Journal of Bioenergetics and Biomembranes, Vol. 32, No. 4, 2000

The Structural and Functional Connection between the Catalytic and Proton Translocating Sectors of the Mitochondrial F_1F_0-ATP Synthase

Sergio Papa,[1,3] Fzaneo Zanotti,[1,2] and Antonio Gaballo[1]

The structural and functional connection between the peripheral catalytic F_1 sector and the proton-translocating membrane sector F_0 of the mitochondrial ATP synthase is reviewed. The observations examined show that the N-terminus of subunit γ, the carboxy-terminal and central region of F_0I-PVP(b), OSCP, and part of subunit *d* constitute a continuous structure, the lateral stalk, which connects the peripheries of F_1 to F_0 and surrounds the central element of the stalk, constituted by subunits γ and δ. The ATPase inhibitor protein (IF_1) binds at one side of the F_1F_0 connection. The carboxy-terminal segment of IF_1 apparently binds to OSCP. The 42L-58K segment of IF_1, which is *per se* the most active domain of the protein, binds at the surface of one of the three α/β pairs of F_1, thus preventing the cyclic interconversion of the catalytic sites required for ATP hydrolysis.

KEY WORDS: F_1F_0-ATP synthase; adenosine triphosphatase-inhibitor protein; synthetic peptides; OSCP.

INTRODUCTION

This paper deals with the structural and functional connection between the catalytic F_1 sector and the proton-translocating F_0 sector of the mitochondrial ATP synthase. The globular F_1 sector, which protrudes in the inner aqueous space (B side), is connected to the membrane integral F_0 sector, by a stalk (Senior, 1990; Cox *et al.*, 1992; Collinson *et al.*, 1994a; Papa *et al.*, 1999). The stalk, which is essential for energy coupling between catalysis in F_1 and proton translocation in F_0, is comprised of F_1 and F_0 subunits (see Table I). Bovine F_1 was resolved at atomic level by X-ray crystallography in 1994 (Abrahams *et al.*, 1994). More recently, the X-ray crystallographic structure of a subcomplex of yeast mitochondrial ATP synthase, showing F_1 connected by γ subunit to 10 copies of subunits *c* has been published (Stock *et al.*, 1999).

Contrary to a previous general view, based on electron microscopy (Fernandez-Moran, 1962; Gogol *et al.*, 1987; Capaldi *et al.*, 1994; Walker and Collinson, 1994), according to which the stalk subunits were conceived to be assembled in a single structure connecting the centers of F_1 and F_0, cross-linking results (Capaldi *et al.*, 1994; Ogilvie *et al.*, 1997) and average analysis of electron microscopy images (Wilkens and Capaldi, 1998; Bottcher *et al.*, 1998; Karrasch and Walker, 1999) indicate that in addition to a central stalk there is a second lateral one connecting the peripheries of F_0 and F_1. In the V-type ATPase there are, apparently, two lateral stalks (Boekema *et al.*, 1999).

The crystal structure of isolated bovine-heart mitochondrial F_1 shows a stem protruding out of the bottom center of the spherical body, which is comprised of hexamer of alternating 3α and 3β subunits (Abrahams *et al.*, 1994; Stock *et al.*, 1999). The stem represents part of the γ subunit. The N- and C-termini of this subunit form a coiled-coil, which penetrates the central cavity in the 3α3β hexamer. The lower

[1] Department of Medical Biochemistry and Biology, University of Bari, 70124 Bari, Italy.
[2] Centre for the Study of Mitochondria and Energy Metabolism, Consiglio Nazionale delle Ricerche, University of Bari, Italy.
[3] To whom all correspondence should be addressed: e-mail: papabehm@cimedoc.uniba.it

0145-479X/00/0800-0401$18.00/0

Table I. The Membrane Topology and Cysteine Residues of F_1F_0-ATPase Subunits from Bovine Heart Mitochondria[a]

Subunits	No. copies	Location	Mass (Da)	N-terminus	C-terminus	Cysteines
F_1						
α	3	External exagon	55164	M	M	C201/C251
β	3	External exagon	51595	M	M	0
γ	1	Internal cavity and stalk	30141	M	M	C91
δ	1	Stalk	15065	M	M	0
ε	1	Stalk	5652	M	M	C18
IF_1	1	Surface	9582	M	M	0
F_0						
F_0I-PVP(b)	1	Stalk-membrane	24670	M	M	C197
ATP6(a)	1	Transmembrane	24815	?	?	0
OSCP	1	Surface F_1 and stalk	20968	M	M	C118
d	1	Stalk-membrane	18603	M	M	C100
g	1	Transmembrane	11328	M	C	0
f	1	Transmembrane	10209	M	C	C72
F_6	1	Stalk-membrane	8958	M	M	0
e	1–2	Transmembrane	8189	M	C	0
c	9–12	Transmembrane	7608	C	C	C64
A6L	1	Transmembrane	7964	C	M	0

[a] The information given in the table represents a summary of data from Walker *et al.*, 1987, Houstek *et al.*, 1988, Oda *et al.*, 1989, Joshi *et al.*, 1990, Guerrieri *et al.*, 1991, Heckman *et al.*, 1991, Belogrudov *et al.*, 1996, and publications quoted therein. M, matrix side, C, cytosolic side.

central part of γ extends throughout the stalk and contacts the polar inner loop of *c* subunits (Stock *et al.*, 1999), whose hydrophobic N- and C- α-helixes span the membrane (Watts *et al.*, 1995; Fillingame, 1997).

Mutational analysis, cross-linking experiments, and X-ray crystallography (Stock *et al.*, 1999) show that γ, together with ε in the *E. coli* (Aggeler *et al.*, 1997) and δ in the mitochondrial ATP synthase (Karrasch and Walker, 1999), constitute the central stalk, which would represent the rotary shaft in the proposed motor of the synthase (Abrahams *et al.*, 1994; Boyer, 1997; Engelbrecht and Junge, 1997; Elston *et al.*, 1998; Oster and Wang, 1999).

The structural and functional organization of F_1 and F_0 subunits in the stalk of mitochondrial ATP synthase have been studied with different approaches: limited proteolysis of F_0 and F_1 subunits, immunodecoration by subunit specific antibodies, crosslinking of near-neighbor subunits, and *in vitro* assembly of stalk complexes from F_0 and F_1 subunits.

Papa and co-workers (Zanotti *et al.*, 1988; Papa *et al.*, 1989) found that, after F_1 removal, trypsin digested a substantial part of the F_0I-PVP(b) subunit from its C-terminus. Collinson *et al.* (1994b) confirmed this and showed that the digestion could reach the Lys120–Arg121 bond, the remaining N-terminal part apparently being shielded from trypsin digestion. The trypsin digestion of F_0I-PVP(b) in F_1-depleted inside-out vesicles of the inner mitochondrial membrane resulted in inhibition of proton conduction in F_0 and loss of oligomycin inhibition of this process (Zanotti *et al.*, 1988). Both processes were completely restored by the addition of the isolated native F_0I-PVP(b) protein to the digested F_0 membrane vesicles. These observations indicate that the membrane-extrinsic C-terminal part of F_0I-PVP(b) has a critical role in the functional organization of the membrane-intrinsic components of the proton channel in F_0 (see also Paul *et al.*, 1992; Schneider and Altendorf, 1984, 1985) and is responsible for the oligomycin inhibition of proton conduction in F_0. The facility with which the isolated F_0I-PVP(b) could be reconstituted with mitochondrial F_0 vesicles in which the endogenous subunit had been truncated by trypsin (Zanotti *et al.*, 1988), indicated that F_0I-PVP(b) is associated with its N-terminal hydrophobic region at the membrane periphery (see Schneider and Altendorf, 1987; Fillingame, 1997) and not in the center of the bundle of the α-helixes of *c* subunits, as previously proposed (Cox *et al.*, 1992). Subsequently atomic force microscopy (AFM) of the *E. coli* ATP synthase provided images showing directly that the *b* subunit is located at one side of the ring of the *c* subunits (Takeyasu *et al.*, 1996; Singh *et al.*,

1996; see also Birkenhager *et al.*, 1995; Fillingame *et al.*, 1998; Capaldi *et al.*, 2000).

