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# Recent advances in stucture-functional studies of mitochondrial factor B

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**Abstract** Since the early studies on the resolution and reconstitution of the oxidative phosphorylation system from animal mitochondria, coupling factor B was recognized as an essential component of the machinery responsible for energy-driven ATP synthesis. At the phenomenological level, factor B was agreed to lie at the interface of energy transfer between the respiratory chain and the ATP synthase complex. However, biochemical characterization of the factor B polypeptide has proved difficult. It was not until 1990 that the N-terminal amino acid sequence of bovine mitochondrial factor B was reported, which followed, a decade later, by the report describing the amino acid sequence of full-length human factor B and its functional characterization. The present review summarizes the recent advances in structure-functional studies of factor B, including its recently determined crystal structure at 0.96 Å resolution. Ectopic expression of human factor B in cultured animal cells has unexpectedly revealed its role in shaping mitochondrial morphology. The supramolecular assembly of ATP synthase as dimer ribbons at highly curved apices of the mitochondrial cristae was recently suggested to optimize ATP synthesis under proton-limited conditions. We propose that the binding of the ATP synthase dimers with factor B tetramers could be a means to enhance the efficiency of the terminal step of oxidative phosphorylation in animal mitochondria.

**Keywords** Factor B · Energy coupling · ATP synthase complex

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## Introduction

Coupling factor B was originally discovered in the laboratory of D. Rao Sanadi, and the manuscript describing its isolation from bovine heart mitochondria and functional characterization was published in *Archives of Biochemistry and Biophysics* (Lam et al. 1967). Chronologically, the discovery of factor B was preceded by identification in the same laboratory of the so-called coupling factor A, explaining the use of a capital letter B to denote the polypeptide. Sanadi's interest in factor B has arisen from the demonstration that $Cd^{++}$ and other divalent metals caused uncoupling of oxidative phosphorylation in rat liver mitochondria (Jacobs et al. 1956). The reversal of uncoupling with either EDTA or small molecule dithiols suggested that protein-based vicinal dithiol is involved in $Cd^{++}$ binding, and triggered a race to isolate a coupling factor sensitive to dithiol-modifying reagents. The studies on factor B and its role in oxidative phosphorylation in animal mitochondria during the subsequent 15 years were summarized in a comprehensive review (Sanadi 1982). The persistent efforts of Sanadi and colleagues culminated in the determination of amino acid sequence of the first 55 residues of the bovine mitochondrial factor B polypeptide with $M_r$ ~22 kDa (Kantham et al. 1990).

The interest in coupling factor B was rekindled a decade later when in 2002 the author of this review together with Youssef Hatefi reported the amino acid sequence of full-length human factor B, the identification of its gene ATP5S on chromosome 14q22.1, and recombinant expression of the human polypeptide and its rigorous functional characterization as a genuine factor B (Belogrudov and Hatefi 2002; Belogrudov 2002). This work has continued at the West Los Angeles VA Medical Center, benefiting from financial support obtained from NIH, and was further expanded to include the cloning, expression, and biophysical

characterization of bovine factor B (Belogrudov 2006; Belogrudov et al. 2006), as well as its crystal structure determination at the atomic resolution of 0.96 Å (Lee et al. 2008). Simultaneously, characterization of factor B's role in cellular bioenergetics commenced (Belogrudov 2007), utilizing modern cell biology techniques, including laser scanning confocal microscopy.

The present review summarizes the recent advances in structure-functional studies of coupling factor B, building upon work performed primarily in the author's laboratory. An attempt is made to provide a critical assessment in light of the earlier data available in the literature. Since most published studies on factor B used bovine heart mitochondria, both as the source for the polypeptide isolation and assay of its coupling activity, the review's scope is limited to topics concerned with oxidative phosphorylation in animal mitochondria isolated from bovine heart tissue.

## Discovery of factor B and its historical background

Because of its key role in the circulation, heart muscle tissue is highly enriched with mitochondria. Among biochemists whose studies were concerned with the fractionation and isolation of enzyme complexes of the mammalian oxidative phosphorylation system, cow hearts have become readily appreciated as a rich source of high quality mitochondria. The heavy fraction of isolated bovine heart mitochondria was found to consist of undamaged organelles that exhibited high P/O ratios (Hatefi and Lester 1958).

