectedly, a c_{10}a-assembly could be isolated with almost quantitive yield suggesting that an intermediate step in the rotating mechanism was frozen under the conditions used. Isolation of dimeric a-subunit and $(c_{10})_2a_2$-complex from dimeric ATP synthase suggested that the a-subunit stabilizes the same monomer–monomer interface that had been shown to involve also subunits e, g, b, i, and h. The natural inhibitor protein Inh1 does not favor oligomerization of yeast ATP synthase. Other candidates for the oligomerization of dimeric ATP synthase building blocks are discussed, e.g. the transporters for inorganic phosphate and ADP/ATP that had been identified as constituents of ATP synthasomes. Independent approaches are presented that support previous reports on the existence of ATP synthasomes in the mitochondrial membrane.

1. Introduction

Mitochondrial ATP synthase, also named F_1F_0-ATP synthase or complex V, is located in the inner mitochondrial membrane together with respiratory chain complexes I–IV. It is a rotary enzyme that uses the electrochemical potential across the inner membrane generated by the respiratory chain complexes to synthesize ATP [1–3]. The extramembranous F_1-part and the F_0-part are linked by central and peripheral stalks [4–7]. The rotor consists of a ring of c-subunits that is associated with the central stalk (F_1-subunits γ, δ, and ε) in yeast and mammalian ATP synthase [8]. Proton-powered rotation of the c-ring with associated central stalk proteins generates torque and conformational changes in the catalytic $\alpha_3\beta_3$ domain of the F_1-part to synthesize ATP [9–11].

ATP synthase is commonly isolated as a functional monomer but this seems not to be the physiological state in mitochondrial and chloroplast membranes. Early electron microscopic analyses of *Paramecium multimicronucleatum* mitochondria showed F_1-complexes arranged as double rows of particles that wind around tubular cristae of the inner mitochondrial membrane [12], and a recent cryo-electron microscopic study of mammalian mitochondria revealed dimer ribbons of ATP synthase shaping the mitochondrial membrane [13]. Isolation of dimeric and higher oligomeric forms of ATP synthase from mitochondria of *Saccharomyces cerevisiae* and mammals [14–18] suggested that dimeric ATP synthase was the building block for the oligomeric structures.

Recent and current investigations try to identify the proteins in the monomer–monomer interface of dimeric ATP synthase and in the dimer–dimer interface of oligomeric ATP synthase. The physiological role of dimeric/oligomeric ATP synthase is not known at present. However, there is strong evidence for a link to mitochondrial morphology, since loss of cristae formation was observed under conditions that destabilized dimerization and oligomerization of ATP synthase [19]. It seems tempting to speculate that formation and decay of supramolecular structures of ATP synthase are dynamic processes linked to formation and loss of cristae. Cristae shape, in turn, might play a regulatory role for the energy metabolism of the cell, since oxidative phosphorylation (OXPHOS) relies on the diffusion of ions and substrates to sites of transport or reaction in the inner membrane and on the number and shape of contact sites of the cristae with the intermembrane space.

2. Subunits and associated proteins

Most subunits of *S. cerevisiae* and bovine ATP synthase are homologous proteins, e.g. F_1-subunits α, β, γ, δ, and ε, subunits a–g, OSCP, and F6 or h, as summarized in Table 1. Some subunits contain transmembrane helices (TMH) as predicted by HMMTOP [20,21] and TMPred [22]. Some subunits are species specific, namely subunits i and k for the yeast enzyme, and coupling factor B [23] and two novel associated proteins, named MLQ and AGP proteins [24] for the mammalian enzyme. ATP synthase can be inhibited by natural

* Corresponding author. Tel.: +49 69 63016933; fax: +49 69 63016970.
E-mail address: wittig@zbc.kgu.de (I. Wittig).

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Table 1
Subunits and proteins associated with yeast (Y) and bovine (B) ATP synthase

	Protein	Gene	Mass (Da)	Amino-terminal sequence	p*I*	Swiss-Prot accession	TMH[a/b]
Y	Su α	*ATP1*	54,944.66	ASTKA	6.73	P07251	0/1
B	Su α	*ATP5A1*	55,263.39	[pca]QKTGT	8.27	P19483	0/0
Y	Su β	*ATP2*	51,126.37	ASAAQ	5.11	P00830	0/0
B	Su β	*ATP5B*	51,705.13	AAQAS	5.00	P00829	1/2
Y	Su γ	*ATP3*	30,616.15	ATLKE	9.06	P38077	0/0
B	Su γ	*ATP5C1*	30,255.71	ATLKD	9.16	P05631	0/1
Y	Su δ	*ATP16*	14,553.48	AEAAA	4.87	Q12165	0/0
B	Su δ	*ATP5D*	15,064.93	AEAAA	4.53	P05630	0/0
Y	Su ε	*ATP15*	6611.39	SAWRK	9.77	P21306	0/0
B	Su ε	*ATP5E*	5651.67	VAYWR	10.09	P05632	0/0
Y	Su 6 or Su a	*ATP6*	27,887.6	SPLDQ	7.84	P00854	7/5
B	Su a	*MT-ATP6*	24,787.91	[formyl]MNENL	9.99	P00847	6/5
Y	Su 4 or b	*ATP4*	23,250.64	MSSTP	7.83	P05626	2/2
B	Su b	*ATP5F1*	24,668.72	PVPPL	9.14	P13619	2/3
Y	Su 5 or OSCP	*ATP5*	20,871.15	ASKAA	9.30	P09457	0/0
B	OSCP	*ATP5O*	20,929.75	FAKLV	9.83	P13621	0/0
Y	Su d	*ATP7*	19,678.41	SLAKS	8.92	P30902	0/0
B	Su d	*ATP5H*	18,561.28	[acetyl]AGRKL	6.02	P13620	0/0
Y	Su g	*ATP20*	12,921.13	MLSRI	9.82	Q12233	1/1
B	Su g	*ATP5L*	11,286.26	[acetyl]AEFVR	9.44	Q28852	0/1
Y	Su f	*ATP17*	10,565.16	VSTLI	9.94	Q06405	1/1
B	Su f	*ATP5J2*	10,165.99	[acetyl]ASVVP	9.87	Q28851	2/1
Y	Su h	*ATP14*	10,407.36	DVIQD	4.24	Q12349	0/0
B	F6	*ATP5J*	8958.09	NKELD	5.41	P02721	0/0
Y	Su e	*ATP21*	10,744.32	[acetyl]STVNV	5.84	P81449	1/1
B	Su e	*ATP5I*	8189.47	VPPVQ	9.52	Q00361	1/0
Y	Su 9 or Su c	*ATP9*	7759.44	[formyl]MQLVL	7.93	P61829	2/2
B	Su c	*ATP5G1*	7608.01	DIDTAA	6.11	P32876	2/2
B	Su c	*ATP5G2*	7608.01	DIDTAA	6.11	P07926	2/2
B	Su c	*ATP5G3*	7608.01	DIDTAA	6.11	Q3ZC75	2/2
Y	Su 8	*ATP8*	5822.26	[formyl]MPQLV	9.99	P00856	1/1
B	Su 8 or A6L	*MT-ATP8*	7936.56	[formyl]MPQLD	9.52	P03929	1/1
Y	Su i	*ATP18*	6687.79	MLKRF	9.70	P81450	1/1
Y	Su k	*ATP19*	7533.77	MGAAY	8.97	P81451	1/1
B	MLQ protein	*MP68*	6834.08	MLQSL	10.00	P14790	1/1
B	AGP or DAPIT	*USMG5*	6303.39	AGPEA	9.84	Q3ZBI7	1/1
B	Coupl. fact. B	*ATP5S*	20,324.55	FWGWL	7.18	P22027	0/0
B	IF_1	*ATPIF$_1$*	9581.5	GSESG	7.17	P01096	0/0
Y	Inh1, IF_1	*INH1*	7383.2	SEGST	9.15	P01097	0/0
Y	Stf1	*STF1*	7286.15	SDGPL	9.00	P01098	0/0
Y	Stf2	*STF2*	9483.33	TRTNK	7.93	P16965	0/0
Y	Sfl2	*TMA10*	9702.38	TRTSK	9.75	Q06177	0/0

