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Review

The peripheral stalk of the mitochondrial ATP synthase

John E. Walker *, Veronica Kane Dickson

The Medical Research Council Dunn Human Nutrition Unit, The Wellcome Trust/MRC Building, Hills Road, Cambridge CB2 2XY, UK

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Abstract

The peripheral stalk of F-ATPases is an essential component of these enzymes. It extends from the membrane distal point of the F_1 catalytic domain along the surface of the F_1 domain with subunit a in the membrane domain. Then, it reaches down some 45 Å to the membrane surface, and traverses the membrane, where it is associated with the a-subunit. Its role is to act as a stator to hold the catalytic $\alpha_3\beta_3$ subcomplex and the a-subunit static relative to the rotary element of the enzyme, which consists of the c-ring in the membrane and the attached central stalk. The central stalk extends up about 45 Å from the membrane surface and then penetrates into the $\alpha_3\beta_3$ subcomplex along its central axis. The mitochondrial peripheral stalk is an assembly of single copies of the oligomycin sensitivity conferral protein (the OSCP) and subunits b, d and F_6. In the F-ATPase in *Escherichia coli*, its composition is simpler, and it consists of a single copy of the δ-subunit with two copies of subunit b. In some bacteria and in chloroplasts, the two copies of subunit b are replaced by single copies of the related proteins b and b′ (known as subunits I and II in chloroplasts). As summarized in this review, considerable progress has been made towards establishing the structure and biophysical properties of the peripheral stalk in both the mitochondrial and bacterial enzymes. However, key issues are unresolved, and so our understanding of the role of the peripheral stalk and the mechanism of synthesis of ATP are incomplete.

Keywords: ATP synthase; Mitochondria; Peripheral stalk; Structure; Function

1. Introduction

The F-ATPases are multisubunit enzyme complexes found in the membranes of mitochondria, eubacteria and chloroplasts [1,2]. Under oxidative conditions, their role is to catalyze the formation of ATP from ADP and inorganic phosphate using energy from a proton motive force across the membrane to drive the reaction. Under anaerobic conditions eubacterial enzymes carry out the reverse reaction and generate the proton motive force using energy liberated by hydrolysis of ATP produced by fermentation, whereas specific inhibitory mechanisms prevent the chloroplast and mitochondrial enzymes from carrying out the reverse reaction.

The F-ATPases consist of two main structural domains, a membrane intrinsic domain known as F_0 and an extrinsic globular domain, called F_1, linked together by a central stalk and a peripheral stalk (Fig. 1). The F_1 domain is the site of catalytic formation of ATP where energy is provided from the proton motive force by a mechanical coupling mechanism in which the central stalk rotates in a clockwise direction as viewed from the membrane. This rotation is driven by the inward passage of protons across the membrane via channels in the single a-subunit in, F_0. which is found in close association with the periphery of a ring (as viewed from above or beneath the membrane) of hydrophobic c-subunits. The passage of protons through the channels releases energy to drive the clockwise rotation of the ring. As the upper surface of the ring is intimately associated with the foot of the central stalk, the ring and the central stalk rotate together. The central stalk extends upwards from the membrane in an exposed region for about 45 Å. Then it penetrates into the spherical globular region of the F_1-catalytic domain, a sphere with an approximate diameter of 100 Å consisting of three α-subunits and three β-subunits arranged in alternation around the axis of the central stalk. In this region, the central stalk consists of an antiparallel coiled-coil of α-helices [3]. One of these helices (the C-terminal region of the γ-subunit) continues upwards beyond the coiled-coil through the

Abbreviations: OSCP, oligomycin sensitivity conferral protein; F_6, factor 6; F-ATPase, F_1F_o-ATP synthase

* Corresponding author.
E-mail address: walker@mrc-dunn.cam.ac.uk (J.E. Walker).

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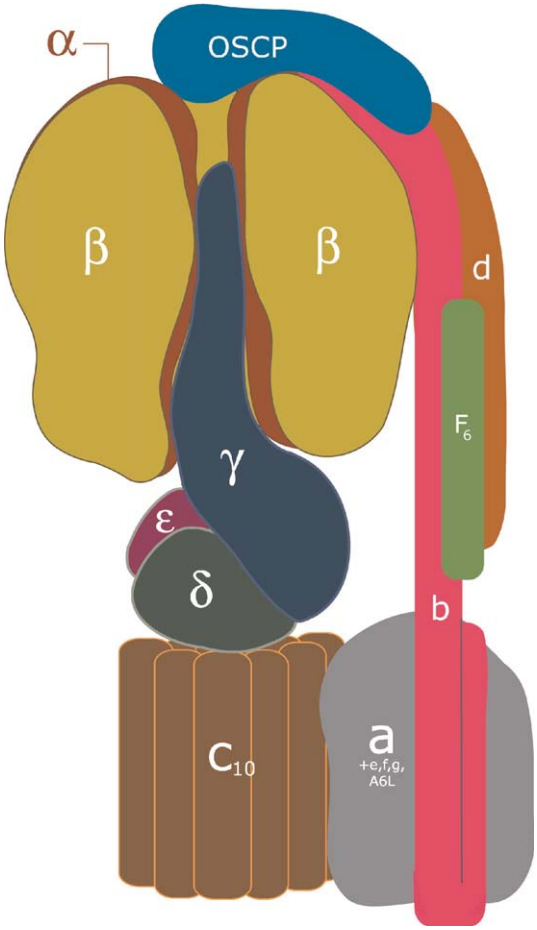