Ultrasonic disintegration of bovine heart mitochondria was found to yield "inside–out" closed vesicles, which derive from the mitochondrial cristae. In such **s**ub**m**itochondrial **p**articles, SMP, the topological orientation of the inner membrane is opposite to that observed in the intact mitochondrion: in SMP, the side of the inner membrane which faced originally the mitochondrial matrix is oriented toward the outside medium, allowing unrestricted access of substrates and inhibitors to the five enzyme complexes of the mitochondrial oxidative phosphorylation system. Inclusion $Mg^{++}$ and/or $Mn^{++}$ in the medium during the sonication procedure resulted in preparations of tightly coupled SMP (Lee 1979). Over the years, these mitochondria-derived membrane vesicles have been successfully used in the studies concerned with the mechanism of ATP synthesis during oxidative phosphorylation (Hatefi 1993).

Early studies on the resolution and reconstitution of oxidative phosphorylation in bovine heart mitochondria established that ATP synthase complex is composed of a catalytic sector $F_1$ and a membrane sector $F_O$, appended with a number of polypeptides collectively termed coupling factors (Sanadi and Joshi 1979). Among these, factor B, $F_6$, and oligomycin-sensitivity conferring protein (OSCP) were found to be required for reconstitution in particular types of depleted SMP (Ernster et al. 1986) partial reactions of oxidative phosphorylation, binding of $F_1$ to $F_O$, and sensitivity of membrane-bound $F_1$ to oligomycin, respectively. Sonic irradiation of heavy bovine heart mitochondria in the medium adjusted to a pH of ~8.8 with **a**mmonia, in the presence of **E**DTA, yielded non-phosphorylating membrane vesicles termed AE-SMP. Surprisingly, treatment of AE-SMP or functionally similar E-SMP with low levels of oligomycin, a specific inhibitor of proton translocation through membrane sector $F_O$, was found to enhance energy-driven reactions catalyzed by the particles, including ATP-driven $NAD^+$ reduction by succinate, ATP-dependent NADH/$NADP^+$ transhydrogenation, ATP-$^{32}P_i$ exchange, and NADH- or succinate-driven ADP phosphorylation (Lee and Ernster 1965; You and Hatefi 1976; Sanadi 1982). An early suggestion that AE-SMP were rendered deficient in $F_1$ content, which could have explained the observed proton leakiness of their membranes, has been ruled out by subsequent studies. Instead, a partially purified from acetone-washed bovine heart mitochondria polypeptide termed factor B was shown to enhance all of the above energy-driven reactions in AE-SMP to significantly higher levels than those attained with oligomycin alone (Lam et al. 1967; Sanadi 1982). Factor B coupling activity was ascribed to the preparations isolated from bovine heart mitochondria that contained polypeptides of 32 kDa (Lam et al. 1967), 47 kDa (Shankaran et al. 1975), 12 kDa (You and Hatefi 1976; Stiggall et al. 1979), and 22 kDa (Kantham et al. 1990).

Although activity assays supported the originally made assignment of factor B as coupling factor, its characterization as a *bona fide* subunit of the ATP synthase complex remained problematic. Factor B was reported to be present at a 1:1 stoichiometry with respect to $F_1$ in $H^+$-ATPase extracted from bovine heart mitochondria with lysolecithin (Hughes et al. 1979); this preparation was also reported to exhibit the highest ATP-$^{32}P_i$ exchange activity, 1,400–1,600 $nmol \times min^{-1} \times mg^{-1}$, among known ATP synthase preparations from animal mitochondria (Hughes et al. 1982). Of note, lysophospholipids, especially lysophosphatidyl choline, are known to induce high positive membrane curvature (Fuller and Rand 2001). Bovine heart mitochondrial complex V was shown to be severely depleted in factor B content and addition of recombinant human factor B increased ATP-$^{32}P_i$ exchange activity of complex V by ~2.5 fold (Belogrudov 2002). A preparation of bovine heart $F_1F_O$-ATPase solubilized and purified using dodecyl-$\beta$-D-maltoside was shown to lack both factor B and the ADP/ATP carrier (Walker et al. 1991). A follow-up study (Lutter et al. 1993) revealed that the isolated enzyme was unable to catalyze ATP-$^{32}P_i$ exchange or ATP synthesis. Binding of $F_1$ to $F_O$ was apparently perturbed since ATPase activity of

the enzyme was poorly sensitive to oligomycin (24%). Taken together, the reported activity assays suggest that the above $F_1F_O$-ATPase preparation apparently comprised an uncoupled and/or damaged enzyme, which raises concerns regarding the validity of its use for determining the stoichiometry of peripheral stalk subunits (Collinson et al. 1996). Although expected to facilitate progress toward crystallization of bovine heart mitochondrial ATP synthase (Lutter et al. 1993), the enzyme preparation and its later modification have thus far failed to yield a high-resolution structure of $F_1F_O$-ATPase.