Mass and p*I* are calculated for the mature proteins without post-translational modifications.
[a/b]Number of transmembrane helices (TMH) as predicted by HMMTOP [19,20] and TMPred [21] (www.expasy.ch). [pca]Pyrrolidone carboxylic acid.

inhibitory proteins Inh1 and IF_1 in yeast and mammals, respectively. Inhibitor binding to isolated enzymes commonly is substoichiometric [25–30]. The inhibitory action of Inh1 is enhanced by two stabilizing proteins, which are termed Stf1 and Stf2 (stabilizing factors 1 and 2). Furthermore, a homologue of Stf2 has been identified in yeast which was named Sfl2 (stabilizing factor like protein 2). Some proteins, as summarized in Table 2, are essential assembly factors [31–33] or required for the expression of subunits of ATP synthase [34–40] that are not found in the fully assembled enzyme.

Monomeric yeast ATP synthase contains 14 different subunits [41], namely subunits α (3), β (3), γ (1), δ (1), ε (1), subunit 6 or a (1), b (1), subunit 9 or c (10), d (1), f (1), h (1), i (1), subunit 8 (1), and oligomycin sensitivity conferring protein OSCP (1) with the assigned stoichiometry. The total protein mass is 572,759 Da or 573,067 Da considering amino-terminal formylation of subunits 8 and 9. Dimeric yeast ATP synthase contains 3 more different subunits, namely subunits e, g, and k that are also known as dimer-specific subunits, since they were associated with the dimeric but not with the monomeric yeast ATP

Table 2
Factors required for assembly and expression of yeast (Y) and bovine (B) ATP synthase

	Protein	Gene	Mass (Da)	Amino-terminal sequence	p*I*	Swiss-Prot accession	TMH[a/b]
Y	FMC1p	*FMC1*	≤18,364	–	–	P40491	0/0
Y	ATP10p	*ATP10*	32,093.91	MQGTF	9.56	P18496	0/0
Y	ATP11p	*ATP11*	≤36,581	–	–	P32453	0/0
Y	ATP12p	*ATP12*	≤36,554	–	–	P22135	0/0
B	AF 2	*ATPAF2*	28,287.15	VPPAE	5.46	Q1LZ96	0/1
Y	ATP22p	*ATP22*	≤79,756	–	–	A6ZYV0	0/2
Y	ATP23p	*ATP23*	26,890.37	MNSSG	7.57	P53722	0/0
Y	Mdm38p	*MDM38*	58,610.83	STDKS	5.80	Q08179	2/2
Y	Aep3p	*AEP3*	70,310.03	MNTLR	9.75	Q12089	0/1
Y	Oxa1p	*OXA1*	40,000.09	NSTGP	9.73	P39952	4/4

Mass and p*I* are calculated for the mature proteins without post-translational modifications.
[a/b]Number of transmembrane helices (TMH) as predicted by HMMTOP [19,20] and TMPred [21] (www.expasy.ch). [pca]Pyrrolidone carboxylic acid.

synthase following blue-native electrophoresis [14]. The total protein mass of the dimer is 1,207,916 Da, assuming a 1:1 stoichiometry for subunits e, g, and k (reviewed in [42]), or 1,208,616 Da considering formylation of subunits 8 and 9 and acetylation of subunit e.

In the bovine enzyme, subunits e and g are relatively tightly bound and isolated with the monomer. The monomeric enzyme contains 15 different protein subunits, namely subunit α (3), β (3), γ (1), δ (1), ε (1), subunit 6 or a (1), b (1), subunit 9 or c (10), d (1), e (1), f (1), g (1), F6 (1), subunit 8 or A6L (1), and OSCP (1). Assuming the assigned stoichiometry, the protein mass is 583,442 Da, or 583,573 Da including the amino-terminal modifications. Two further proteins have recently been found associated with rat and bovine ATP synthase, the MLQ protein or 6.8 kDa mitochondrial proteolipid, and the AGP or DAPIT protein [24] that increase the total protein mass of the isolated monomeric complex to 596,579 Da, or to 596,711 Da together with the amino-terminal modifications. Coupling factor B is required to restore the energy coupling activity of ATP synthase [23]. In contrast to the MLQ and AGP proteins it is not isolated with the enzyme.

3. Modules and assembly intermediates

Hydrophilic F_1-subcomplex containing subunits α, β, γ, δ, and ε can be removed from the mitochondrial inner membrane as a stable subcomplex. Racker and Kagawa demonstrated that OSCP is required to reconstitute the F_1-domain with the stripped inner mitochondrial membrane to form the active, oligomycin-sensitive holo-complex [43]. This suggested that the holo-enzyme contains two major parts, the F_1-domain, comprising subunits α, β, γ, δ, and ε, and the F_0-domain comprising the remainder of subunits (Fig. 1A). Today, depiction of the domains and modules of ATP synthase are more complicated: the rotor, for example, contains some subunits of the F_1-domain, namely subunits γ, δ, and ε that form the central stalk, and also a ring of c-subunits assigned to the F_0-part. An associate of this rotor with the catalytic $\alpha_3\beta_3$ headpiece of the F_1-domain, abbreviated as F_1-c complex, had been isolated from yeast and even crystallized [8]. Similarly, an F_1-c subcomplex with bound inhibitor protein IF_1 has been identified in human mitochondria as an assembly intermediate or dead end product in the biosynthesis of ATP synthase [44].

Recent work by Walker and colleagues [45,46] using recombinant expression and purification of modified bovine subunits b, d, and F6 led to the crystallization of a peripheral stalk subcomplex b–d-F6. It was possible to dock this structure, the structure of the amino-terminal domain of OSCP, and the structure of the F_1-c_{10} complex into the 32 Å structure obtained by cryo-electron microscopy of single particles of the intact enzyme [6], and to present a model for a complex comprising $\alpha_3\beta_3$, OSCP, F6, and the peripheral stalk subunits b and d [46,47]. The possibility to remove $\alpha_3\beta_3$, OSCP, and h (the F6 homologue) from yeast ATP synthase suggested that these four subunits stabilize the rotor in the active holo-enzyme by their association with the peripheral stalk [48]. Dissection of ATP synthase between the rotor subunit γ and the $\alpha_3\beta_3$ part of the stator is not surprising considering the rotary mechanism but it was for the first time that the lability of the protein–protein interaction in this dynamic part of the enzyme could be shown immediately on the protein level.