Immunodecoration with subunit specific antibodies, of the bovine ATP synthase in mitoplasts and inside-out vesicles of the inner mitochondrial membrane, show that the F_0 subunits F_0I-PVP(b), *d*, F_6, and OSCP are exposed at the matrix, but not at the cytosolic side of the inner mitochondrial membrane, while subunits *c* and *a* are occluded to their antibodies on both sides (Heckman *et al.*, 1991). A6L appears to be anchored to the membrane with its N-terminal region, the C-terminal part being exposed at the matrix side (Heckman *et al.*, 1991) (see Table I). Subunits *f* and *g* were found to be both exposed with the N-terminus at the matrix side and the C-terminus at the cytosolic side of the membrane, while the *e* subunit appeared to be exposed essentially at the cytosolic side (Belogrudov *et al.*, 1996).

Collinson *et al.* (1994a) overexpressed in *E. coli* the entire F_0I-PVP(b) subunit, as well as its C-terminal domain, F_6, OSCP, and the *d* subunit and studied their association *in vitro*. They obtained an assembly of a stoichiometric quaternary complex of OSCP–F_6–*d* and the entire or C-terminal part of subunit F_0I-PVP(b). This quaternary complex produced a stoichiometric pentameric complex with the isolated F_1-ATPase. It was concluded that the quaternary OSCP–F_0I-PVP(b)–*d*–F_6 complex obtained *in vitro* constitutes the essential part of the stalk in the native F_1F_0-ATP synthase.

THE CONTRIBUTION OF THE N-TERMINUS OF SUBUNIT α TO THE F_1, F_0 CONNECTION

In the *E.coli*, the *b* subunit extends with the central and carboxy-terminal part throughout the stalk and is apparently connected by the δ subunit to the N-terminus of the α (Dunn *et al.*, 1980; Mendel-Hartvig and Capaldi, 1991; Ogilvie *et al.*, 1997; Wilkeus *et al.* 1997) or of the α and β subunits (Wilkens *et al.*, 2000). It has been proposed that in the mitochondrial ATP synthase the F_0I-PVP(b) subunit would have a similar location (Hundal *et al.*, 1983).

In the X-ray crystallographic structure of the bovine F_1, the N-terminal regions of subunits α and β detect a dimple at the top of the α3β3 hexagon, which is opposite to the contact side of F_1 with F_0 (Abrahams *et al.*, 1994). Electron microscopy analysis shows that in mitochondrial (Karrasch and Walker, 1999), as well as in the *E.coli* F_1F_0 complex (Wilkens and Capaldi, 1998; Wilkens *et al.*, 2000), this dimple is occupied by a cap (see Fig. 1).

Limited trypsin digestion of isolated F_1, selectively removes, under mild digestion conditions, 15 and 7 amino acids from the N-termini of the α and β subunits, respectively (Walker *et al.*, 1985; Xu *et al.*, 1998). The cooperativity for ATP hydrolysis by soluble F_1 was impaired by this trypsin digestion, but the affinity for AMP–PNP and GTP hydrolysis were not influenced. The inhibition of ATP hydrolysis by ADP was attenuated by trypsin digestion. Trypsin digestion of F_1 did not affect its capacity to bind to F_0 nor did it alter the sensitivity of ATP hydrolysis in the F_1F_0

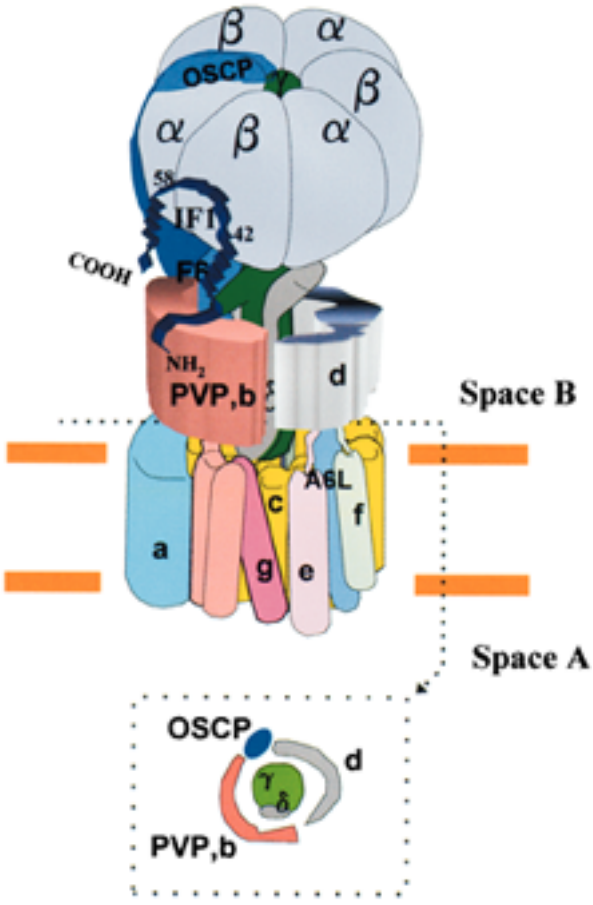

Fig. 1. Tentative oligomeric structure of the mitochondrial F_1F_0-ATP synthase complex. The F_1 structure is a sketch based on the crystal structure of bovine F_1 (Abrahams *et al.*, 1994). The structure of F_0 and stalk(s) is drawn on the basis of the observations reviewed and their interpretation in this paper. The model shows also the IF_1 protein on the surface of the F_1-F_0 connection, bound to OSCP and at an α–β interface.

reconstituted system to oligomycin and *N,N′*-dicyclohexylcarbodiimide (DCCD). The cleavage of the α and β subunits did, on the other hand, impair the ATP-driven proton pumping in the reconstituted F_1F_0 complex, the inhibition by F_1 of passive proton conduction in F_0, and the inhibition of passive proton conduction in F_0 by AMP–PNP binding to F_1. These results (Xu *et al.*, 1998) show that the limited cleavage of the N-termini of the α and β subunits, located at the top of F_1, results in decoupling of catalysis from proton transport.

In MgATP-submitochondrial particles, which consist of inside-out vesicles of the inner mitochondrial membrane with a functionally competent F_1F_0-ATP synthase, as well as in the isolated F_1F_0-ATP synthase complex from bovine heart, the N-terminus of the α subunits was found to be shielded against digestion by mild treatment with trypsin, while the N-terminus of the three β subunits was still cleaved off (Xu *et al.*, 2000). Under these conditions, there was no impairment of the ATPase activity, of its oligomycin sensitivity, and of ATP-driven proton pumping (Xu *et al.*, 2000).