This brief overview suggests that the extent to which proton translocation through $F_O$ in the purified enzyme is uncoupled from ATP synthesis at $F_1$ could be related to the loss of either some polypeptide components, including factor B, or of bound phospholipids, or both. To meet the goal of obtaining the crystal structure of ATP synthase complex, development of new or improvement of the existing purification protocols is needed. To preserve the intact oligomeric state and subunit composition of the ATP synthase complex, its solubilization from the mitochondrial membranes and purification progress should rely on the ATP synthesis activity assay.

## Factor B structure-function relationships

The amino acid sequences of human and bovine mature factor B polypeptides comprise 175 residues and share 90% sequence identity (Belogrudov and Hatefi 2002; Belogrudov 2006). Each polypeptide contains six cysteine residues, of which Cys92 and Cys94 are juxtaposed in the linear amino acid sequence. Factor B homologs have been found in all animal species whose genomes have been sequenced thus far, as well as in invertebrates. No factor B counterpart was identified in yeasts or prokaryotes. A human polypeptide encoded by FLJ10241 cDNA (accession number BAA91503) shares 30% sequence identity with human factor B (Belogrudov and Hatefi 2002); homologous polypeptides were subsequently identified in other animal species, suggesting that the factor B family is composed of two members.

Western blot analysis with anti-human factor B antibody revealed that AE-SMP are depleted in factor B content (Belogrudov and Hatefi 2002). Recombinant human or bovine factor B restored oxidative phosphorylation and its partial reactions in reconstituted AE-SMP (Belogrudov and Hatefi 2002; Belogrudov 2002; Belogrudov 2006; Belogrudov 2008), providing unequivocal evidence that the cloned mitochondrial polypeptide is indeed coupling factor B. Proteomic analyses have identified factor B in bovine (Belogrudov 2008) and mouse heart (Zhang et al. 2008) mitochondria, and the distribution of the polypeptide across 14 mouse tissues has recently been reported (Pagliarini et al. 2008).

A comprehensive structure-function analysis of bovine factor B was reported (Belogrudov 2008). Briefly, we demonstrated that the C-terminal domain, which is composed of four leucine-rich repeats (LRR), lacked coupling activity of the full-length polypeptide, and that factor B mutants with truncated N-terminal amino acids exhibited diminished coupling activity. Cross-linking studies that used a factor B mutant harboring a biosynthetically incorporated unnatural photoreactive amino acid analog at Trp2 position yielded cross-links between factor B and subunits *e* and *g* of the ATP synthase membrane sector $F_O$, as well as the ADP/ATP carrier. The interaction between factor B and the carrier was confirmed by co-sedimentation of both polypeptides in sucrose density gradients. Recombinant factor B was shown to co-sediment with the membrane sector $F_O$ and to block passive proton diffusion through $F_O$ reconstituted in liposomes. Together, these results suggest that the N-terminal domain and, in particular, the proximal N-terminal amino acids are important for coupling activity and protein-protein interactions of factor B.

Three polypeptides, which are known today as subunits *e*, *f* and *g*, have been found to co-purify with bovine heart $F_1F_O$-ATPase and its membrane sector $F_O$ (Walker et al. 1991; Collinson et al. 1994). They contain 70, 87 and 102 amino acid residues, respectively. The hydropathy profiles of the polypeptides suggest that each is a single-span membrane protein. The topological orientation of subunits *e*, *f* and *g* within the mitochondrial inner membrane was investigated based on the pattern of proteolytic digestion with trypsin and chymotrypsin in SMP and mitoplasts (Belogrudov et al. 1996). A model was suggested in which the N-termini of each polypeptide orient toward the mitochondrial matrix while the C-termini face the cytoplasmic side of the inner membrane. Interestingly, the C-terminal half of subunit *e*, which emerges from lipid bilayer into the intermembrane space, was predicted to form a coiled-coil. Cross-linking experiments have demonstrated the formation of *e*-*e*, *e*-*g*, *g*-*f*, and *f*-A6L dimers (Belogrudov et al. 1996).