Another dynamic region of the enzyme is the interface of c-ring and a-subunit in the membrane. According to the common mechanistic view, protons are passed to subunit c via the a-subunit. This requires close neighborship of the two proteins for a short intermediate step and this reversible interaction must allow for unhindered rotation [49,50]. Experimental evidence for by order of magnitude higher conductance of F_0 compared to F_0F_1 suggesting low internal resistance in F_0 has been presented by the group of Junge [51]. Therefore, it seemed not surprising that immediate interactions of the two proteins had not been shown for the yeast or mammalian enzymes. Disassembling yeast ATP synthase by low amounts of SDS we could electrophoretically isolate for the first time and with nearly quantitative yield

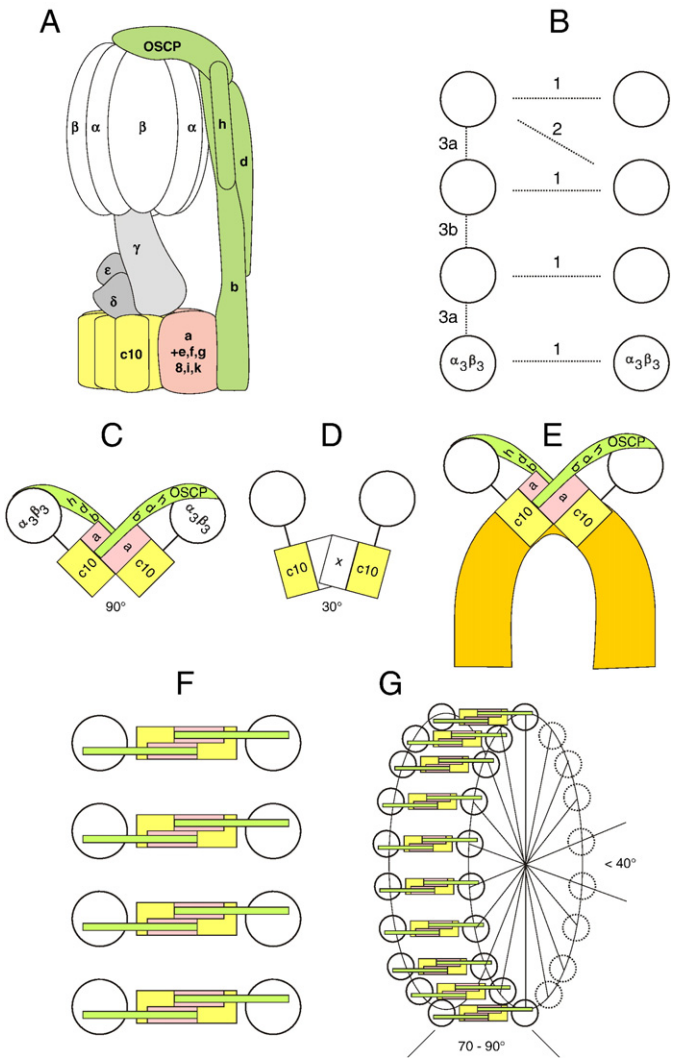

Fig. 1. Model of the subunit organization in monomeric and oligomeric mitochondrial ATP synthase. (A) Monomeric ATP synthase, modified from [46,47]. The F_1-domain contains the catalytic $\alpha_3\beta_3$ headpiece (white) and the central stalk (grey; subunits γ, δ, and ε). The yeast F_0-domain comprises a ring of 10 c-subunits (bright yellow), subunits a, 8, e, f, g, i and k (pink; the transmembrane parts), and the peripheral stalk (green; subunits b, d, h, and OSCP). The rotor is made up of the central stalk and the c-ring. The remainder of the subunits is assigned to the stator. (B) Dimer ribbons of mammalian ATP synthase (circles represent $\alpha_3\beta_3$ headpieces) in the mitochondrial inner membrane, adapted from [13]. Numbers label potential alternatives to form dimeric building blocks. (C) Larger distances between two F_1-domains (1 and 2 in B) seem compatible with larger angles (e.g. 70–90°) between the monomeric ATP synthase. The dimer-interface is postulated to be formed by subunit a and stabilized by subunits e and g (not shown; see A for location). (D) Short distances between two F_1-domains (3a,b in B) seem compatible with smaller angles (e.g. 0–40°). Proteins at the interface of two monomers (marked x) are unknown. (E) ATP synthase dimer inducing strong curvature of a membrane (dark yellow), adapted from [13]. (F) Dimer ribbons of ATP synthase viewed from the mitochondrial matrix side. (G) Dimer ribbons of ATP synthase defining the shape of a small vesicle, adapted from [13]. Large angles (70–90°) and small angles (<40°) between neighboring ATP synthase monomers have been identified in electronmicroscopic analyses of dimeric ATP synthase (see Section 4).

the c-ring with associated subunit a [48]. The quantitative aspect suggested that an intermediate step with reversible subunit c/a interaction was stabilized and trapped under the experimental conditions used.

4. Monomer–monomer interface in dimeric ATP synthase

Analysis of yeast dimeric ATP synthase revealed three subunits e, g, and k [14] in addition to those 14 subunits that had been isolated with the monomeric form [41]. Later on, also higher oligomeric forms of

ATP synthase were detected, especially using the mild detergent digitonin and clear native electrophoresis (CNE) instead of blue-native electrophoresis (BNE) [16–18]. Null mutants of subunits e and g assembled active ATP synthase that appeared monomeric in BNE, and the mutants showed abnormal mitochondrial cristae morphology, expressed as onion like structures of the inner mitochondrial membrane [19,52]. Aberrant mitochondrial morphology similar to null mutants of subunits e and g was also observed with incorporation of a subunit γ-DsRed fusion protein into the F_1-domain. DsRed tetramers thereby seem to link F_1-domains together and thus disturb the correct structural organization of the ATP synthase into oligomers similar to null mutants of subunits e and g [53]. Since deletion of subunit k had no apparent effect on the dimeric state of ATP synthase, subunits e and g were the first subunits identified as important components of the monomer–monomer interface in dimeric ATP synthase. Subunits e and g clearly stabilize the dimer but are not essential for dimer formation in the membrane [54,48].

Cross-linking studies identified subunits e and g as proteins of the dimerization/oligomerization interface, since e–e and g–g homo-dimers and e–g hetero-dimers were found [16,55–57]. Mutating the GXXXG dimerization motif in the transmembrane domains of subunits g and e destabilized the dimeric form of ATP synthase and impaired cristae morphology [16,56–58]. The GXXXG motif in the subunit e transmembrane domain is required for the stability of subunit g and to stabilize ATP synthase dimers [16], whereas a specific coiled-coil motif seems to favor subunit e homo-dimers [56]. The N-terminal anchor region of subunit e, and not the coiled-coil region at the C-terminus is important for normal cristae formation [56–58]. In the absence of subunit e, subunit g is not assembled into the complex and is degraded [14,16,19,52]. Subunit e–e and g–g homo-dimers have previously been identified only with oligomeric but not with dimeric ATP synthase [16,57] suggesting that both subunits could be involved not only in dimerization but also in the oligomerization of ATP synthase. Recent data suggest that another not yet identified small protein might assist subunits e and g with their dimerizing role [58].

The observation of subunit b homo-dimers upon cross-linking in the mitochondrial membrane suggested subunit b as another candidate involved in the dimerization interface [59,60], since monomeric yeast ATP synthase contains a single copy of subunit b [60]. Initially it was thought that subunits e and g stabilize the ATP synthase dimer and subunit b favors the oligomerization. However, cross-links of subunits e–b and g–b suggested that all three proteins stabilize the same monomer–monomer interface in dimeric ATP synthase (Fig. 1A, C,F) ([61], and Schägger, H., unpublished results). In vivo FRET experiments also revealed close proximity of two peripheral stalks subunit b in the membrane suggesting that the peripheral stalks participate in the interaction of two neighboring ATP synthase complexes [54].