It is conceivable that in the F_1F_0 complex, F_0 subunit(s) cover and/or induce a close configuration of the N-terminus of the α subunits, thus establishing interactions essential for energy coupling in the ATP synthase. The N-terminal region of subunits α could contribute to hold the C- and N-terminus of γ in the functional central position and/or provide a docking site for F_0 subunit(s), which contribute the lateral stalk (see Fig. 1).

THE CONTRIBUTION OF F_0 SUBUNITS TO THE STALK STRUCTURES

After removal of F_1 from the F_0 sector in the membrane, in addition to the F_0I-PVP(b) subunit, also OSCP and F6 can be cleaved by trypsin (Houstek *et al.*, 1988; Heckmann *et al.*, 1991; Collinson *et al.*, 1994b), with loss of oligomycin sensitivity of proton conduction in F_0 and of the ATPase activity of F_1 reconstituted with the digested F_0 sector. While oligomycin sensitivity of the first reaction was restored by the addition of F_0I-PVP(b) alone, restoration of the oligomycin sensitivity of the ATPase activity required, in addition to F_0I-PVP(b), also addition of OSCP and F6 (Guerrieri *et al.*, 1991). OSCP, rather than being by itself responsible for the oligomycin sensitivity of ATP hydrolysis appears, thus, to contribute to this effect by promoting correct assembly in the stalk of other F_0 proteins, like the hydrophilic portion of F_0I-PVP(b).

Cleavage of subunits α, OSCP, and *d*, produced in MgATP-SMP by more drastic treatment with trypsin, did not impair the ATPase activity, but suppressed ATP-driven proton pumping (Xu *et al.*, 2000). OSCP was easily and completely digested by trypsin in inside-out vesicles of the inner mitochondrial membrane, containing F_0 deprived of F_1. Addition of F_1 to these vesicles protected OSCP from trypsin digestion. The protective effect was abolished if, before reconstitution, the N-termini of subunits α and β were cleaved by trypsin in the soluble F_1 (Xu *et al.*, 2000). Since there are dozens of trypsin cleavage sites distributed all over the OSCP sequence (Ovchinnikov *et al.*, 1984), it is unlikely that the short N-terminal segments of subunits α and β, cleaved off by trypsin, can cover all OSCP. It is possible that interaction with these domains induces assembly of OSCP in the F_1F_0 complex in a position or configuration, which prevents trypsin digestion.

Reconstitution of soluble F_1 with inner membrane vesicles containing F_0 devoid of OSCP (AUSMP), but possibly also of other peripheral subunits, left the N-terminus of subunit α easily accessible to trypsin digestion. Adding back OSCP protected, but only part of the N-termini of subunits α against cleavage by trypsin (Xu *et al.*, 2000). Evidently, in the native F_1F_0 complex, other F_0 subunits, in addition to OSCP, are also anchored to the N-termini of subunits α and contribute to the lateral stalk. In the isolated F_1, trypsin can cleave subunit γ in smaller fragments (Xu *et al.*, 1998). The trypsin cleavage sites of subunit γ can be located, on the basis of crystallographic data (Abrahams *et al.*, 1994; see also Hausrath *et al.*, 1999) and predicted folding (Garnier *et al.*, 1996; Watts *et al.*, 1996), in a position intermediate between the N- and C-termini and the contact region of γ with the peripheral loop of *c* subunits. In the ATP synthase complex in inside-out MgATP particles, as well as in the isolated F_1F_0 complex, subunit γ, is completely inaccessible to digestion by trypsin (Xu *et al.*, 2000). This finding seems difficult to be reconciled with structural models in which the α3β3 hexamer of F_1 is connected to F_0 by a single lateral stalk, confined at one side of the complex, leaving most of the central part of the (rotating) subunit γ uncovered (Junge *et al.*, 1997; Wilkens and Capaldi, 1998; Elston *et al.*, 1998). It is conceivable that OSCP, the carboxyl region of F_0I-PVP(b), the trypsin cleavable part of subunit *d*, and eventually

other F_0 subunits, like A6L and F6 (Collinson *et al.*, 1994c; Papa *et al.*, 1999; Xu *et al.*, 2000), which are, in part, exposed at the F_1 side of F_0 (Papa *et al.*, 1999), constitute a single (Wilkens and Capaldi, 1998; Bottcher *et al.*, 1998; Karrasch and Walker, 1999) or more (Boekema *et al.*, 1999; Ubbink-Kok *et al.*, 2000) lateral stalk(s), surrounding the γ and δ subunits located in the central stalk (Fig.1). Such a ring structure, which can correspond to the collar structure seen in average electron microscopy images to constitute the foot of the stalk sitting on the membrane sector (Karrasch and Walker, 1999), could be formed in the *E.coli* by the lobes of the carboxyl region of the two *b* subunits. It is possible that the recent average analysis of electron microscopy images of ATP synthase and V-ATPase has so far revealed only part(s) of the lateral stalk.

FUNCTIONAL IMPACTS OF CROSSLINKING OF STALK SUBUNITS

Cysteine residues in the mitochondrial F_1F_0 subunits are very limited in number (see Table I) (Papa *et al.*, 1999). Diamide, which oxidizes vicinal thiol groups with the formation of disulfide bridges, can be conveniently used to analyze the nearest-neighbor relationship of cysteine domains in the F_1F_0 subunits and the functional impact of their relative immobilization by disulfide crosslinking (Zanotti *et al.*, 1985, 1988; Gaballo *et al.*, 1998).

Diamide treatment of the F_1F_0-ATP synthase in inside-out submitochondrial particles, containing the full complement of F_1F_0 subunits (ESMP), in the absence of a respiratory $\Delta\mu_{H^+}$, as well as of isolated F_0 reconstituted with F_1 or γ subunit was found to result in disulfide crosslinking between cysteine 197, in the carboxy-terminal region of the F_0I-PVP(b) subunit, and cysteine 91, at the carboxyl end of a small α-helix of subunit γ (Gaballo *et al.*, 1998). In submitochondrial particles respiring with succinate, the F_0I-PVP(b) and γ crosslinking caused dramatic enhancement of oligomycin-sensitive decay of the respiratory $\Delta\mu_{H^+}$ (Table II), which was associated with decoupling of respiratory ATP synthesis. It can be noted that diamide did not exert any inhibitory effect on respiration and on the H^+/e^- ratio for proton pumping, which was rather enhanced (see Gaballo *et al.*, 1998). These effects are consistent with the view that F_0I-PVP(b) and F_1-γ are components of the stator and the rotor of the proposed rotary motor, respectively. The fact that the carboxy-terminal region of F_0I-PVP(b) and the short α-helix of F_1-γ can form a direct disulfide bridge shows that these two protein domains are, at least in the resting state of the enzyme, in direct contact.