The subsequent detailed studies of these polypeptides employed the yeast *Saccharomyces cerevisiae* and have recently been reviewed (Stuart 2008). Yeast subunits *e* and *g* have been proposed to play a role in the formation of ATP synthase dimers (Arnold et al. 1998), and down-regulation of their expression was found to correlate with changes in the morphology of mitochondrial cristae (Arselin et al. 2004). In contrast to their yeast counterparts (Arnold et al. 1998), bovine subunits *e* and *g* are present in both monomeric and dimeric forms of ATP synthase (Meyer et al. 2007; Belogrudov 2008; Bisetto et al. 2008) and thus cannot be considered as dimer-specific subunits as is the

case for the yeast enzyme. The animal subunit *g* contains a completely conserved acidic residue in the middle of its transmembrane domain (Hong and Pedersen 2004), whereas the intermembrane space located C-terminus of subunit *f* harbors two His residues.

In the membrane sector $F_O$ of bacteria and chloroplasts, the proton-translocating pathway is thought to be formed by a ring of oligomeric subunit *c*, with each subunit contributing a critical acidic residue that lies in the middle of the lipid bilayer, and two half-channels, located at the interface between subunit *a* and the subunit *c* ring (Vik et al. 2000; Fillingame et al. 2003). The entry half-channel is proposed to deliver protons from the periplasmic side of the membrane to the subunit *c* ring, the unidirectional rotation of which brings the protons in proximity to the exit half-channel for release on the cytoplasmic side of the membrane. Aqueous access and release pathways in subunit *a* are proposed to mediate proton transport to and from the proton-binding site in subunit *c* (Angevine et al. 2007).

In addition to the described pathway, we propose that the membrane sector $F_O$ of animal mitochondria could harbor a second, latent proton-translocating pathway, to the assembly of which supernumerary subunits *e*, *f*, *g*, and A6L, as well as the ADP/ATP carrier, could contribute their transmembrane segments. We further propose that the latent proton-translocating pathway is occluded with the N-terminal α-helix of factor B (see below) from the matrix membrane side in coupled mitochondria. The C-terminal tails of subunits *e* and *f*, which emerge at the cytoplasmic side of the inner membrane, are enriched in protonatable groups and could act as "proton-collecting antenna" that feeds protons into the pathway. Displacement of factor B from the membrane surface in AE-SMP is proposed to activate the second proton-translocating pathway and to short-circuit the protonmotive force. Because the AE-SMP could be de-coupled with either oligomycin or dicyclohexylcarbodiimide (DCCD) (Belogrudov and Hatefi 2002; Belogrudov 2006), the entry proton half-channels could be partially shared between the two proton-translocating pathways.

## Crystal structure of factor B

We determined the crystal structures of wild-type bovine factor B and a Gly3-to-Glu mutant at 2.9 Å and 0.96 Å resolutions, respectively, in collaboration with the Stroud laboratory at UCSF (Lee et al. 2008). The Gly3-to-Glu substitution occurs in factor B homologs identified in rodents and was introduced in bovine factor B by site-directed mutagenesis. The crystal structure revealed an oblong, oval-shaped molecule composed of two domains: a unique N-terminal globular domain and the C-terminal domain comprising four leucine-rich repeats, LRR (Fig. 1). The first ten N-terminal residues fold as a short α-helix that extends away from the body of the molecule. The N-terminal α-helix is suggested to anchor factor B to the matrix side of the mitochondrial inner membrane, thus orienting the rest of the polypeptide molecule in a lateral direction along the membrane surface. The LRR motif is found in a variety of cellular proteins where it is proposed to mediate protein-protein interactions (Kobe and Kajava 2001). The presence of the cysteine-containing LRR motif in the mitochondrial inner membrane protein such as factor B is unique. Unexpectedly, the high resolution structure has revealed a bound $Mg^{++}$ ion, demonstrating for the first time that factor B is a metalloprotein.