Fig. 1. The subunit organisation in mitochondrial F_1F_o-ATPase. Subunits are colour coded and labelled. F_1 is the globular catalytic domain made of subunits α, β and the three central stalk subunits, γ, δ and ε. The F_0 domain is comprised of the c oligomer (10 copies in yeast mitochondrial ATP synthase), subunit a, and the peripheral stalk subunits b, d, F_6 and OSCP. The so-called minor subunits (e, f, g, and A6L) and are not shown individually, but they all span the membrane and are probably present in a 1:1:1:1 stoichiometry. The rotor is made up of the central stalk and the c-ring. The remainder of the subunits make up the stator. F_1 is shown with one α subunit removed for clarity. The inhibitor protein (IF_1) is also not shown; it binds in a catalytic α/β interface near the bottom of $(\alpha\beta)_3$.

$\alpha_3\beta_3$ domain, so that the C-terminal residue is exposed at the top of the structure. This penetrating region of the central stalk is asymmetrical, and as it rotates, it brings about a series of conformational changes in the three catalytic sites, which lie mainly in β-subunits at interfaces with α-subunits. During each 360° rotation of the central stalk, each catalytic site is taken in turn through a series conformational states defined by the asymmetry of the central stalk where ADP and phosphate bind, ATP forms and ATP is released. The structures of each of these states and of intermediate transition states have been defined [3–5].

This review concerns the peripheral stalk. As its name implies, the peripheral stalk lies at the edge of the multisubunit assembly made of F_1, central stalk and the F_0 subunits a and c. It extends from the top (membrane distal) region of F_1, along the external surface of the $\alpha_3\beta_3$ domain, and then reaches down, into and across the membrane, where it is associated with subunit a. Its role is to act as stator to counter the tendency of the $\alpha_3\beta_3$ complex to follow the rotation of the central stalk and attached c-ring, and to anchor the a-subunit [6]. It is one of the least studied parts of the enzyme, and its structure, biophysical properties and exact roles in the enzyme complex have yet to be properly established. This review will concentrate mainly upon the peripheral stalk in the mitochondrial enzyme where greatest progress has been made towards establishing its structure, but reference will also be made to the bacterial peripheral stalk where there is also significant progress towards the structure, and where the functions of specific regions have been explored by mutagenesis and biophysical approaches.

Peripheral stalks are also a feature of the V-ATPases and A-ATPases (vacuolar and archaeal ATPases, respectively) [7–9]. Significant, but somewhat less progress has been made so far in establishing their structures and functions, and the peripheral stalks in these enzymes will not be reviewed here.

2. Discovery of the peripheral stalk

2.1. The central stalk

More than 40 years ago, the so-called lollipop or mushroom structures were discovered in abundance in association with everted inner membranes from bovine mitochondria by electron microscopic examination in negative stain (Fig. 2) [10–12]. This observation preceded the demonstration that the lollipops are the sites of ATP synthesis [11]. It was the first observation that the domain later known as the F_1-ATPase (the lollipop head) was attached to the membrane by a slender stem, more recently termed the central stalk [12]. In contrast, discovery of the peripheral stalk in F-ATPases is a relatively recent event.

2.2. Subunit b

When the sequence of the *unc* operon encoding the subunits of the F-ATPase from *Escherichia coli* was determined in the early 1980s [13–16], it was found that between the genes for the a- and c- subunits lay a gene encoding a protein that seemed to correspond in molecular weight to a subunit named b that had been detected in the F-ATPase from *E. coli* [17]. Its sequence suggested that its N-terminal region traversed the membrane in a single α-helical span, and that the rest of the protein protruded from the membrane in a highly charged α-helical structure [18]. This topology suggested that the protein was important for binding the F_1 domain to the membrane. The F_1 domain protected the protein from proteolysis, but it became susceptible when F_1 was removed. These observations were consistent with the model. The F-ATPase from *E. coli* contained two copies of subunit b [19], and subsequently, they were found to form a homodimer [20]. Other eubacterial species [21,22], and also chloroplasts, contained two related proteins, b and b′ (known as subunits I and II in chloroplasts [23,24]), in a heterodimeric arrangement [25]. The subunit composition and structure of the central stalk were not known at the time, and it was assumed that because b was required for binding F_1 to F_0 that it was in this central region of the complex.

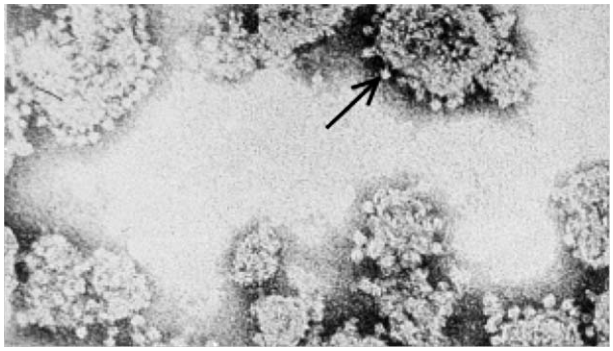

Fig. 2. Electron micrograph of sub-mitochondrial particles. Everted inner mitochondrial membranes lined with lollipop structures, later shown to be the ATP synthase. From [12].