When submitochondrial particles were treated with diamide in the presence of $\Delta\mu_{H^+}$ generated by respiration, no crosslinking between F_0I-PVP(b) and γ subunits (Gaballo *et al.*, 1998), nor the associated effects on proton conduction and ATP synthesis were observed (see Table II). Crosslinking was restored in respiring ESMP by $\Delta\mu_{H^+}$ collapsing agents as well as by DCCD or oligomycin. These observations indicate that $\Delta\mu_{H^+}$ decay through F_0 induces a relative motion and/or a separation of the F_0I-PVP(b) subunit and γ, which places the single cysteine residues, present in each of the two subunits, at a distance at which they cannot be engaged in disulfide bridging.

Treatment of isolated F_0 with diamide produced crosslinking of OSCP with subunit *d*. Diamide-induced crosslinking of OSCP with subunit *d* had no significant effect on transmembrane proton conduction in F_0 reconstituted in liposomes (Xu *et al.*, 2000).

Extending the study of the impact of diamide on the functions of the F_1F_0 complex in ESMP, it was found in our laboratory that the disulfide cross-linking of subunits γ and F_0I-PVP(b), had no effect on the ATPase activity and proton translocation from the F_1, B side to the opposite A side, when the latter was driven by either ATP hydrolysis or a diffusion membrane potential (Gaballo *et al.*, 1999).

The experiments summarized in Fig. 2 show that anaerobic diamide treatment of ESMP, under conditions in which it induces disulfide crosslinking of γ and F_0I-PVP(b), resulted in marked enhancement of the oligomycin-sensitive proton translocation from the A to the B space, induced by a positive membrane potential inside the vesicles. Oligomycin-sensitive proton translocation through F_0 in the opposite direction from the B to the inner A space, induced by K^+ diffusion potential of an opposite sign was, on the contrary, unaffected by diamide treatment.

Figure 3 shows that anaerobic treatment of ESMP with diamide has no effect on proton translocation from the B (F_1-side) to the A space driven by ATP hydrolysis, under conditions in which the treatment accelerates the subsequent backflow of protons from the A to the B side. Kinetic analysis of ATP-driven proton translocation in ESMP monitored by ACMA fluorescence or neutral red absorbance showed that diamide treatment had no effect on the V_{max} and apparent K_m for proton translocation driven by ATP hydroly-

Table II. Differential Effects of Diamide on Succinate Respiratory Rate, Rate of $\Delta\mu\Delta\mu_{H^+}$ Decay, H^+/e^- and P/O Ratios in ESMP, and in MgATP–Submitochondrial Particles[a]

	Respiration Rate (ng O/min/mg)	Rate $\Delta\mu_{H}{}^{+}$ decay ng H^+/mg/min	H^+/e^-	ATP synthesis nmolATP/mg/min	P/O
ESMP	115 ± 9.6	69.5 ± 5.8	1.22 ± 0.20	29.3 ± 2.3	0.26 ± 0.04
ESMP + diamide[b]	129 ± 12.9	195.0 ± 19.5	3.09 ± 0.61	20.6 ± 1.3	0.16 ± 0.02
ESMP + diamide[c]	114 ± 8.8	71.9 ± 5.5	1.27 ± 0.19	30.3 ± 1.1	0.27 ± 0.03
MgATP − SMP	103 ± 7.8	34.0 ± 2.6	0.67 ± 0.10	71.4 ± 4.1	0.70 ± 0.09
MgATP − SMP + diamide[b]	114 ± 8.3	46.8 ± 3.4	0.83 ± 0.12	25.6 ± 3.2	0.23 ± 0.04
MgATP − SMP + diamide[c]	95 ± 5.9	30.3 ± 1.9	0.64 ± 0.08	63.2 ± 3.8	0.67 ± 0.08

[a] ESMP (3 mg protein /ml) or MgATP-SMP (3 mg protein/ml) were incubated in the succinate-reaction mixture containing: 200 mM sucrose, 30 mM KCl, 20 mM succinate, pH 7.4 at 25°C. Anaerobic or respiring particles were incubated 2 min with 2 mM diamide. To measure respiration-driven proton uptake and the following anaerobic relaxation of the respiratory proton gradient, the particles suspension was supplemented with valinomycin and catalase and respiration activated with pulses of H_2O_2. To measure P/O ratios of oxidative phosphorylation, particles (1 mg protein/ml) were incubated in a mixture containing: 200 mM sucrose, 10 mM K-succinate, 3 mM $MgCl_2$, 1 mM EDTA, 10 mM K-phosphate (pH 7.4), 20 mM glucose, 5 units hexokinase, and 300 μM p^1,p^5-Di (Adenosin-5)-pentaphosphate (to inhibit adenylate kinase), 300 μM MgADP was added and the respiratory rate measured for 5 min. The values reported in the table are the means of four experiments ± S.E.M.

[b] Submitochondrial particles pretreated with diamide in the anaerobic state.

[c] Submitochondrial particles pretreated with diamide in the succinate respiring state.

sis. Furthermore, direct spectrophotometric analysis of the kinetics of ATP hydrolysis showed that diamide treatment had no effect on the apparent V_{max} and K_m of this process (Gaballo *et al.*, 1999). It was concluded that diamide-induced disulfide crosslinking of F_0I-PVP(b) and F_1-γ, separate components of the stator and rotor of the proposed ATP synthase motor, respectively, results, as expected, in promotion of diffusion through F_0 from the A side to the B, F_1-side of the respiratory proton gradient, and decoupling of oxidative phosphorylation. The lack of any apparent effect of diamide-induced crosslinking of these two subunits on the ATPase activity and ATP-driven proton translocation from the B to the A side was, on the same grounds, unexpected. The finding that proton translocation from the B to the A side was unaffected by diamide treatment of ESMP, also when driven by a K^+ diffusion potential, in the absence of catalytic activity of F_1, shows that the promotion of H^+ release from the F_0 channel into the B space, caused by F_0I-PVP(b) and F_1-γ crosslinking, does not affect proton translocation in the reverse direction from the B to the A side. It can be mentioned, in this respect, that mutation of Leu156 to Arg in the a subunit (ATPase 6) in NARP patients has been found to be associated with decoupling of oxidative phosphorylation, in the absence of any effect on proton pumping driven by ATP hydrolysis (Baracca *et al.*, 2000). It is conceivable that there are different rate-limiting steps in the two directions of proton translocation through F_0.

Chemical crosslinking in the F_1F_0 complex (Duncan *et al.*, 1995; Aggeler *et al.*, 1997) and photochemistry experiments in the isolated F_1 sector have provided definite evidence showing that ATP hydrolysis promotes rotation of γ (Sabbert *et al.*, 1996; Noji *et al.*, 1997) and ε (Kato-Yamada *et al.*, 1998), relative to the α3β3 hexagon with generation of a torsional force (Cherepanov *et al.*, 1999). Evidence showing also ATP-driven rotation of *c* subunits in the isolated F_1F_0 complex has more recently been presented (Sambongi *et al.*, 1999; Panke *et al.* 2000; see, however, Tsunoda *et al.*, 2000).

The mechanism by which rotation of γ will make the *c* ring rotate, during ATP-driven proton pumping and by which $\Delta\mu_{H^+}$-driven rotation of the *c* ring induces rotation of γ during ATP synthesis, is not known. It can be speculated that electrostatic, acid–base interactions between polar residues in the hydrophylic loop of *c* with polar residues in γ and ε are involved in the coupling process (Fillingame, 1997; Cherepanov *et al.*, 1999). It might be possible that in the cross-linked enzyme, at least, the electrostatic interactions elicited by ATP hydrolysis directly promote injection of protons into the B side of the F_0 channel and their translocation to the A side, with possible promotion of rotation of the *c* ring.