In the crystal structure, the sulfur atoms of the cysteine pairs Cys33/Cys71 and Cys94/Cys123 are separated by 3.7 Å and 4.7 Å, respectively, and could potentially form disulfide bridges. Cys94 and Cys123 are located within the LRR-containing C-terminal domain and apparently

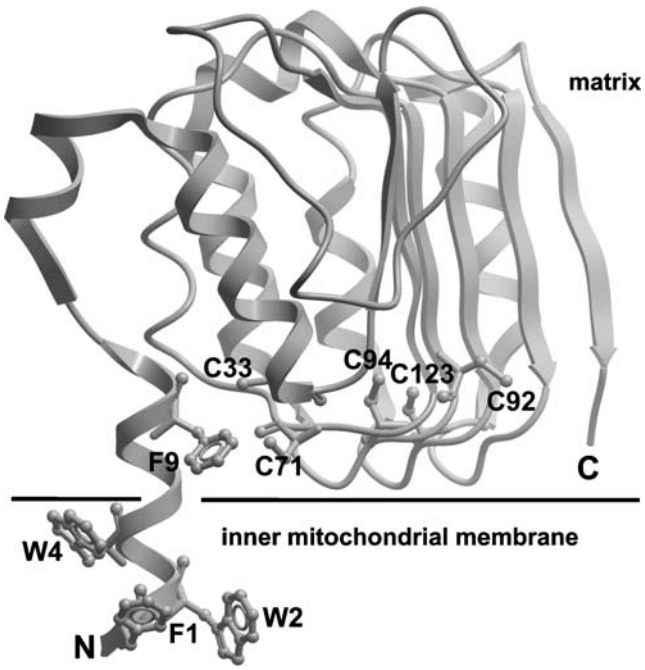


**Fig. 1** Ribbon model of the crystal structure of a bovine factor B Gly3-Glu mutant refined to a 0.96 Å resolution (PDB code 3e4g). The figure depicts the factor B molecule viewed head-on from the N-terminal domain. N and C indicate the $NH_2$- and COOH-termini of the polypeptide. The side chains of Phe1, Trp2, Trp4, Phe9, as well as Cys33, Cys71, Cys92, Cys94, and Cys123 are shown in ball-and-stick presentation. Cys101, which is located at the convex side of the protein, is not indicated. The N-terminal α-helix is proposed to anchor factor B to the matrix side of the mitochondrial inner membrane, thus orienting the rest of the polypeptide molecule in a lateral direction along the membrane surface. The depth of the α-helix insertion into the membrane is yet to be determined. For clarity, the tightly bound $Mg^{++}$ ion is omitted. F, phenylalanine; W, tryptophan; C, cysteine. The figure was prepared with ICM Browser Pro software (Molsoft L.L.C., La Jolla, CA)

have a structural role, as their thiols form hydrogen bonds with nearby residues. The Cys33/Cys71 pair is located near the N-terminal α-helix. We isolated recombinant bovine factor B with a Cys33-Cys71 disulfide bond and found that the oxidized polypeptide associates with AE-SMP with an approximately 3-fold lower affinity ($K_d$=352 nM) than the reduced polypeptide ($K_d$=113 nM) (Belogrudov, unpublished data). In mitochondria, Cys33-Cys71 disulfide formation could result from elevated levels of reactive oxygen species and could proceed through a mechanism in which one of the thiols is oxidized by hydrogen peroxide to sulfenic acid as a reaction intermediate (Bindoli et al. 2008). The rate of the disulfide reduction by glutathione, a major reducing agent in the mitochondrial matrix, could be affected by poor accessibility of glutathione to the disulfide, especially in the membrane-bound factor B, resulting in increased steady-state levels of oxidized polypeptide. Additionally, the formation of a stable factor B-glutathionyl mixed disulfide during reduction of Cys33-Cys71 disulfide with glutathione could perturb the interaction of the nearby N-terminal α-helix with $F_O$ subunits *e* and *g*. Thus, a change in Cys33/Cys71 redox status could be linked to an increase in proton conductance through the latent proton translocating pathway proposed above and uncoupling of respiration from ATP synthesis.

Soaking crystals with inhibitors of the factor B coupling activity $Cd^{++}$ or phenylarsine oxide has revealed that the former ligates Cys101 while the latter covalently modifies Cys71.