In 1987, the F-ATPase from bovine mitochondria was found to contain a protein, that was also named subunit b, with an N-terminal membrane spanning hydrophobic domain of two anti-parallel α-helices attached to a highly charged and α-helical extrinsic region [26]. The sequence of this protein was at best very weakly related to the eubacterial and chloroplast b- and b′-subunits, but the topological similarity to the bacterial b protein suggested that it might fulfil a similar role in the mitochondrial enzyme. A second protein in the bovine mitochondrial enzyme, named subunit d, also seemed to be likely to be an α-helical protein that might interact with subunit b, but it lacked an N-terminal membrane anchoring segment [26]. The stoichiometries of subunits b and d had not been established at this stage, but later it was shown that the assembled F-ATPase complex contained one copy of each protein [27]. One possibility was that bovine subunits b and d together might be equivalent to two bacterial b-subunits [26]. Subunits b and d have also been characterized in the F-ATPase from mitochondria of *Saccharomyces cerevisiae*, where sometimes subunit b is known as subunit 4 [28]. They are related in sequence to their bovine counterparts, and the yeast b-subunit has a similar topology to the bovine homologue.

2.3. The bovine OSCP and F_6 and bacterial subunit δ

In a series of classical reconstitution experiments, Racker and colleagues demonstrated in the 1960s that the OSCP was required for reassembling the F_1 domain onto stripped mitochondrial inner membranes (membranes from which F_1 domains had been removed) so as to re-form a fully active and oligomycin sensitive membrane bound ATPase [11]. Another protein, F_6, was also required for oxidative phosphorylation, but not for inhibitor-sensitive passage of protons through F_0 [29–31]. Subsequently, it was shown that the OSCP was the homologue of the bacterial δ-subunit [18]. Mild trypsinolysis of mitochondrial F_1 removes residues 1–15 of α-subunits and residues 1–7 of the β-subunits [32], and this treatment prevents OSCP from binding to the F_1 domain [33]. Thus, the N-terminal regions of either or both proteins appear to be required for binding of OSCP. Mild trypsinolysis of the *E. coli* F_1-ATPase has similar effects on α- and β-subunits and prevents binding of δ-subunits [34]. However, the locations of the OSCP and the bacterial δ-subunit and of the N-terminal regions of α- and β-subunits in the intact F-ATPase complexes were not known. Even with the emergence of b-subunits, there was no evidence of a direct physical association between OSCP (or bacterial δ-subunits) and b-subunits, and none was proposed at this stage. Later, the bacterial b- and δ-subunits were shown to interact via their C-terminal regions [35]. In addition, subunit b interacted strongly with the F_1 domain in the presence of the δ-subunit in a Mg^{2+}-dependent way [36], and weakly in the absence of the δ-subunit [37,36].

Similarly, the structural role of F_6 and its location in the mitochondrial complex were obscure.

2.4. Emergence of the peripheral stalk

In the early 1990s, the availability of recombinant forms of bovine subunits b, d, OSCP and F_6, and fragments of them, allowed the possible interactions between these subunits to be explored by in vitro reconstitution experiments [6]. Thus, a quaternary sub-complex containing subunits b′, d, OSCP and F_6 formed readily (b′, residues 79–214 of subunit b, lacks the membrane spanning hydrophobic N-terminal region). This sub-complex, referred to as the "stalk" sub-complex formed a 1:1 complex with the F_1 domain [6]. There was one copy of each of the four "stalk" subunits in the separate stalk complex, in the F_1-"stalk" complex and in the intact F_1F_o-ATPase [27]. At first, because subunits b, d and OSCP (and bacterial b and subunit δ) were thought to part of the central stalk, it was assumed also that

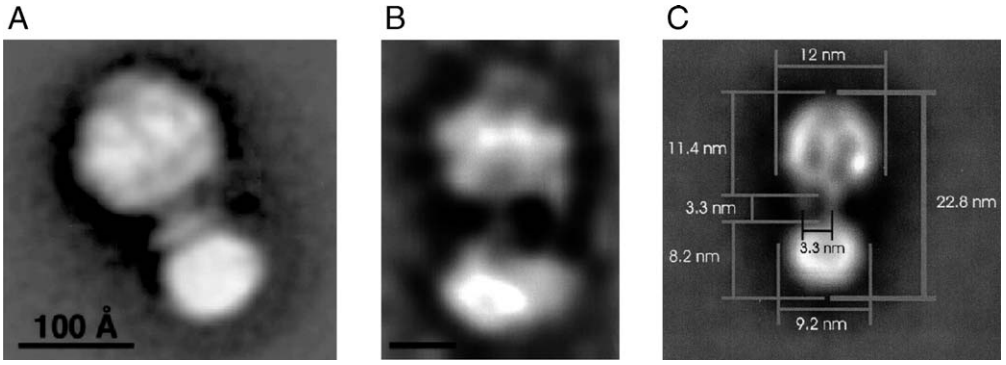

Fig. 3. Electron microscopic images of ATP synthase from various organisms. The images were obtained by averaging negatively stained single particles. The peripheral stalk can be seen in each image from (left) bovine mitochondria [45], from *E. coli* [46], and (right) chloroplasts [47].