Evidence for the involvement of further peripheral stalk proteins, especially of subunit h, in the dimerization of ATP synthase came from the identification of homo-dimeric subunit h cross-links [55,62]. Similar to subunit b, a single copy of subunit h is present in monomeric yeast ATP synthase [62]. Even in null mutants of subunits e or g, subunit h can still form cross-linked homo-dimers that stabilize the ATP synthase dimer [55,62].

Isolation of subunit i–i homo-dimers following cross-linking in the membrane [55,63,64] and chemical cross-links like e–b, g–b, h–b, and i–e ([61,62], and Schägger, H., unpublished results) suggested that at least 5 proteins are neighbors in the same monomer–monomer interface of dimeric ATP synthase, namely subunits e, g, b, h, and i [55].

Recently, dimeric a-subunit could be isolated from dimeric yeast ATP synthase in SDS gels without using chemical cross-linking, and dimeric a-subunit was postulated to bridge two c-rings [48]. These findings and the very high number of predicted transmembrane helices (5–7 TMH depending on the program used; see Table 1) suggested that the a-subunit may constitute the most important basis for the interaction of two monomeric ATP synthase complexes as depicted in Fig. 1C. All other protein components in this interface contain only one TMH (subunits e, g, and i), or two (subunit b), or zero TMH (subunit h). Protein–protein interactions in the membrane may be strengthened by extramembranous interactions in the matrix and intermembrane spaces.

Preferential in-membrane interaction of two ATP synthase monomers became evident also from independent approaches: (i) electron microscopic single particle analysis of isolated ATP synthase dimers from the alga *Polytomella* and from bovine heart clearly showed that the dimerization interface includes only the membranous F_0 [65] or the F_0 and F_1/peripheral stalk parts [66]. Some particles had lost one of the two F_1-headpieces [65]. This did not destroy the overall structure but preserved a stable assembly with two membrane parts joining at an angle around 70° in the *Polytomella* enzyme (similar to Fig. 1C). Such particles with one or two F_1-headpieces lost were observed also with the yeast enzyme [67]. However, two angles around 35–40° and 70–90° were observed. This suggested that two dimer types exist in oligomeric ATP synthase (exemplified in Fig. 1B–G): a dimeric building block named "true dimer" [67] with a large (70–90°) angle between two associated monomers (similar to Fig. 1C), and a small angle (<40°) "pseudo dimer" (similar to Fig. 1D). (ii) Tight interaction of the membrane domains of two monomers was also shown by

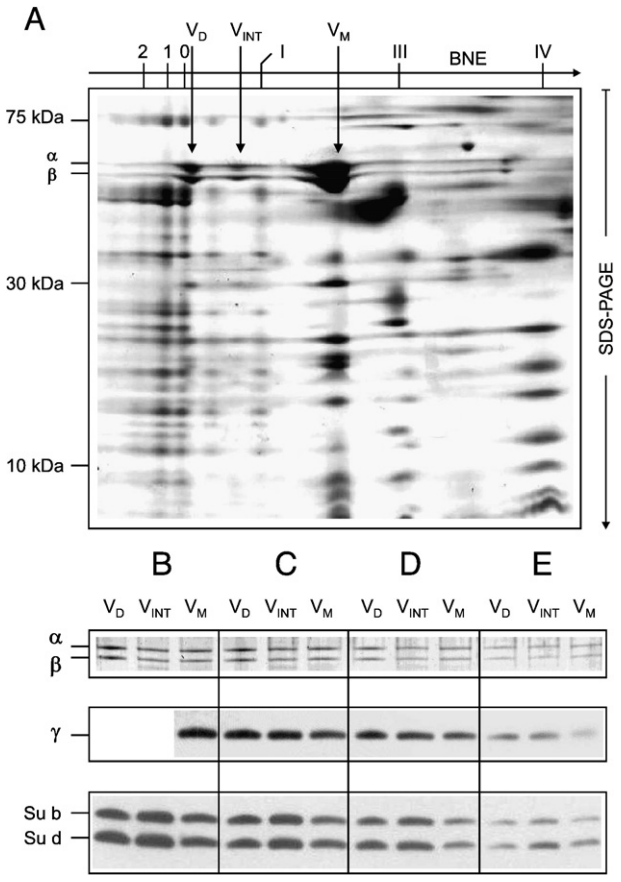

Fig. 2. Dimerization of mammalian mitochondrial ATP synthase (complex V) does not require interaction of F_1-headpieces, as revealed by detection of a fragment of dimeric ATP synthase (V_{INT}) that missed one of the two F_1-domains. V_M, V_D, V_{INT}, monomeric, dimeric, and intermediate-size ATP synthase. I, III, IV, respiratory complexes I, III, and IV; 0, 1, 2, respiratory supercomplexes containing monomeric complex I, dimeric complex III, and zero (0), one (1), or two (2) copies of complex IV. (A) Rat heart mitochondria were solubilized by digitonin, and the mitochondrial complexes were resolved by BNE. Subunits of complexes were then resolved by 2-D SDS-PAGE. (B–E) The protein load to gel wells in (B) was the highest (100%). The load was reduced by 25% in (C), by 50% in (D), and by 75% in (E). Signal intensities of peripheral stalk subunits b and d (Su b and d) in V_{INT} were increased by a factor of 1.9±0.2 (n=4) relative to V_M and V_D but the amounts of F_1-subunits α, β, and γ were comparable. This is explained by loss of one F_1-subcomplex from dimeric ATP synthase.

disassembling dimeric yeast ATP synthase under various conditions [48]. Two F_1-parts were removed from dimeric ATP synthase leaving a residual complex with dimeric F_0/peripheral stalk domains. (iii) Following separation of digitonin-solubilized complexes from mammalian mitochondria by BNE, a protein complex with intermediate size between monomeric and dimeric ATP synthase was often observed (V_{INT} in Fig. 2A). This band contained all subunits of ATP synthase but with altered stoichiometry, since one of the two F_1-parts was dissociated from the dimeric ATP synthase, as demonstrated by quantitative western blots (Fig. 2B–E). Recovery of a stable V_{INT}-complex upon dissociation of one of the two F_1-parts from dimeric ATP synthase demonstrated a tight interaction of two monomers by their F_0/peripheral stalk domains also in the mammalian enzyme.

Another protein that must be considered as a candidate protein to link two monomers together is the natural inhibitor protein IF_1. This protein had been shown to link soluble bovine F_1-subcomplexes [68,69]. Since the inhibitory action of IF_1 depends on the pH, it seemed possible that IF_1 can regulate the ATP synthase activity and also the monomeric or dimeric state. This possibility could be excluded for yeast. Formation of the yeast F_1F_0-ATP synthase dimeric complex did not require the ATP inhibitor protein Inh1 and associated proteins Stf1, Stf2, and Sfl2 [70]. In contrast, promotion of dimerization by IF_1 has been reported for bovine ATP synthase [71]. Potential involvement of Inh1 for the formation of oligomeric structures of yeast ATP synthase was analyzed next.