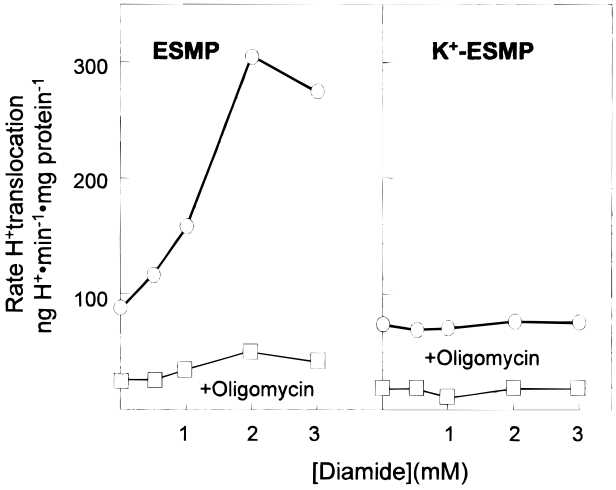

Fig. 2. Differential effects of diamide treatment on proton conduction in inside-out bovine submitochondrial particles (ESMP) and ESMP loaded with KCl (K^+–ESMP). ESMP (3mg/ml) were incubated in a succinate reaction medium, pH 7.4, in the presence of a constant stream of N_2. K^+-preloaded ESMP were incubated under the same conditions in the succinate reaction mixture without KCl. Once anaerobiosis was reached, diamide was added at the concentrations reported in the figure and, after 2 min, the reaction was stopped by centrifugation at 105,000 × *g*. ESMP and K^+-ESMP were resuspended in the same medium. ESMP, treated with diamide (○), were incubated for 2 min in 150 mM KCl. K^+-ESMP, treated with diamide (●), were incubated for 2 min in 250 mM sucrose. H^+ release in ESMP and H^+ uptake in K^+-ESMP were initiated by the addition of 2 μg/mg particle protein valinomycin. ESMP or K^+-ESMP treated with diamide (□) were incubated for 2 min with oligomycin (2 μg/mg particle protein) before adding valinomycin. Redrawn from Gaballo *et al.*, 1999.

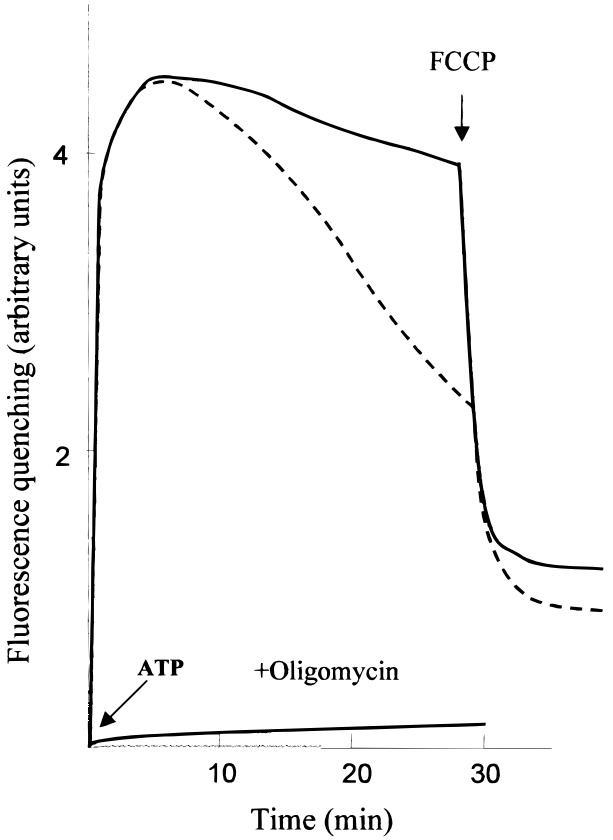

Fig. 3. Effect of diamide on ATP-driven H^+ uptake. Proton translocation driven by ATP hydrolysis was monitored by ACMA fluorescence quenching in ESMP preincubated for 2 min, under a constant stream of N_2 in the succinate medium in the absence (continous trace) or presence (dashed trace) of 2 mM diamide. Where indicated, oligomycin (2 μg/mg particle protein) and FCCP (2 μM) were added. Reproduced with permission from Gaballo *et al.*, 1999.

THE DOCKING SITES OF THE ATPase INHIBITOR PROTEIN

In mitochondria, the F_1F_0 complex is made to function essentially as ATP synthase by natural protein inhibitors, the best known of which is the ATPase inhibitor protein, IF_1 (Harris and Das, 1991; Lebowitz and Pedersen, 1996; Green and Grover, 2000). IF_1 associates reversibly in a 1:1 stoichiometry with the F_1F_0 complex in the membrane, as well as with the soluble F_1 moiety (Harris and Das, 1991). IF_1 is displaced from the complex in the membrane by the respiratory transmembrane electrochemical gradient (PMF) (Pedersen *et al.*, 1981; Harris and Das, 1991). The ΔpH component of the PMF, in particular, the matrix pH appears to be the critical factor for the binding and the inhibitory activity of IF_1. Thus IF_1 inhibits ATP hydrolysis, but is without significant effect on oxidative phosphorylation. In our laboratory it has been found that IF_1 inhibits also passive proton conduction by the F_1F_0 complex (Papa *et al.*, 1996) (see Table III). In the light of the new findings on the structure of the central and lateral stalks and of the proposed rotary mechanism of ATP synthase/hydrolase, knowledge of the binding site(s) of IF_1 is of particular interest. Crosslinking studies in yeast mitochondria show that the binding site of IF_1 to the F_1F_0 complex encompass both the α and β subunits (Mimura *et al.*, 1993) and probably includes the DELSEED sequence at residues β-394–400 (Jackson and Harris, 1988). This sequence of the three β subunits of F_1 is supposed to establish contact with the γ subunit during catalysis (Abrahams *et al.*, 1994).

As shown from the data summarized in Table III, the binding and inhibitory affinity of IF_1 is considerably higher in the particulate F_1F_0 complex than in the

Table III. Inhibitory Activities of ATP Hydrolysis and Passive Transmembrane H^+ Conduction in F_1F_0 Complex Exhibited by Natural IF_1, Synthetic IF_1 Peptide, and Recombinant IF_1[a]

Peptides activity	$I_{50}(\mu M)$[b]				
	F_0F_1-SMP			F_1	
	ATPase activity[d]		H^+ conduction	ATPase[d]	
	(21 °C)	(37 °C)	(21 °C)[e]	(21 °C)	(37 °C)
IF_1-(1–84)(n)[c]	0.64	0.036	0.46	1.25	0.84
IF_1-(1–84)(r)[c]	0.64	0.027	0.51	1.15	0.76
IF_1-(22–46)(cs)[c]	No effect	No effect	No effect	1.43	1.51
IF_1-(42–58)(cs)[c]	0.22	0.009	1.56	0.81	0.66