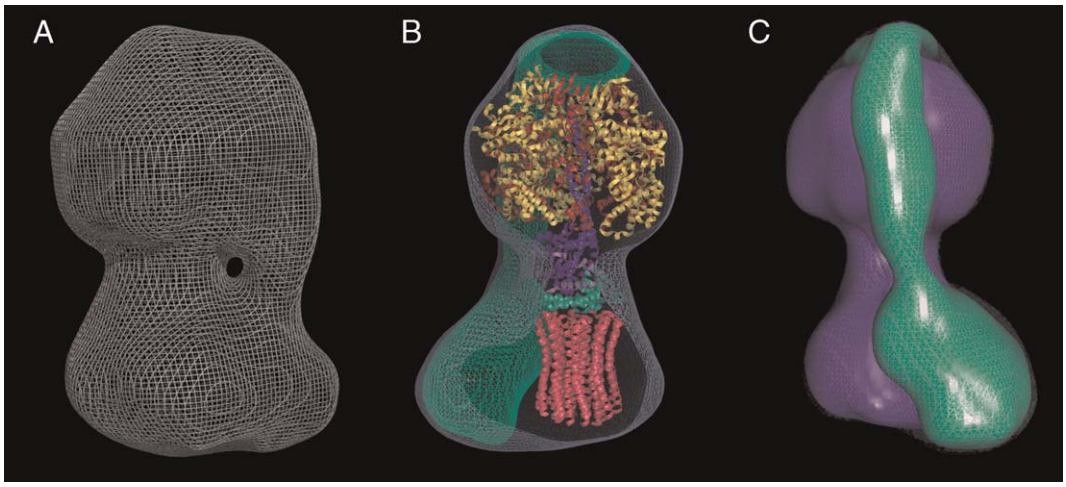

Fig. 4. Cryo-EM reconstruction of the F-ATPase from bovine heart *mitochondria*. (A) The electron density map; (B) the map with the structure of the yeast F_1c_{10} complex docked in it; and (C) with the density for F_1c_{10} in blue, and the remaining green density contains the peripheral stalk (subunits OSCP, b, d and F_6) and the F_0 subunits, a, e, f, g, A6L. From [48].

the "stalk" sub-complex was associated with the central stalk [38]. However, a series of observations made this view untenable. In 1994, the structure of the F_1-domain showed that the γ-subunit was the main component of the central stalk [3], and although the δ- and ε-subunits were not resolved in this early structure, it seemed likely that they were associated with a region of the γ-subunit that was also unresolved, at the foot of the central stalk, as it was eventually shown [39]. In the meantime, in vitro reconstitution experiments with recombinant bovine δ- and ε-subunits showed that they formed a heterodimer, and that crucially, this δ·ε subcomplex did not interact with the "stalk" [40]. It had also been shown that bovine b, d, OSCP and F_6 could be cross-linked chemically to the α- and β-subunits of the F_1 domain, but not to γ- and δ-subunits [41]. These findings led to the conclusion that the central stalk and "stalk" complexes were separate entities that did not interact directly in the complete F-ATPase complex [40]. It had also become clear from the structure of the bovine F_1-ATPase that the N-terminal regions of the α-subunits, and possibly of the β-subunits, that interacted with the OSCP were at the "top" of F_1. This membrane-distal location of the OSCP, its presence in the "stalk" complex and the idea that the "stalk" and central stalk were separate entities, led directly to the proposal that the "stalk" complex is peripherally located in the F-ATPase complex [27]. The concept of the rotary mechanism of the ATPase involving the rotation of the γ-subunit (and the presumptively attached δ- and ε-subunits), then led to the original proposal that the peripheral stalk "serves as a 'stator' to counter the tendency of the $\alpha_3\beta_3$ subcomplex to rotate in response to a rotation of the γ-subunit within $\alpha_3\beta_3$ during catalysis" [27].

Before the introduction of the concept the peripheral stalk, it had been proposed that some of the "supernumerary" subunits of