5. Dimer–dimer interface in oligomeric ATP synthase

Considerably less is known about the dimer–dimer interface (schematically depicted in Fig. 1B,F,G). Natural inhibitor Inh1 and associated proteins Stf1, Stf2, and Sfl2 have been dismissed as important linkers of dimers in yeast, since all null mutants contained normal amounts of higher oligomeric ATP synthase, as analyzed by CNE [48]. However, this might be specific for yeast. The bovine enzyme is under current investigation in order to verify or dismiss a potentially oligomerizing function of bovine IF_1 or of the AGP and MLQ proteins, two novel proteins associated with bovine ATP synthase [24]. Subunits e and g are candidate proteins for the dimer–dimer interface in addition to their participation in the monomer–monomer interface, since oxidation of mitochondrial membranes generated disulfide-bridged g–g and e–e homo-dimers with oligomeric but not with dimeric ATP synthase [16,57]. Also subunits f, Su 8 (A6L), and transmembrane helices of the a-subunit not involved in the monomer–monomer interface are candidates for the dimer–dimer interface. Further candidates are the carriers for inorganic phosphate (PIC) and for ADP/ATP (ANT) that have been described to form a supercomplex with the ATP synthase, the ATP synthasome [72]. If major amounts of ATP synthase are in fact assembled into ATP synthasomes, the arrangement of F_1-headpieces into helical double rows [12] or dimer ribbons of ATP synthase [13], as observed by electron microscopy, needs new interpretation: the double rows would then represent oligomerized dimeric ATP synthasomes instead of dimeric ATP synthase building blocks.

6. ATP synthasome

Isolation of ATP synthasomes from rat liver mitochondrial membranes was first reported by Pedersen and coworkers [73]. The particle showed monomeric ATP synthase with an oblong basepiece but no peripheral stalk was detected. Immuno-electron microscopic studies also pointed to the existence of ATP synthasomes in mitochondrial membranes [72]. However, the ANT is rather abundant in mitochondria and therefore it is not easy to differentiate between true protein complexes and just hydrophobic interactions and aggregations induced during isolation. We used the mild detergent digitonin for solubilization of bovine heart mitochondria and CNE (Fig. 3A) or BNE (Fig. 3B) to separate mitochondrial (super)complexes and to verify the existence of ATP synthasomes. The mild conditions of CNE are optimal for the isolation of oligomeric active ATP synthase [17,74]. BNE can yield comparable results if very low digitonin/protein ratios are used ([75], Fig. 3B). The various forms of ATP synthase were identified by in-gel ATP hydrolysis assay in the CNE and BNE gels [76].

Following solubilization by digitonin (2 g/g protein), 1-D separation by CNE, 2-D SDS-PAGE, and western blotting, a specific antibody was used to identify ANT at the positions of monomeric and oligomeric complexes (Fig. 3A, lower panel). Some strong signal intensity also extended to the mass range below monomeric ATP synthase, i.e. to the right side of monomeric ATP synthase (M). This strong signal can be explained by the migration of individual ANT (p*I* 9.8) not attached to ATP synthase, if a negative charge shift was induced on the protein for example by binding acidic lipids like cardiolipin (not determined so

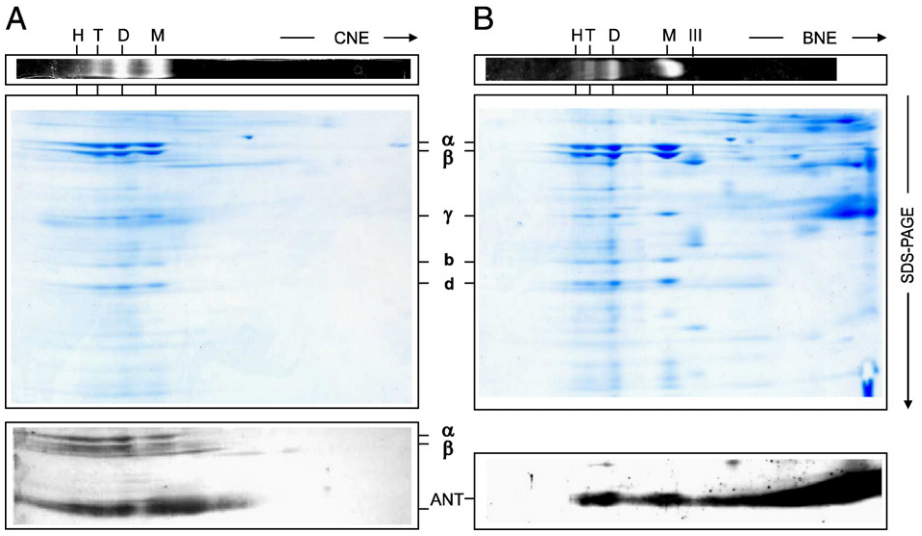

Fig. 3. The ADP/ATP translocator (ANT) interacts with monomeric and oligomeric ATP synthase. M, D, T, H, monomeric, dimeric, tetrameric, and hexameric ATP synthase, respectively; III, respiratory complex III. Bovine heart mitochondria were solubilized by digitonin (2 g/g protein) and separated by (A) CNE and (B) BNE. Monomeric and oligomeric ATP synthase forms were identified by in-gel ATP hydrolysis assay in the native gels (upper panels). Similar strips from 1-D native gels were processed by 2-D SDS-PAGE and Coomassie-stained (central panels) or blotted on PVDF membranes for immunodetection using a polyclonal ANT antibody (lower panels). Peripheral stalk subunits b and d and F_1-subunits α and β are assigned. Detection of hexameric ATP synthase (H) in the upper and central panels of figure part B but not in the lower panel of figure part B is explained by the use of similar but not identical 1-D BNE gels.

far). Comparable results with ANT bound to all oligomeric forms of ATP synthase were also obtained using more digitonin to solubilize membranes for CNE (4 g/g and 8 g/g; immunoblots not shown).

The situation is different for BNE. Negatively charged Coomassie dye binds to protein surfaces and imposes a charge shift on proteins. This makes even basic proteins migrating towards the anode. Therefore, most ANT was fast migrating to the anode as individual protein and finally was found near the electrophoretic front, i.e. on the right side of 2-D BN/SDS gels and 2-D immunoblots (Fig. 3B, lower panel). Some signal intensity was also observed at the positions of monomeric (M), dimeric (D), and tetrameric (T) ATP synthase. Using more digitonin to solubilize membranes for BNE (4 g/g and 8 g/g protein) dissociated oligomeric ATP synthase into the monomeric form, and binding of ANT to the monomeric ATP synthase could no longer be detected (not shown).

Together, the results using CNE and BNE support previous reports on the physiological association of ATP synthase and ANT and the existence of ATP synthasomes that assemble to supramolecular structures in the mitochondrial membrane. This is also in line with the observation that ATP synthase and ANT are predominantly located in the cristae membrane and show a similar distribution [77].

Viral mitochondria-localized inhibitor of apoptosis (vMIA) which is one of the two antiapoptotic proteins encoded by the human cytomegalovirus (CMV) affects mitochondrial morphology and ATP synthesis via the ATP synthasome in addition to its antiapoptotic effect. Upon vMIA expression in human cell lines, mitochondria looked rounder and smaller, the mitochondrial network was fragmented, and the cristae number was reduced [78]. A direct interaction of vMIA with constituents of the ATP synthasome, as reported for the ANT [79,80] can only take place at sites where both mitochondrial membranes are connected, since the ATP synthasome and vMIA are located in the inner and outer mitochondrial membranes, respectively. The dramatic changes in mitochondrial morphology and ATP synthesis by vMIA thus may be based on specific alterations in a relatively few contact sites that contain ATP synthasomes and potentially are central for the organization of the supramolecular structure of ATP synthase or ATP synthasomes.