[a] The recombinant IF_1 was a generous gift of Prof. John Walker, Cambridge, U.K. From Papa *et al.*, 1996 and unpublished data.
[b] I_{50}, concentrations of peptides giving half-maximal inhibition of ATP hydrolysis and H^+ passive conduction.
[c] n, natural; cs, chemical synthesis; r, recombinant.
[d] IF_1 depleted submitochondrial particles (1 mg/ml) or soluble F_1 (0.1 mg/ml) were incubated in a mixture containing: 200 mM sucrose, 10 mM Tris/acetate, 1 mM K-EDTA, 6 mM $MgCl_2$, 1 mM ATP, pH 6.7 in the absence or presence of IF_1 in the concentration range 1 nM and 3 μM for 10 min at 21°C or 37°C. An aliquot of the suspension containing 50 μg particle protein (or 5 μg soluble F_1) was then added to 1 ml of ATPase assay mixture. ATP hydrolysis was determined by measuring the decrease in the absorbance of NADH at 340 nm. For experimental details see Papa *et al.*, (1996).
[e] Particles (10 mg/ml) were incubated in the above mixture in the absence or in the presence of IF_1 in a range of concentrations between 0.1 μM and 3 μM for 10 min at 21°C. An aliquot of the suspension containing 3 mg particle protein were diluted in a mixture containing: 200 mM sucrose, 30 mM KCl, 20 mM succinate as respiratory substrate, 0.5 μg valinomycin/mg particle protein, 0.2 mg/ml purified catalase, pH 7.5, followed by incubation in a glass vessel, under a constant stream of N_2, at 21°C. H^+ conduction was determined by following potentiometrically the anaerobic release of the respiratory proton gradient activated by repetitive pulses of H_2O_2.

purified F_1 moiety (Papa *et al.*, 1996; van Raaij *et al.*, 1996). The same applies to the synthetic peptide with the 42L–58K sequence of IF_1, which was found in our laboratory to be the segment with the highest inhibitory activity for ATP hydrolysis (Table III) (Papa *et al.*, 1996). It can, on the other hand, be noted that the peptide with the sequence 22F–46K of IF_1, which was proposed to represent the active domain (Stout *et al.*, 1993), while exhibiting in the isolated F_1, an inhibitory affinity comparable to that of IF_1 was uneffective on the membrane bound F_0F_1 complex (Papa *et al.*, 1996; Van Raaij et al 1996). These findings suggest that F_0 subunit(s) can contribute and/or promote the binding of IF_1 to the ATP synthase. In order to identify this (these) subunit(s), EDC-induced crosslinking of IF_1 with F_1F_0 subunits was analyzed in our laboratory, following, with specific antibodies, the native subunits and their cross-linking products (Raho *et al.*, in preparation). The results of these experiments (Fig. 4), show that EDC induces crosslinking of IF_1 with both the α/β subunits and OSCP. At saturating concentration of EDC, all IF_1 was crosslinked to the α/β subunits, whose initial amount was reduced by one third, and to OSCP, whose level was reduced by two-thirds, respectively (Fig. 4). EDC-induced crosslinking of IF_1 was found to be associated with inhibition of the

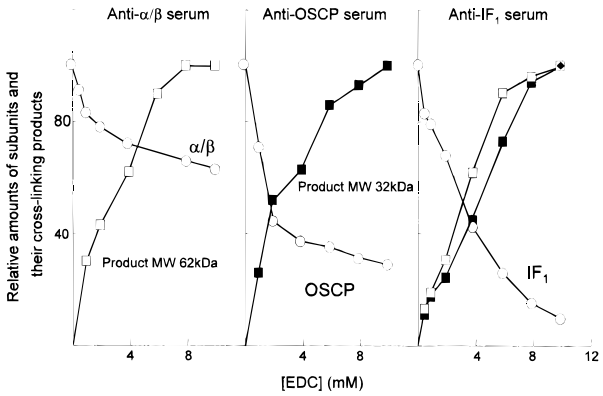

Fig. 4. Immunoblotting analysis of EDC-induced crosslinking of IF_1 with α/β and OSCP in MgATP submitochondrial particles. MgATP-SMP (2 mg/ml) were treated at 25°C at pH 7.4 with increasing amounts of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), which catalyzes intermolecular crosslinking with amide formation (Packer *et al.*, 1979), at the concentrations reported in the figure. After 10 min of incubation, the reaction was stopped by centrifugation at 105,000 × *g*. Control and EDC-treated materials were subjected to SDS/PAGE on a 12–15% gradient polyacrylamide, electrotransferred to nitrocellulose and immunodecorated with rabbit anti-F_1, OSCP, and IF_1 IgG's. Symbols: ○ amount of subunits (% of the control); □ cross-linking products between IF_1 and α/β subunits (presented as % of the maximum amount of the product produced); ■ cross-linking product between IF_1 and OSCP subunit.

ATPase activity and enhancement of proton conduction by the F_1F_0 complex. These results, which are in perfect agreement with the reported stoichiometry ratios of 3α, 3β, 1-OSCP, 1-IF_1 (Walker *et al.*, 1985), show that IF_1 can bind, through two distinct domains, simultaneously to one of the three α/β pairs and to OSCP. Selective trypsin digestion of the carboxy-terminal region of IF_1 associated to the membrane-bound F_1F_0 complex showed that this domain was involved in the crosslinking of IF_1 with OSCP. EDC-promoted crosslinking of IF_1 with the α/β subunits was, on the other hand, unaffected by trypsin digestion of the carboxy-terminal region of IF_1, indicating that the central and/or NH_2 terminus are involved in the binding of IF_1 to the α/β subunits.

CONCLUSIONS

The data reviewed show that the N-terminus of subunit α, OSCP, the central -C-terminal region of F_0I-PVP(b), and part of subunit *d* constitute a continuous structure, the lateral stalk, which connects F_1 to F_0 and is peripherally located with respect to the central stalk contributed by subunits γ and δ (Fig.1). The N-terminus of subunit α is located at the distal pole of the α3β3 hexamer of F_1. OSCP extends from the F_1F_0 junction along the lateral surface of the α3β3 hexamer to cover the N-terminus of subunit α. The latter can provide an anchoring site for OSCP. A number of observations reviewed here indicate that the lateral stalk is extended around and covers the central stalk. A ringlike structure will optimize the rotation of the central stalk along the main axis, perpendicular to the plane of the membrane, preventing its disordered tumbling.

The inhibitor protein IF_1 is located at one side of the lateral stalk (Fig. 5). In the absence of PMF, IF_1 binds with the carboxy-terminal segment to OSCP and with the 42–58 segment and the N-terminus to one of the three α/β pairs, thus blocking the cyclic interconversion of the three catalytic sites required for ATP hydrolysis. The superficial location of IF_1 could allow simultaneous binding of dimeric IF_1 to dimeric F_1F_0 complexes (Arnold *et al.*, 1998) as proposed by Cabezon *et al.* (2000).