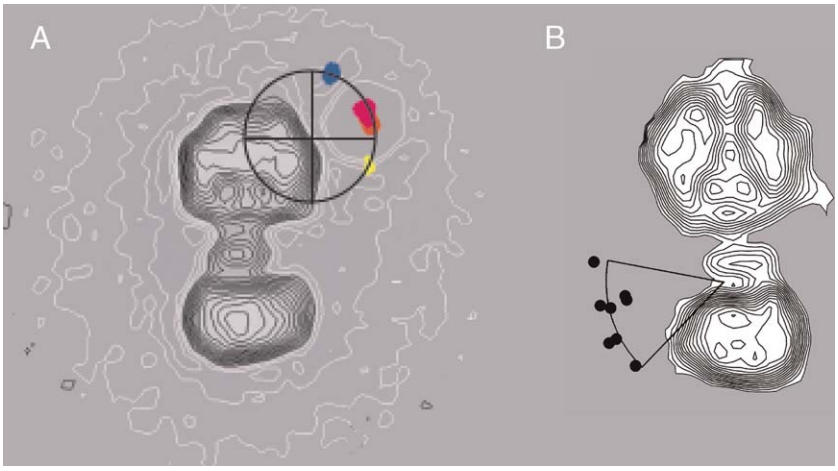

Fig. 5. Localisation of the C-termini of the OSCP and subunit h (F_6) in the F-ATPase from *S. cerevisiae*. Biotinylation signals were fused to the C-terminals of (A) the OSCP and (B) subunit h. They became biotinylated in vivo and avidin was attached to the purified complexes in vitro. The complexes were visualised by negatively stained single particle electron microscopy. The range of avidin positions from a series of class averages can be triangulated back to a single point on the surface of the F-ATPase particle. From [50,51].

the mitochondrial F-ATPase (including the OSCP, b, d and F_6) form a mosaic that is equivalent to the structure of the bacterial δ- and b-subunits [26]. Thus, in the proposal of the concept of the peripheral stalk in the mitochondrial enzyme, it was implicit that a similar feature involving their δ- and b-subunits was present in bacterial and chloroplast F-ATPases also, as was then proposed in the spinach chloroplast [42–44].

2.5. Electron microscopic observation of peripheral stalks in F-ATPases

The proposal that F-ATPases had a peripheral stalk feature provoked a number of investigations by electron microscopic examination of single particles of the F-ATPase complexes from various species combined with averaging of the images. Thus, evidence was obtained for the presence of the peripheral stalk in negatively stained single particles of the F-ATPase from bovine mitochondria and other sources [1,45–47] (Fig. 3). Whilst these experiments provided direct evidence for the peripheral stalk, it became clear that the images produced by averaging several thousand particles in each case were not accurate representations of the F-ATPase complexes; they lacked detail and they were too symmetrical. The first problem is inherent in the limited resolution attainable by averaging negatively stained particles. The second is likely to arise, at least in part, from the averaging process itself where the inclusion in each case [1,45–47] of mirror symmetric images may have forced the 3D images to have a mirror plane of symmetry [48]. A compounding difficulty is that the particles have a strongly preferred orientation on the electron microscope grids where their long axis lies in the plane of the grid, and therefore these 3D-reconstructions were based solely on side views [48].

The way around these problems was to conduct the single particle analysis in vitreous ice [48] and to exclude mirror symmetry operations from the reconstruction [48]. The three dimensional model obtained from this cryo-electron microscopic analysis, albeit at the relatively modest resolution of about 32 Å, has allowed accurate structures of the F_1 domain and of the F_1–c_{10} complex to be docked into the model of F-ATPase, revealing much more clearly the full extent and shape of the peripheral stalk. In the model, the peripheral stalk extends from the top of the F_1 domain, along the external surface of the $\alpha_3\beta_3$ complex, down to the membrane surface and into the membrane domain itself, where a substantial part of the peripheral stalk lies alongside and in close proximity to the c-ring (Fig. 4). This membrane embedded region contains subunits a and A6L, and the membrane spanning regions of subunits e, f and g [49]. Apart from revealing the overall structure of the peripheral stalk, the additional importance of this 3D-model is that it provides an accurate template into which high resolution structures of additional individual subunits or of subcomplexes of the F-ATPase, determined by solution NMR studies or by X-ray crystallography, can be docked, so as to build up an accurate high resolution model of the peripheral stalk, and eventually of the entire enzyme complex. As part of this process, it will be important to improve the resolution of the cryo-em 3D-model itself.

3. The structure of the peripheral stalk

3.1. Locations of subunits in the peripheral stalk in intact F-ATPases

The C-termini of two peripheral stalk subunits, OSCP and F_6, in the mitochondrial enzyme from *S. cerevisiae* were located by creating strains of the organism where a linker sequence followed by a biotinylation signal was fused to the C-terminus

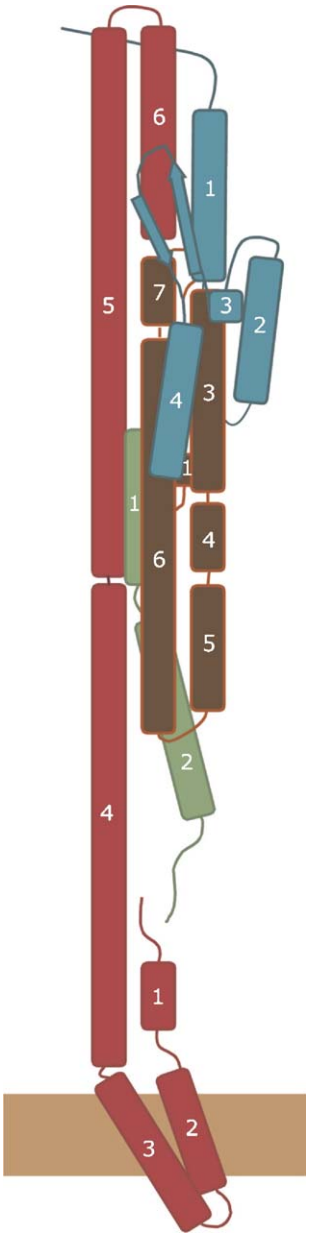

Fig. 6. Model of the arrangement of the subunits in the peripheral stalk of mitochondrial ATP synthase based on biochemical data and secondary structure predictions. Rectangles, arrows and lines represent α-helices (numbered from N- to C-termini), β-strands and random coil residues, respectively. The arrangement is based on peptide array studies of inter-subunit interactions in the context of other biochemical data. Subunits b, d and F_6 are magenta, brown and green, respectively; the C-terminal domain of the OSCP is cyan. Secondary structure predictions were carried out using PSIpred [74].