## Factor B as a component of dimeric/oligomeric ATP synthase complex

In contrast to most enzyme complexes of the mitochondrial oxidative phosphorylation system whose oligomerization properties were thoroughly investigated, until recently there has been little interest in assigning functional significance to oligomeric species of the ATP synthase. In fact, the purification protocols of the enzyme developed in the Walker laboratory over the past two decades have emphasized selection of its monomeric form. The situation has changed dramatically since the recent study by Strauss et al. (2008), in which analysis of bovine heart and rat liver mitochondrial fragments with electron cryo-tomography revealed the existence of rows composed of dimeric/oligomeric ATP synthase molecules at the apices of the mitochondrial cristae.

Transmission electron microscopy has long been a method of choice in oxidative phosphorylation studies. The technique has provided insights into the ultrastructural organization of mitochondria, SMP, and even individual ATP synthase molecules (Fernandez-Moran et al. 1964), which, described at the time as the "elementary particles," were seen to assemble into ordered arrays on the surface of mitochondrial cristae. It was not, however, until electron tomography was applied to mitochondria, as pioneered by Frey's and Mannella's laboratories (Frey and Mannella 2000; Frey et al. 2006; Mannella 2006), that the complex organization of the inner membrane became fully appreciated.

Electron tomography of thick-sectioned specimens followed by three-dimensional reconstruction has revealed four topologically distinct regions within the mitochondrial inner membrane: (a) the inner boundary membrane, which lies opposite to the outer mitochondrial membrane, (b) the roughly circular crista junction, through which the inner boundary membrane projects into the mitochondrial matrix to form (c) the flat, lamellar regions of the mitochondrial crista and (d) along its perimeter, the folding back membrane regions characterized by high curvature (Frey and Mannella 2000). It is at these curved regions that Strauss et al. (2008) have found rows encompassing up to 80 dimers of ATP synthase molecules.

In their study, Strauss *et al.* used sonically disrupted bovine heart and rat liver mitochondria that along with the intact organelles, also contained a heterogeneous population of tubular and vesicular SMP. Within individual membrane-bound dimers, two ATP synthase molecules associate at the angle of ~70° and 55°–95° in rat and bovine heart preparations, respectively; the variability in the angular association of the latter could be due to partial displacement of factor B during the sonication procedure. The distance between $F_1$ sectors and the centers of $F_O$ within a dimer were calculated to be ~28 nm and ~13 nm, respectively. The distance between the ATP synthase dimers along the ribbon was ~12 nm.

Crystals of wild-type factor B contain four molecules per asymmetric unit (Lee et al. 2008). Biophysical analysis revealed that in solution factor B forms oligomers, which were erroneously identified as trimers rather than tetramers (Belogrudov et al. 2006). In the crystals, two factor B dimers associate to form a tetramer with approximate dimensions of 10.2 nm×2.8 nm×6.4 nm. The dimers are assembled at ~90°, with all four N-terminal α-helixes being oriented in approximately the same direction, allowing them to interact with the inner membrane. Based on these data, as well as the results of previous cross-linking experiments (Belogrudov 2008), we have proposed (Lee et al. 2008) that the tetrameric species of factor B could bind to the ATP synthase dimer ribbons discovered by Strauss et al. (2008) from the mitochondrial matrix either between the two ATP synthase molecules constituting an ATP synthase dimer or at the interface between two adjacent ATP synthase dimers. Such a location of the membrane-bound factor B could maximize its efficiency in blocking a proton leak that may occur at the interface between the two angularly assembled ATP synthase molecules.

The molecular mechanisms underlying the biogenesis of highly curved apices of mitochondrial cristae are poorly understood. Electron microscopic analysis of detergent-solubilized bovine heart ATP synthase dimers (Minauro-Sanmiguel et al. 2005) suggests that the dimers themselves may possess intrinsic phospholipid bilayer-bending properties. The role of factor B dimers and tetramers in sensing or stabilizing membrane curvature, similar to that proposed for N-Bar domain-containing proteins (McMahon and Gallop 2005), remains to be elucidated.