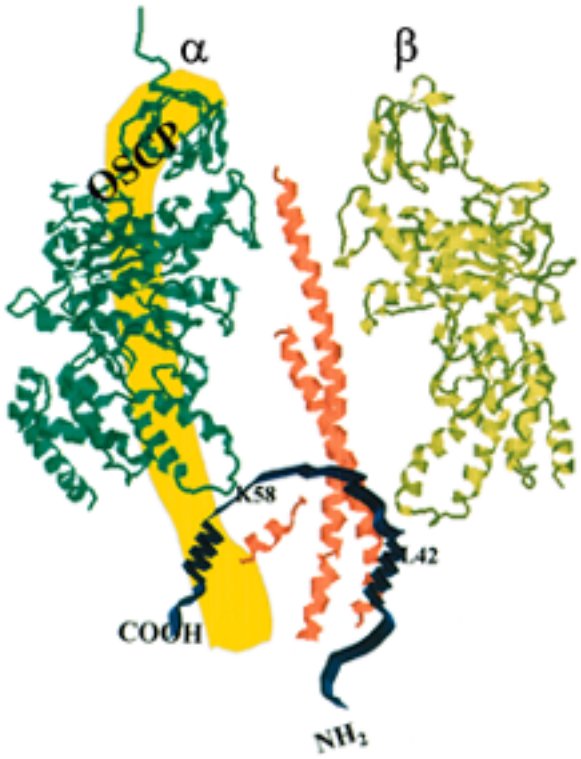

Fig. 5. Details of the location of the IF_1 protein in the ATP synthase. The three-dimensional structures of α, β, and γ are drawn from the PDB coordinates of the crystal structure of the bovine F_1 (Abrahams *et al.*, 1994) using the RAS Mol 2.6 Program. The mass of OSCP, ∞ yellow, is shown to extend from the stalk along the surface of an α subunit to cover its N-terminus. The IF_1 protein with long extended segments, which predominate in the absence of PMF (Lebowitz and Pedersen, 1996), is shown to be attached at the surface of F_1 and OSCP, with the carboxy-terminus bound to the latter and the L42-K58 segment located at an α–β interface in the empty configuration.

ACKNOWLEDGMENTS

This work was supported by grants from the National Project on Bioenergetics and Biomembranes of the Italian Ministry for the University and Scientific and Technological Research (MURST) and the finalized Project for Biotechnology of the Italian Research Council (CNR, Rome) Project No.99.00430.PF49. Dr Antonio Gaballo is supported by a research grant from the University of Bari within the CEE operative program 94/99 “Ricerca, Sviluppo Tecnologico ed Alta Formazione.”

REFERENCES

Abrahams, J. P., Leslie, A. G. W., Lutter, R., and Walker, J. E. (1994). *Nature (London)* **370**, 621–628.
Aggeler, R., Ogilvie, I., and Capaldi, R. A. (1997). *J. Biol. Chem.* **272**, 19621–19624.