of the subunit of interest. The proteins became biotinylated in vivo, and avidin was bound to the biotin in vitro in the purified enzyme complex. The avidin molecules associated with the F-ATPase complex were visualised readily by averaging single particles observed by electron microscopy in negative stain. In this way, the C-terminus of the OSCP was determined to be approximately 90 Å from the top of ATP synthase along the side of F_1 (see Fig. 5A; [50]) and that of the C-terminus of F_6 (known as subunit h in *S. cerevisiae*) was localised near to the membrane domain of ATP synthase close to the region where the peripheral stalk enters the membrane (see Fig. 5B; [51]).

Evidence for the location of the *E. coli* δ-subunit was obtained by labelling the enzyme with a monospecific antibody for the subunit (epitope unknown), and then by comparing the 3D reconstructions of negatively stained images from electron microscopy of the labelled and unlabelled enzymes. An additional region of density in the labelled enzyme relative to the unlabelled enzyme was attributed to the antibody [52]. It provided evidence that the δ-subunit (or at least the region containing the epitope) was on the top of the F-ATPase.

3.2. Intersubunit interactions in the peripheral stalk

In a series of reconstitution experiments with bovine OSCP, b′, d and F_6, it was shown that the OSCP interacts via its C-terminal domain with an N-terminal segment of subunit b (residues 121–214), and that subunit b interacts with both subunits d and F_6 [6]. The binary complexes OSCP·b′ and b′·d and the ternary complexes OSCP·b′·d or b′·d·F_6 were isolated, but the binary and ternary complexes were less stable than the quaternary complex OSCP·b′·d·F_6 [6], As the complexes were simplified further by omitting components, and as the individual components were shortened by deletion, the susceptibility of the complexes to limited proteolysis increased (J. A. Silvester and Walker, unpublished results). However, one particular ternary complex consisting of residues 79–184 of subunit b, residues 1–124 of subunit d and the whole of F_6 crystallized spontaneously at 4 °C (J. A. Silvester and J. E. Walker, unpublished work). The X-ray diffraction properties of these crystals have been improved, and the structure of most of this sub-complex has been solved by X-ray crystallography (V. Kane Dickson, A. G. W. Leslie and J. E. Walker, unpublished results).

Inter-subunit interactions in the bovine peripheral stalk have also been studied by use of peptide arrays. These experiments have uncovered associations within segments of the OSCP with regions of subunit b (residues 119–145 of the OSCP with residues 193–214 of subunit b), and with regions of subunit d (residues 94–125 of the OSCP with residues 16–40 of subunit d; residues 94–125 of the OSCP with residues 149–160 of subunit d; residues 174–190 of the OSCP with residues 76–120 of subunit d). They also provided evidence for an association between residues 80–120 of subunit d with residues 202–214 of subunit b, and between residues 76–100 of subunit d and residues 54–60 of F_6 (V. Kane Dickson and J. E. Walker, unpublished results). These data, in combination with predictions