7. Functional roles of dimeric and oligomeric ATP synthase

Mitochondrial ATP synthase can be isolated as a monomeric, fully active and oligomycin-sensitive enzyme. Immediate roles of dimerization and oligomerization for the catalytic ATP synthase activity therefore are unlikely. Several advantages for physiological dimerization and oligomerization of the ATP synthase are conceivable: (i) Connecting two stator parts in a dimeric enzyme can stabilize the holo-enzyme structure, in particular since dynamic rotor/stator interactions must continuously be closed and opened which facilitates dissociation of protein components and modules. (ii) An angular association of two ATP synthase monomers by membrane/peripheral stalk domains can induce bending of membranes. Therefore, arranging multiple dimers as dimer ribbons [13] or as helical double rows [12] can favor cristae formation and stability. (iii) Extended cristae enlarge the surface area available for respiratory chain complexes and ATP synthase. (iv) Altered arrangement of respiratory chain complexes, ANT, and ATP synthase relative to each other may interfere with fast metabolite/substrate channelling and/or efficient cooperation of complexes. In this way the supramolecular organization of the ATP synthase would indirectly affect the overall flux through the respiratory chain and result in a lower membrane potential. Stable association of ATP synthase to oligomeric structures could thus be essential for the maintenance of bioenergetically fully competent mitochondria [81].

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References

[1] Y. Hatefi, The mitochondrial electron transport and oxidative phosphorylation system, Ann. Rev. Biochem. 54 (1985) 1015–1069.
[2] M. Saraste, Oxidative phosphorylation at the fin de siècle, Science 283 (1999) 1488–1493.
[3] P.D. Boyer, The ATP synthase — a splendid molecular machine, Ann. Rev. Biochem. 66 (1999) 717–749.
[4] C. Gibbons, M.G. Montgomery, A.G. Leslie, J.E. Walker, The structure of the central stalk in bovine F_1-ATPase at 2.4 Å resolution, Nat. Struct. Biol. 11 (2000) 1055–1061.
[5] S. Karrasch, J.E. Walker, Novel features in the structure of bovine ATP synthase, J. Mol. Biol. 290 (1999) 379–384.
[6] J.L. Rubinstein, J.E. Walker, R. Henderson, Structure of the mitochondrial ATP synthase by electron microscopy, EMBO J. 22 (2003) 6182–6192.
[7] J.L. Rubinstein, V.K. Dickson, M.J. Runswick, J.E. Walker, ATP synthase from *Saccharomyces cerevisiae*: location of subunit h in the peripheral stalk region, J. Mol. Biol. 345 (2005) 513–520.
[8] D. Stock, A.G. Leslie, J.E. Walker, Molecular architecture of the rotary motor in ATP synthase, Science 286 (1999) 1700–1705.
[9] D. Sabbert, S. Engelbrecht, W. Junge, Intersubunit rotation in active F-ATPase, Nature 381 (1996) 623–625.
[10] H. Noji, R. Yasuda, M. Yoshida, K. Kinosita, Direct observation of the rotation of F_1-ATPase, Nature 386 (1997) 299–302.
[11] H. Seelert, A. Poetsch, N.A. Dencher, A. Engel, H. Stahlberg, D.J. Muller, Structural biology. Proton-powered turbine of a plant motor, Nature 405 (2000) 418–419.
[12] R.D. Allen, C.C. Schroeder, A.K. Fok, An investigation of mitochondrial inner membranes by rapid-freeze deep-etch techniques, J. Cell Biol. 108 (1989) 2233–2240.
[13] M. Strauss, G. Hofhaus, R.R. Schröder, W. Kühlbrandt, Dimer ribbons of ATP synthase shape the inner mitochondrial membrane, EMBO J. 27 (2008) 1154–1160.
[14] I. Arnold, K. Pfeiffer, W. Neupert, R.A. Stuart, H. Schägger, Yeast mitochondrial F_1F_0-ATP synthase exists as a dimer: identification of three dimer-specific subunits, EMBO J. 17 (1998) 7170–7178.
[15] H. Schägger, K. Pfeiffer, Supercomplexes in the respiratory chains of yeast and mammalian mitochondria, EMBO J. 19 (2000) 1777–1783.
[16] G. Arselin, M.F. Giraud, A. Dautant, J. Vaillier, D. Brèthes, B. Coulary-Salin, J. Schaeffer, J. Velours, The GxxxG motif of the transmembrane domain of subunit e is involved in the dimerization/oligomerization of the yeast ATP synthase complex in the mitochondrial membrane, Eur. J. Biochem. 270 (2003) 1875–1884.
[17] I. Wittig, H. Schägger, Advantages and limitations of clear native polyacrylamide gel electrophoresis, Proteomics 5 (2005) 4338–4346.
[18] F. Krause, N.H. Reifschneider, S. Goto, N.A. Dencher, Active oligomeric ATP synthases in mammalian mitochondria, Biochem. Biophys. Res. Commun. 329 (2005) 583–590.
[19] P. Paumard, J. Vaillier, B. Coulary, J. Schaeffer, V. Soubannier, D.M. Mueller, D. Brèthes, J.P. di Rago, J. Velours, The ATP synthase is involved in generating mitochondrial cristae morphology, EMBO J. 21 (2002) 221–230.
[20] G.E. Tusnády, I. Simon, Principles governing amino acid composition of integral membrane proteins: application to topology prediction, J. Mol. Biol. 283 (1998) 489–506.
[21] G.E. Tusnády, I. Simon, The HMMTOP transmembrane topology prediction server, Bioinformatics 17 (2001) 849–850.
[22] K. Hofmann, W. Stoffel, TMbase — a database of membrane spanning proteins segments, Biol. Chem. Hoppe-Seyler 374 (1993) 166.
[23] G.I. Belogrudov, Y. Hatefi, Factor B and the mitochondrial ATP synthase complex, J. Biol. Chem. 277 (2002) 6097–6103.
[24] B. Meyer, I. Wittig, E. Trifilieff, M. Karas, H. Schägger, Identification of two proteins associated with mammalian ATP synthase, Mol. Cell. Proteomics 6 (2007) 1690–1699.
[25] T. Hashimoto, Y. Yoshida, K. Tagawa, Regulatory proteins of F_1F_0-ATPase: role of ATPase inhibitor, J. Bioenerg. Biomembr. 22 (1990) 27–38.
[26] N. Ichikawa, Y. Yoshida, T. Hashimoto, N. Ogasawara, H. Yoshikawa, F. Imamoto, K. Tagawa, Activation of ATP hydrolysis by an uncoupler in mutant mitochondria lacking an intrinsic ATPase inhibitor in yeast, J. Biol. Chem. 265 (1990) 6274–6278.
[27] A. Akashi, Y. Yoshida, H. Nakagoshi, K. Kuroki, T. Hashimoto, K. Tagawa, F. Imamoto, Molecular cloning and expression of a gene for a factor which stabilizes formation of inhibitor-mitochondrial ATPase complex from *Saccharomyces cerevisiae*, J. Biochem. (Tokyo) 104 (1988) 526–530.
[28] Y. Yoshida, T. Sato, T. Hashimoto, N. Ichikawa, S. Nakai, H. Yoshikawa, F. Imamoto, K. Tagawa, Isolation of a gene for a regulatory 15-kDa subunit of mitochondrial F_1F_0-ATPase and construction of mutant yeast lacking the protein, Eur. J. Biochem. 192 (1990) 49–53.
[29] T. Hashimoto, Y. Yoshida, K. Tagawa, Simultaneous bindings of ATPase inhibitor and 9K protein to F_1F_0-ATPase in the presence of 15K protein in yeast mitochondria, J. Biochem. (Tokyo) 108 (1990) 17–20.
[30] H. Iwatsuki, Y.M. Lu, K. Yamaguchi, N. Ichikawa, T. Hashimoto, Binding of an intrinsic ATPase inhibitor to the F_1F_0 ATPase in phosphorylating conditions of yeast mitochondria, J. Biochem. (Tokyo) 128 (2000) 553–559.
[31] S.H. Ackerman, A. Tzagoloff, Identification of two nuclear genes (ATP11, ATP12) required for assembly of the yeast F_1-ATPase, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 4986–4990.
[32] L. Lefebvre-Legendre, J. Vaillier, H. Benabdelhak, J. Velours, P.P. Slonimski, J.P. di Rago, Identification of a nuclear gene (FMC1) required for the assembly/stability of yeast mitochondrial F(1)-ATPase in heat stress conditions, J. Biol. Chem. 276 (2001) 6789–6796.