Arnold, I., Pfeiffer, K., Neupert, W., Stuart, R. A., and Shagger, H. (1998). *EMBO J.* **17**, 7170–7178.
Baracca, A., Barogi, S., Carelli, V., Lenaz, G., and Solaini, G. (2000). *J. Biol. Chem.* **275**, 4177–4182.
Belogrudov, G. I., Tomich, J. M., and Hatefi, Y. (1996). *J. Biol. Chem.* **271**, 20340–20345.
Birkenhager, R., Hoppert, M., Deckers-Hebestreit, G., Mayer, F., and Altendorf, K. (1995). *Eur. J. Biochem.* **230**, 58–67.
Boekema, E. J., Van Breemen, J. F. L., Brisson, A., Ubbink-Kok, T., Konings, W. N., and Lolkema, J. S. (1999). *Nature (London)* **401**, 37–38.
Bottcher, B., Schwarz, L., and Graber, P. (1998). *J. Mol. Biol.* **281**, 757–762.
Boyer, P. D. (1997). *Annu. Rev. Biochem.* **66**, 717–749.
Cabezon, E., Butler, P. J. G., Runswick, M. J., and Walker, J. E. (2000). *J. Biol. Chem.*, **275**, 25460–25464.
Capaldi, R. A., Aggeler, R., Turina, P., and Wilkens, S. (1994). *Trends Biochem. Sci.* **19**, 284–289.
Capaldi, R. A., Schulenberg, B., Murray, J., and Aggeler, R. (2000). *J. Exp. Biol.* **203**, 29–33.
Cherepanov, D. A., Mulkidjanian, A. Y., and Junge, W. (1999). *FEBS Lett.* **449**, 1–6.
Collinson, I. R., van Raaij, M. J., Runswick, M. J., Fearnley, I. M., Skehel, J. M., Orris, G. L., Miroux, B., and Walker, J. E. (1994a). *J. Mol. Biol.* **242**, 408–421.
Collinson, I. R., Fearnley, I. M., Skehel, J. M., Runswick, M. J., and Walker, J. E. (1994b). *Biochem. J.* **303**, 639–645.
Collinson, I. R., Runswick, M. J., Buchanan, S. K., Fearnley, I. M., Skehel, J. M., van Raaij, M. J., Griffiths, D. E., and Walker, J. E. (1994c). *Biochemistry* **33**, 7971–7978.
Cox, G. B., Devenish, R. J., Gibson, F., Howitt, S. M., and Nagley, P. (1992). In *Molecular Mechanism in Bioenergetics* (Ernster, L., ed.). Elsevier, Amsterdam, The Netherlands, pp. 283–315.
Duncan, T. M., Bulygin, V. V., Zhou, Y., Hutcheon, M. L., and Cross, R. L. (1995). *Proc. Natl. Acad. Sci. USA* **92**, 10964–10968.
Dunn, S. D., Heppel, L. A., and Fullmer, C. S. (1980). *J. Biol. Chem.* **255**, 6891–6896.
Elston, T., Wang, H., and Oster, G. (1998). *Nature (London)* **391**, 510–513.
Engelbrecht, S. and Junge, W. (1997). *FEBS Lett.* **414**, 485–491.
Fernandez-Moran, H. (1962). *Circulation* **26**, 1039–1065.
Fillingame, R. H. (1996). *Curr. Opin. Struct. Biol.* **6**, 491–498.
Fillingame, R. H. (1997). *J. Exp. Biol.* **200**, 217–224.
Fillingame, R. H., Jones, P. C., Jiang, W., Valiyaveetil, F. I., and Dmitriev, O. Y. (1998). *Biochim. Biophys. Acta* **1365**, 135–142.
Gaballo, A., Zanotti, F., Raho, G., and Papa, S. (1999). *FEBS Lett.* **463**, 7–11.
Gaballo, A., Zanotti, F., Solimeo, A., and Papa S., (1998). *Biochemistry* **37**, 17519–17526.
Garnier J., Gibrat, J. F., and Robson, B. (1996). *Methods Enzymol.* **266**, 540–553.
Gogol, E. P., Lucken, U., and Capaldi, R. A. (1987). *FEBS Lett.* **219**, 274–278.
Green, D. W. and Grover, G. J. (2000). *Biochim. Biophys. Acta* **1458**, 343–355.
Guerrieri, F., Zanotti, F., Capozza, G., Colaianni, G., Ronchi, S., and Papa, S., (1991). *Biochim. Biophys. Acta* **1059**, 348–354.
Harris, D. A. and Das, A. M. (1991). *Biochem. J.* **280**, 561–573.
Hausrath, A. C., Gruber, G., Matthews, B. W., and Capaldi, R. A. (1999). *Proc. Natl. Acad. Sci. USA* **96**, 13697–13702.
Heckman, C., Tomich, J. M., and Hatefi, Y. (1991). *J. Biol. Chem.* **266**, 13564–13571.
Houstek, J., Kopecky, J., Zanotti, F., Guerrieri, F., Jirillo, E., Capozza, G., and Papa, S. (1988). *Eur. J. Biochem.* **173**, 1–8.
Hundal, T., Norling, B., and Ernster, L. (1983). *FEBS Lett.* **162**, 5–10.
Jackson, P. J. and Harris, D. A. (1988). *FEBS Lett.* **229**, 224–228.
Joshi, S. and Burrows, R. (1990). *J. Biol. Chem.* **265**, 14518–14525.
Junge, W., Lill, H., and Engelbrecht, S. (1997). *Trends Biochem. Sci.* **22**, 123–126.
Karrasch, S. and Walker, J. E. (1999). *J. Mol. Biol.* **290**, 379–384.
Kato-Yamada, Y., Noji, H., Yasuda, R., Kinosita, K., and Yoshida, M. (1998). *J. Biol. Chem.* **273**, 19375–19377.
Lebowitz, M. S. and Pedersen, P. L. (1996). *Azch. Biochem. Biophys.* **330**, 342–354.
Mendel-Hartvig, J. and Capaldi, R. A. (1991). *Biochim. Biophys. Acta* **1060**, 115–124.
Mimura, H., Hashimoto, T., Yoshida, Y., Ichikawa, N., and Tagawa, K. (1993). *J. Biochem.* **113**, 350–354.
Noji, H., Yasuda, R., Yoshida, M., and Kinosita, K. (1997). *Nature (London)* **386**, 299–302.
Oda, T., Futaky, S., Kitagawa, K., Yoshihara, Y., Tani I., and Higuti, T. (1989). *Biochem. Biophys. Res. Commun.* **165**, 449–456.
Ogilvie, I., Aggeler, R., and Capaldi, R. A. (1997). *J. Biol. Chem.* **272**, 16652–16656.
Oster, G. and Wang, H. (1999). *Structure* **7**, R 67–R72.
Ovchinnikov, Y. A., Modyanov, N. N., Grinkevich, V. A., Aldanova, N. A., Trubetskaya, O. E., Nazimov, I. V., Hundal, T., and Ernster, L. (1984). *FEBS Lett.* **166**, 19–22.
Packer, L., Tristram, S., Herz, J. M., Russel, C., and Borders, C. L. (1979). *FEBS Lett.* **108**, 243–248.
Panke, O., Gumbiowski, K., Junge, W., and Engelbrecht (2000). *FEBS Lett.* **472**, 34–38.
Papa, S., Guerrieri, F., Zanotti, F., Houstek, J., Capozza, G., and Ronchi, S. (1989). *FEBS Lett.* **249**, 62–66.
Papa, S., Zanotti, F., Cocco, T., Perrucci, C., Candita, C., and Minuto, M. (1996). *Eur. J. Biochem.* **240**, 461–467.
Papa, S., Xu, T., Gaballo, A., and Zanotti, F. (1999). In *Frontiers of Cellular Bioenergetics: Molecular Biology, Biochemistry and Physiopathology* (Papa, S., Guerrieri, F., Tager, J. M., eds.), Plenum Press, London, New York, pp. 459–487.
Paul, M. F., Guerin, B., and Velours, J. (1992). *Eur. J. Biochem.* **205**, 163–172.
Pedersen, P. L., Schwerzmann, K., and Cintron, N. M. (1981). *Curr. Top. Bioenerge.* **11**, 149–199.
Raho, G., Zanotti, F., Gaballo, A., Vuolo, R., and Papa, S. (2000). In preparation.
Sabbert, D., Engelbrecht, S., and Junge, W. (1996). *Nature (London)* **381**, 623–625.
Sambongi, Y., Iko, Y., Tanabe, M., Omote, H., Iwamoto-Kihara, A., Ueda, I., Yanagida, T., Wada, Y., and Futai, M. (1999). *Science* **286**, 1722–1724.
Schneider, E. and Altendorf, K. (1984). *Proc. Natl. Acad. Sci. USA* **81**, 7279–7283.
Schneider, E. and Altendorf, K. (1985). *Eur. J. Biochem.* **153**, 105–109.
Schneider, E. and Altendorf, K. (1987). *Microbiol. Rev.* **51**, 477–497.
Senior, A. E. (1990). *Annu. Rev. Biophys. Biophys. Chem.* **19**, 7–41.
Singh, S., Turina, P., Bustamante, C. J., Keller, D. J., and Capaldi, R. A. (1996). *FEBS Lett.* **397**, 30–34.
Stock, D., Leslie, A. G. W., and Walker, J. E. (1999). *Science* **286**, 1700–1705.
Stout, J. S., Partridge, B. E., Dibbern, D. A., and Schuster, S. M. (1993). *Biochemistry* **32**, 7496–7502.
Takeyasu, K., Omote, H., Nettikadan, S., Tokumasu, F., Iwamoto-Kihara, A., and Futai, M.(1996). *FEBS Lett.* **392**, 110–113.
Tsunoda, S. P., Aggeler, R., Noji, H., Kinosita, K., Yoshida, M., and Capaldi, R. A. (2000). *FEBS Lett.* **470**, 244–248.
Ubbink-Kok, T., Boekema, E. J., van Breemen, J. F., Brisson, A., Konings, W. N., and Lolkema, J. S. (2000). *J. Mol. Biol.* **296**, 311–321.
van Raaij, M. J., Orris, G. L., Montgomery, M. G., Runswick, M. J., Fearnley, I. M., Skehel, J. M., and Walker, J. E. (1996). *Biochemistry* **35**, 15618–15625.

Walker, J. E. and Collinson, I. R. (1994). *FEBS Lett.* **346**, 39–43.
Walker, J. E., Fearnley, I. M., Gay, N. J., Gibson, B. W., Northrop, F. D., Powell, S. J., Runswick, M. J., Saraste, M., and Tybulewicz, W. L. J. (1985). *J. Mol. Biol.* **184**, 677–701.
Walker, J. E., Runswick, M. J. and Poulter, L., (1987) *J. Mol. Biol.* **197**, 89–100.
Watts, S. D., Zhang, Y., Fillingame, R. H., and Capaldi, R. A. (1995). *FEBS Lett.* **368**, 235–238.
Watts, S. D., Tang, C., and Capaldi, R. A. (1996). *J. Biol. Chem.* **271**, 28341–28347.
Wilkens, S. and Capaldi, R. A. (1998). *Nature (London)* **393**, 29.
Wilkens, S., Dunn, S. D., Chandler, J., Dahlquist, F. W., and Capaldi, R. A. (1997). *Nature Struct. Biol.* **4**, 198–201.
Wilkens, S., Zhou, J., Nakayama, J., Dunn, S. D., and Capaldi, R. (2000). *J. Mol. Biol.* **295**, 387–391.
Xu, T., Candita, C., Amoruso, G., and Papa, S. (1998). *Eur. J. Biochem.* **252**, 155–161.
Xu, T., Zanotti, F., Gaballo, A., Raho, G., and Papa, S. (2000). *Eur. J. Biochem.* **267**, 4445–4455.
Zanotti, F., Guerrieri, F., Scarfò, R., Berden, J., and Papa, S. (1985). *Biochem. Biophys. Res. Commun.* **132**, 985–990.
Zanotti, F., Guerrieri, F., Capozza, G., Houstek, J., Ronchi, S., and Papa, S. (1988). *FEBS Lett.* **237**, 9–14.