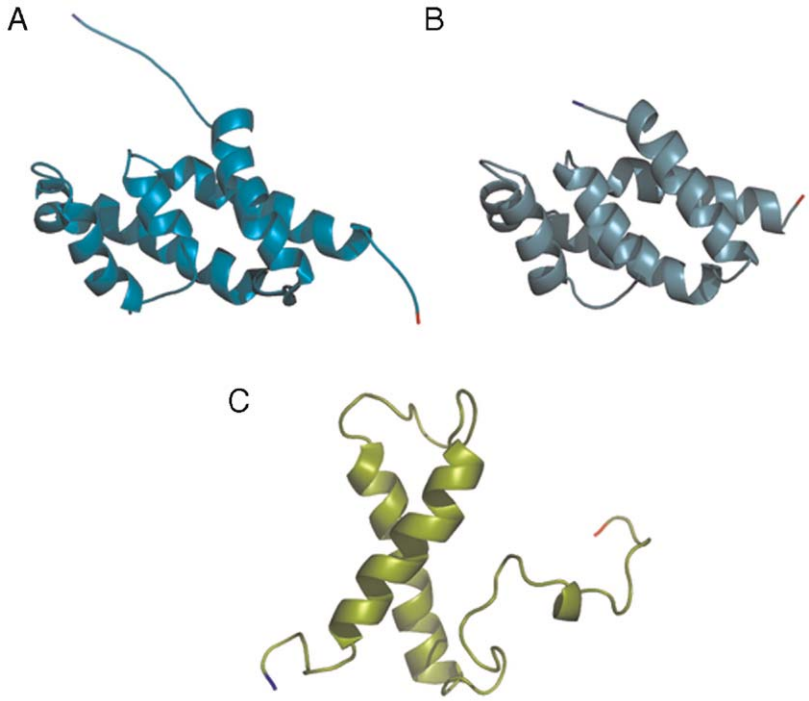

Fig. 7. NMR solution structures of subunits of the peripheral stalks of ATP synthases: the N-terminal domains of the bovine mitochondrial OSCP and of its bacterial homologue subunit δ, and the bovine F_6 subunit are shown. The sequences of the N-terminal domains of the OSCP and the δ are homologous and the domains have very similar folds (see also Fig. 10). Part A, residues 1–121 of bovine OSCP (PDB code 2BO5, [53]); part B, residues 1–134 of the *E. coli* δ-subunit (PDB code 1ABV, [54]); and part C, residues 1–76 of bovine F_6 (PDB code 1VZS, [55]). N- and C-termini are blue and red, respectively.

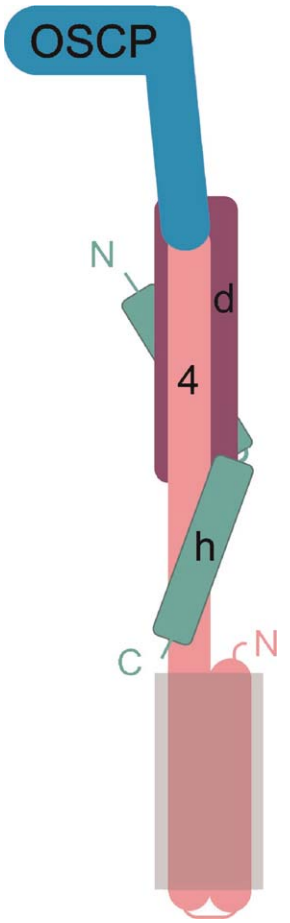

Fig. 8. Model of the F-ATPase with subunit F_6 in an extended conformation in the peripheral stalk. F_6 (green) forms an α-helical hairpin in solution as determined by NMR (see Fig. 8), but is likely to interact with other peripheral stalk subunits in an extended conformation with its C-terminus near to the membrane based on the C-terminal localisation of subunit h in yeast (see also Fig. 6). From [51].

of the secondary structures of the subunits, have led to a more detailed proposal for the arrangement of the subunits in the mitochondrial peripheral stalk (Fig. 6).

3.3. Structures of individual peripheral stalk subunits

3.3.1. The OSCP and the bacterial δ-subunit

The sequences of the bovine OSCP and the bacterial δ-subunits are 190 and 177 residues long, respectively, and they are 27.7% identical. Secondary structure prediction methods indicate that the OSCP consists of a series of α-helical regions between residues 1–112, followed by first, a β-structure, and then three α-helices interspersed with three β-strands. The region following residue 112 is susceptible to proteolysis, and so the protein appears to consist of two separate domains, as does the *E. coli* δ-subunit. The structures of the N-terminal domains of both the bovine OSCP (PDB code 2BO5; [53]), and the *E. coli* δ-subunit (PDB code 1ABV; [54]) have been determined by solution NMR studies. They both consist of a bundle of six α-helixes and their folds are extremely similar (Fig. 7A and B). Solution NMR studies of the C-terminal domains of both proteins indicate that they are unfolded.

3.3.2. F_6

The structure of recombinant bovine F_6 was determined by solution ^{1}H NMR studies (PDB code 1VZS; [55]). The solution structure is highly flexible, and consists of two α-helical regions from residues 7–22 and 34–51 that cross over in a loosely packed hydrophobic region (Fig. 7C). The N- and C-terminal regions and the linker connecting the α-helices are largely unstructured, and the range of structures observed was reminiscent of a molten globule state. Therefore, it was proposed that this structure might not represent the structure of F_6 assembled in the peripheral stalk in the intact F-ATPase complex [55]. In the light of the C-terminal localisation of yeast F_6 (subunit h) close to the membrane surface [51], it seemed more likely that F_6 assembled in the peripheral stalk would have an extended linear conformation oriented approximately along the long axis of the peripheral stalk, with the N-terminus nearer the top and the C-terminus pointing towards the membrane (Fig. 8) [51].

The sequences of bovine F_6 and its yeast homologue, subunit h, are 14.5% identical [56]. The yeast protein is 16 residues longer than the bovine protein.

3.3.3. The E. coli subunit b

The *E. coli* subunit b is 156 residues in length. It has been divided into four functional domains [57]. They are in order from C- to N-terminus, the δ-subunit binding domain, the dimerisation domain, the tether region and the membrane domain (see Fig. 9). The structure of the membrane spanning region (residues 1–33) has been solved by solution ^{1}H NMR in organic solvents, and, as expected, the sequence is α-helical (1B9U; [58]). The structure of residues 62–122, which contains the