Recent studies have concluded that the protonmotive force in energy-transducing membranes is likely to be conserved via ejected protons that diffuse laterally along the phospholipid bilayer toward the proton acceptor, without entering the bulk media phase (Branden et al. 2006; Mulkidjanian et al. 2006). Numerical simulations performed by Strauss et al. (2008) suggest that the highest proton concentration could be attained at the apex of intracrystal membrane leaflet where protons are consumed by the ATP synthase dimers organized in ribbons. To extend these insights into the mechanism of energy transfer and conservation, we further propose that the mitochondrial inner membrane may harbor a heterogeneous population of the ATP synthase molecules, and polypeptide composition, supramolecular organization and functional state of a given population of the enzyme is specified by the allocation to particular inner membrane regions.

## Ectopic expression of human factor B in animal cells

Stable or transient expression of human factor B fused with green fluorescent protein (GFP) (Belogrudov 2007) in mammalian cell lines has caused an alteration in mitochondrial morphology (Belogrudov, unpublished data). LLC-$PK_1$ cells stably expressing factor B-GFP fusion contained thinned filamentous mitochondria with a balloon-like structure at one of the poles, while transient expression in HeLa, HEK293, and HL-1 cells resulted in fragmented, balloon/button-shaped mitochondria. Transmission electron microscopy of LLC-$PK_1$ cells stably expressing factor B-GFP fusion revealed the presence of elongated tubular cristae running along the longitudinal diameter of the organelle.

## Factor B and human disease

The incidence of obesity and associated with it diabetes has reached epidemic proportions in the U.S. and other developed countries. Obesity is also the highest risk factor for cardiovascular disorders, which are the leading cause of death in the U.S., and cancer. Drugs aimed at modulating the coupling efficiency of oxidative phosphorylation are being intensively investigated. Suppression of factor B gene transcript using RNAi technologies could lead to uncoupling of respiration from ATP synthesis in mitochondria, with excess consumed energy being expended as heat, provided that the polypeptide has a reasonably short half-life in the organelle. Identification of small molecules that interfere with the binding of factor B to the mitochondrial inner membrane could offer an additional avenue for therapeutics development. Their rational design is expected to benefit from recently determined high resolution structure of bovine factor B.

Mitochondrial fragmentation has been reported in cells undergoing apoptosis (Youle and Karbowski 2005). Drugs directed at the polypeptides involved in mitochondrial fission and fusion (Chan 2006) could be expected to modulate the rate of cell death. Stable expression of factor B-GFP fusion protein in HEK293 cells results in mitochondria with altered morphology and enhanced resistance of the cells to chemotherapeutic drug etoposide (Belogrudov, unpublished data). It remains to be seen whether alterations in mitochondrial morphology observed in the cells with ectopic expression of factor B could be exploited as a therapeutic modality in a disease setting.

Finally, aging and age-associated degenerative diseases have long been linked to mitochondrial dysfunction, with reduced capacity for ATP synthesis and increased generation of reactive oxygen species. The role of factor B in disease etiology remains to be explored.

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## References

Angevine CM, Herold KA, Vincent OD, Fillingame RH (2007) J Biol Chem 282:9001–9007
Arnold I, Pfeiffer K, Neupert W, Stuart RA, Schägger H (1998) EMBO J 17:7170–7178
Arselin G, Vaillier J, Salin B, Schaeffer J, Giraud MF, Dautant A, Brethes D, Velours J (2004) J Biol Chem 279:40392–40399
Belogrudov GI (2002) Arch Biochem Biophys 406:271–274
Belogrudov GI (2006) Arch Biochem Biophys 451:68–78
Belogrudov GI (2007) Arch Biochem Biophys 461:95–103
Belogrudov GI (2008) Arch Biochem Biophys 473:76–87
Belogrudov GI, Hatefi Y (2002) J Biol Chem 277:6097–6103
Belogrudov GI, Tomich JM, Hatefi Y (1996) J Biol Chem 271:20340–20345