[33] S.H. Ackerman, A. Tzagoloff, ATP10, a yeast nuclear gene required for the assembly of the mitochondrial F_1–F_0 complex, J. Biol. Chem. 265 (1990) 9952–9959.
[34] K.G. Helfenbein, T.P. Ellis, C.L. Dieckmann, A. Tzagoloff, ATP22, a nuclear gene required for expression of the F_0 sector of mitochondrial ATPase in *Saccharomyces cerevisiae*, J. Biol. Chem. 278 (2003) 19751–19756.
[35] T.P. Ellis, K.G. Helfenbein, A. Tzagoloff, C.L. Dieckmann, Aep3p stabilizes the mitochondrial bicistronic mRNA encoding subunits 6 and 8 of the H^+-translocating ATP synthase of *Saccharomyces cerevisiae*, J. Biol. Chem. 279 (2004) 15728–15733.
[36] M.J. Payne, E. Schweizer, H.B. Lukins, Properties of two nuclear pet mutants affecting expression of the mitochondrial oli1 gene of *Saccharomyces cerevisiae*, Curr. Genet. 19 (1991) 343–531.
[37] X. Zeng, W. Neupert, A. Tzagoloff, The metalloprotease encoded by ATP23 has a dual function in processing and assembly of subunit 6 of mitochondrial ATPase, Mol. Biol. Cell. 18 (2007) 617–626.
[38] C. Osman, C. Wilmes, T. Tatsuta, T. Langer, Prohibitins interact genetically with Atp23, a novel processing peptidase and chaperone for the F_1F_0-ATP synthase, Mol. Biol. Cell. 18 (2007) 627–635.
[39] A.E. Frazier, R.D. Taylor, D.U. Mick, B. Warscheid, N. Stoepel, H.E. Meyer, M.T. Ryan, B. Guiard, P. Rehling, Mdm38 interacts with ribosomes and is a component of the mitochondrial protein export machinery, J. Cell Biol. 172 (2006) 553–564.
[40] L. Jia, M.K. Dienhart, R.A. Stuart, Oxa1 directly interacts with Atp9 and mediates its assembly into the mitochondrial F_1F_0-ATP synthase complex, Mol. Biol. Cell 18 (2007) 1897–1908.
[41] I. Arnold, K. Pfeiffer, W. Neupert, R.A. Stuart, H. Schägger, ATP synthase of yeast mitochondria. Isolation of subunit j and disruption of the ATP18 gene, J. Biol. Chem. 274 (1999) 36–40.
[42] J. Velours, G. Arselin, The *Saccharomyces cerevisiae* ATP synthase, J. Bioenerg. Biomembr. 32 (2000) 383–390.
[43] Y. Kagawa, E. Racker, Partial resolution of the enzymes catalyzing oxidative phosphorylation. IX. Reconstruction of oligomycin-sensitive adenosine triphosphatase, J. Biol. Chem. 241 (1966) 2467–2474.
[44] R. Carrozzo, I. Wittig, F.M. Santorelli, E. Bertini, S. Hofmann, U. Brandt, H. Schägger, Subcomplexes of human ATP synthase mark mitochondrial biosynthesis disorders, Ann. Neurol. 59 (2006) 265–275.
[45] J.A. Silvester, V.K. Dickson, M.J. Runswick, A.G. Leslie, J.E. Walker, The expression, purification, crystallization and preliminary X-ray analysis of a subcomplex of the peripheral stalk of ATP synthase from bovine mitochondria, Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 62 (2006) 530–533.
[46] V.K. Dickson, J.A. Silvester, I.M. Fearnley, A.G. Leslie, J.E. Walker, On the structure of the stator of the mitochondrial ATP synthase, EMBO J. 25 (2006) 2911–2918.
[47] J.E. Walker, V.K. Dickson, The peripheral stalk of the mitochondrial ATP synthase, Biochim. Biophys. Acta 1757 (2006) 286–296.
[48] I. Wittig, J. Velours, R.A. Stuart, H. Schägger, Characterization of domain-interfaces in monomeric and dimeric ATP synthase, Mol. Cell. Proteomics 7 (2008) 995–1004.
[49] H. Ueno, T. Suzuki, K. Kinosita Jr., M. Yoshida, ATP-driven stepwise rotation of F_0F_1-ATP synthase, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 1333–1338.
[50] P. Dimroth, C. von Ballmoos, T. Meier, Catalytic and mechanical cycles in F-ATP synthases, EMBO Rep. 7 (2006) 276–282.
[51] B.A. Feniouk, M.A. Kozlova, D.A. Knorre, D.A. Cherepanov, A.Y. Mulkidjanian, W. Junge, The proton-driven rotor of ATP synthase: ohmic conductance (10 fS), and absence of voltage gating, Biophys. J. 86 (2004) 4094–4109.
[52] G. Arselin, J. Vaillier, B. Salin, J. Schaeffer, M.-F. Giraud, A. Dautant, D. Brèthes, J. Velours, The modulation in subunits e and g amounts of yeast ATP synthase modifies mitochondrial cristae morphology, J. Biol. Chem. 279 (2004) 40392–40399.
[53] P.D. Gavin, M. Prescott, S.E. Luff, R.J. Devenish, Cross-linking ATP synthase complexes in vivo eliminates mitochondrial cristae, J. Cell. Sci. 117 (2004) 2333–2343.
[54] P.D. Gavin, M. Prescott, R.J. Devenish, Yeast F_1F_0-ATP synthase complex interactions in vivo can occur in the absence of the dimer specific subunit e, J. Bioenerg. Biomembranes 37 (2005) 55–66.
[55] R. Fronzes, T. Weimann, J. Vaillier, J. Velours, D. Brèthes, The peripheral stalk participates in the yeast ATP synthase dimerization independently of e and g subunits, Biochemistry 45 (2006) 6715–6723.
[56] V. Everard-Gigot, C.D. Dunn, B.M. Dolan, S. Brunner, R.E. Jensen, R.A. Stuart, Functional analysis of subunit e of the F_1F_0-ATP synthase of the yeast *Saccharomyces cerevisiae*: importance of the N-terminal membrane anchor region, Eukaryot. Cell 4 (2005) 346–355.
[57] D.M. Bustos, J. Velours, The modification of the conserved GXXXG motif of the membrane-spanning segment of subunit g destabilizes the supramolecular species of yeast ATP synthase, J. Biol. Chem. 280 (2005) 29004–29010.
[58] S. Saddar, R.A. Stuart, The yeast F_1F_0-ATP synthase. Analysis of the molecular organization of subunit g and the importance of a conserved GXXXG motif, J. Biol. Chem. 280 (2005) 24435–24442.
[59] C. Spannagel, J. Vaillier, G. Arselin, P.V. Graves, X. Grandier-Vazeille, J. Velours, Evidence of a subunit 4 (subunit b) dimer in favor of the proximity of ATP synthase complexes in yeast inner mitochondrial membrane, Biochim. Biophys. Acta 1414 (1998) 260–264.