Belogrudov GI, Schirf V, Demeler B (2006) Biochim Biophys Acta 1764:1741–1749
Bindoli A, Fukuto JM, Forman HJ (2008) Antioxid Redox Signal 10:1549–1564
Bisetto E, Picotti P, Giorgio V, Alverdi V, Mavelli I, Lippe G (2008) J Bioenerg Biomembr 40:257–267
Branden M, Sanden T, Brzezinski P, Widengren J (2006) Proc Natl Acad Sci USA 103:19766–19770
Chan DC (2006) Annu Rev Cell Dev Biol 22:79–99
Collinson IR, Runswick MJ, Buchanan SK, Fearnley IM, Skehel JM, van Raaij MJ, Griffiths DE, Walker JE (1994) Biochemistry 33:7971–7978
Collinson IR, Skehel JM, Fearnley IM, Runswick MJ, Walker JE (1996) Biochemistry 35:12640–12646
Ernster L, Hundal T, Sandri G (1986) Methods Enzymol 126:428–433
Fernandez-Moran H, Oda T, Blair PV, Green DE (1964) J Cell Biol 22:63–100
Fillingame RH, Angevine CM, Dmitriev OY (2003) FEBS Lett 555:29–34
Frey TG, Mannella CA (2000) Trends Biochem Sci 25:319–324
Frey TG, Perkins GA, Ellisman MH (2006) Annu Rev Biophys Biomol Struct 35:199–224
Fuller N, Rand RP (2001) Biophys J 81:243–254
Hatefi Y (1993) Eur J Biochem 218:759–767
Hatefi Y, Lester RL (1958) Biochim Biophys Acta 27:83–88
Hong S, Pedersen PL (2004) J Bioenerg Biomembr 36:515–523
Hughes J, Joshi S, Murfitt RR, Sanadi DR (1979) In: Lee CP, Schatz G, Ernster L (eds) Membrane bioenergetics. Addison-Wesley, Boston, pp 81–95
Hughes J, Joshi S, Torok K, Sanadi DR (1982) J Bioenerg Biomembr 14:287–295
Jacobs EE, Jacob M, Sanadi DR, Bradley L (1956) J Biol Chem 223:147–156
Kantham L, Raychowdhury R, Ogata KK, Javed A, Rice J, Sanadi DR (1990) FEBS Lett. 277:105–108
Kobe B, Kajava AV (2001) Curr Opin Struct Biol 11:725–732
Lam KW, Warshaw JB, Sanadi DR (1967) Arch Biochem Biophys 119:477–484
Lee CP (1979) Methods Enzymol 55:105–112
Lee CP, Ernster L (1965) Biochem Biophys Res Commun 18:523–529
Lee JK, Belogrudov GI, Stroud RM (2008) Proc Natl Acad Sci USA 105:13379–13384
Lutter R, Saraste M, van Walraven HS, Runswick MJ, Finel M, Deatherage JF, Walker JE (1993) Biochem J 295:799–806
Mannella CA (2006) Biochim Biophys Acta 1763:542–548
McMahon HT, Gallop JL (2005) Nature 438:590–596
Meyer B, Wittig I, Trifilieff E, Karas M, Schägger H (2007) Mol Cell Proteomics 6:1690–1699
Minauro-Sanmiguel F, Wilkens S, García JJ (2005) Proc Natl Acad Sci USA 102:12356–12358
Mulkidjanian AY, Heberle J, Cherepanov DA (2006) Biochim Biophys Acta 1757:913–930
Pagliarini DJ, Calvo SE, Chang B, Sheth SA, Vafai SB, Ong SE, Walford GA, Sugiana C, Boneh A, Chen WK, Hill DE, Vidal M, Evans JG, Thorburn DR, Carr SA, Mootha VK (2008) Cell 134:112–123
Sanadi DR (1982) Biochim Biophys Acta 683:39–56
Sanadi DR, Joshi S (1979) Methods Enzymol 55:380–383
Shankaran R, Sani BP, Sanadi DR (1975) Arch Biochem Biophys 168:394–402
Stiggall DL, Galante YM, Kiehl R, Hatefi Y (1979) Arch Biochem Biophys 196:638–644
Strauss M, Hofhaus G, Schroder RR, Kuhlbrandt W (2008) EMBO J 27:1154–1160
Stuart RA (2008) J Bioenerg Biomembr 40:411–417
Vik SB, Long JC, Wada T, Zhang D (2000) Biochim Biophys Acta 1458:457–466
Walker JE, Lutter R, Dupuis A, Runswick MJ (1991) Biochemistry 30:5369–5378
You KS, Hatefi Y (1976) Biochim Biophys Acta 423:398–412
Youle RJ, Karbowski M (2005) Nat Rev Mol Cell Biol 6:657–663
Zhang J, Li X, Mueller M, Wang Y, Zong C, Deng N, Vondriska TM, Liem DA, Yang JI, Korge P, Honda H, Weiss JN, Apweiler R, Ping P (2008) Proteomics 8:1564–1575