[60] J. Velours, P. Paumard, V. Soubannier, C. Spannagel, J. Vaillier, G. Arselin, P.V. Graves, Organisation of the yeast ATP synthase F(0): a study based on cysteine mutants, thiol modification and cross-linking reagents, Biochim. Biophys. Acta 1458 (2000) 443–456.
[61] V. Soubannier, F. Rusconi, J. Vaillier, G. Arselin, S. Chaignepain, P.V. Graves, J.M. Schmitter, J.L. Zhang, D. Mueller, J. Velours, The second stalk of the yeast ATP synthase complex: identification of subunits showing cross-links with known positions of subunit 4 (subunit b), Biochemistry 38 (1999) 15017–15024.
[62] R. Fronzes, S. Chaignepain, K. Bathany, M.F. Giraud, G. Arselin, J.M. Schmitter, A. Dautant, J. Velours, D. Brèthes, Topological and functional study of subunit h of the F_1F_0-ATP synthase complex in yeast *Saccharomyces cerevisiae*, Biochemistry 42 (2003) 12038–12049.
[63] M.F. Giraud, P. Paumard, V. Soubannier, J. Vaillier, G. Arselin, B. Salin, J. Schaeffer, D. Brèthes, J.P. di Rago, J. Velours, Is there a relationship between the supramolecular organization of the mitochondrial ATP synthase and the formation of cristae? Biochim. Biophys. Acta 1555 (2002) 174–180.
[64] P. Paumard, G. Arselin, J. Vaillier, S. Chaignepain, K. Bathany, J.M. Schmitter, D. Brèthes, J. Velours, Two ATP synthases can be linked through subunits i in the inner mitochondrial membrane of *Saccharomyces cerevisiae*, Biochemistry 41 (2002) 10390–10396.
[65] N.V. Dudkina, J. Heinemeyer, W. Keegstra, E.J. Boekema, H.-P. Braun, Structure of dimeric ATP synthase from mitochondria: an angular association of monomers induces the strong curvature of the inner membrane, FEBS Lett. 579 (2005) 5769–5772.
[66] F. Minauro-Sanmiguel, S. Wilkens, J.J. Garcia, Structure of dimeric mitochondrial ATP synthase: novel F_0 bridging features and the structural basis of mitochondrial cristae biogenesis, Proc. Natl. Acad. Sci.U. S. A. 102 (2005) 12356–12358.
[67] N.V. Dudkina, S. Sunderhaus, H.P. Braun, E.J. Boekema, Characterization of dimeric ATP synthase and cristae membrane ultrastructure from Saccharomyces and Polytomella mitochondria, FEBS Lett. 580 (2006) 3427–3432.
[68] E. Cabezon, P.J.G. Butler, M.J. Runswick, J.E. Walker, Modulation of the oligomerization state of the bovine F_1-ATPase inhibitor protein, IF_1, by pH, J. Biol. Chem. 275 (2000) 25460–25464.
[69] E. Cabezón, I. Arechaga, P. Jonathan, G. Butler, J.E. Walker, Dimerization of bovine F_1-ATPase by binding the inhibitor protein, IF_1, J. Biol. Chem. 275 (2000) 28353–28355.
[70] M. Dienhart, K. Pfeiffer, H. Schägger, R.A. Stuart, Formation of the yeast F_1F_0-ATP synthase dimeric complex does not require the ATPase inhibitor protein Inh1, J. Biol. Chem. 277 (2002) 39289–39295.
[71] J.J. García, E. Morales-Ríos, P. Cortés-Hernandez, J.S. Rodríguez-Zavala, The inhibitor protein (IF_1) promotes dimerization of the mitochondrial F_1F_0-ATP synthase, Biochemistry 45 (2006) 12695–12703.
[72] C. Chen, Y. Ko, M. Delannoy, S.J. Ludtke, W. Chiu, P.L. Pedersen, Mitochondrial ATP synthasome: three-dimensional structure by electron microscopy of the ATP synthase in complex formation with carriers for Pi and ADP/ATP, J. Biol. Chem. 279 (2004) 31761–31768.
[73] Y.H. Ko, M. Delannoy, J. Hullihen, W. Chiu, P.L. Pedersen, Mitochondrial ATP synthasome. Cristae-enriched membranes and a multiwell detergent screening assay yield dispersed single complexes containing the ATP synthase and carriers for Pi and ADP/ATP, J. Biol. Chem. 278 (2003) 12305–12309.
[74] H. Schägger, W.A. Cramer, G. von Jagow, Analysis of molecular masses and oligomeric states of protein complexes by blue native electrophoresis and isolation of membrane protein complexes by two-dimensional native electrophoresis, Anal. Biochem. 217 (1994) 220–230.
[75] I. Wittig, H.P. Braun, H. Schägger, Blue-Native PAGE, Nature Protocols 1 (2006) 416–428.
[76] E. Zerbetto, L. Vergani, F. Dabbeni-Sala, Quantification of muscle mitochondrial oxidative phosphorylation enzymes via histochemical staining of blue native polyacrylamide gels, Electrophoresis 18 (1997) 2059–2064.
[77] F. Vogel, C. Bornhövd, W. Neupert, A.S. Reichert, Dynamic subcompartmentalization of the mitochondrial inner membrane, J. Cell Biol. 175 (2006) 237–247.
[78] D. Poncet, A.L. Pauleau, G. Szabadkai, A. Vozza, S.R. Scholz, M. Le Bras, J.J. Brière, A. Jalil, R. Le Moigne, C. Brenner, G. Hahn, I. Wittig, H. Schägger, C. Lemaire, K. Bianchi, S. Souquère, G. Pierron, P. Rustin, V.S. Goldmacher, R. Rizzuto, F. Palmieri, G. Kroemer, Cytopathic effects of the cytomegalovirus-encoded apoptosis inhibitory protein vMIA, J. Cell Biol. 174 (2006) 985–996.
[79] V.S. Goldmacher, L.M. Bartle, A. Skaletskaya, C.A. Dionne, N.L. Kedersha, C.A. Vater, J.W. Han, R.J. Lutz, S. Watanabe, E.D. Cahir McFarland, E.D. Kieff, E.S. Mocarski, T. Chittenden, A cytomegalovirus-encoded mitochondria-localized inhibitor of apoptosis structurally unrelated to Bcl-2, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 12536–12541.
[80] H.L. Vieira, A.S. Belzacq, D. Haouzi, F. Bernassola, I. Cohen, E. Jacotot, K.F. Ferri, C. El Hamel, L.M. Bartle, G. Melino, C. Brenner, V. Goldmacher, G. Kroemer, The adenine nucleotide translocator: a target of nitric oxide, peroxynitrite, and 4-hydroxynonenal, Oncogene 20 (2001) 4305–4316.
[81] C. Bornhövd, F. Vogel, W. Neupert, A.S. Reichert, Mitochondrial membrane potential is dependent on the oligomeric state of F_1F_0-ATP synthase supracomplexes, J. Biol. Chem. 281 (2006) 13990–13998.