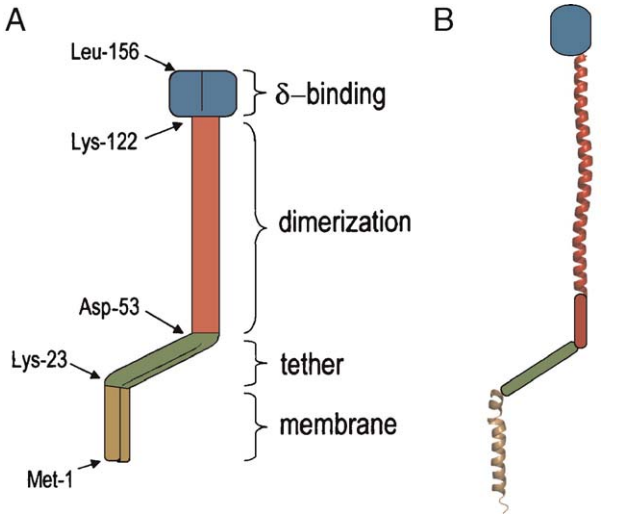

Fig. 9. The proposed quadripartite structure of the *E. coli* subunit b. (A) The arrangement of domains of the homodimer of subunit b with labelled residues at the junctions between domains. The b subunit is predominantly α-helical (adapted from [57]). (B) A monomer of subunit b including the NMR-determined structure of the membrane domain (PDB code 1B9U, [58]) and the X-ray crystallographic structure of the dimerisation domain (PDB code 1L2P; [59]).

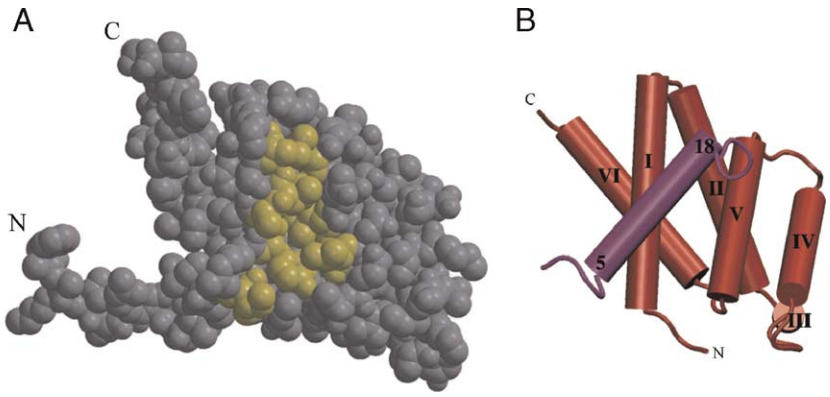

Fig. 10. The interaction of the N-terminal residues of an α-subunit with the N-terminal domain of the OSCP in mitochondria, or the δ-subunit in *E. coli*. (A) Residues in helices 1 and 5 of the bovine OSCP N-terminal domain that interact with a peptide consisting of residues 1–17 the α-subunit are yellow [53]; (B) the solution structure of the complex of the α-peptide (purple) from *E. coli* F-ATPase with the N-terminal domain of the δ-subunit (red) [61].

"dimerisation domain", has been solved by X-ray crystallography. The structure is monomeric and is also α-helical (PDB code 1L2P; [59]). It has been proposed that the two b-subunits interact via a right-handed coiled-coil [59].

3.3.4. Mitochondrial subunits b and d

Despite considerable effort, no region of subunits b and d from bovine and yeast mitochondria has been found to be suitable for solution NMR studies. Nor have crystals been grown from any part of either protein in isolation.

3.4. Building up the structure of the peripheral stalk

The low resolution cryo-em structure of the bovine F-ATPase provides a frame-work in which to build up a detailed structure of the complex from the atomic structures of its constituent domains. Hence, the structures of the F_1 domain [39] and of the F_1–c_{10} complex [60] were docked into the cryo-em structure, revealing the extent of the peripheral stalk, including its membrane domain (see Fig. 4; [48]). Similarly, as shown in Fig. 11, the structure of the N-terminal domain of the bovine OSCP [53] has been docked into additional density above the crown region on top of the F_1 domain of the cryo-em model of the intact F-ATPase. This location is consistent with other data (see above). By addition of N-terminal peptides from the α- and β-subunits of F_1-ATPase, it has been shown that a single α-peptide (residues 1–20), and not the β-peptide (residues 1–15 or 3–37), binds to the N-terminal domain of the OSCP in a groove between helices 1, 2 and 5 [53], and a similar mode of binding has been proposed for a similar region of the α-subunit of F_1-ATPase from *E. coli* with the N-terminal domain of the δ-subunit (Fig. 10) [61]. Two orientations of the N-terminal domain of the OSCP are possible, either with the N-terminal region of an α-subunit sandwiched between the domain and the top of the F_1 domain, or with the binding groove exposed above F_1 with N-terminal region of an α-subunit reaching over and interacting with this exposed upper surface of the OSCP domain. Marginally, the latter orientation appears to fit the density better, and so currently, it is the slightly preferred orientation (Fig. 11). At present, there is no clear evidence of other specific contacts between the N-terminal domain of the OSCP and other regions of F_1-ATPase, and in the *E. coli* F-ATPase, it has been proposed that this single contact with the N-terminal region of an α-subunit is sufficient to resist the torque generated by the rotary mechanism [62]. It remains to be established whether this is so in the mitochondrial enzyme.

As other detailed structures, such as that of the peripheral stalk sub-complex (V. Kane Dickson, A. G. W. Leslie and J. E. Walker, unpublished results), are determined, they also will be used to interpret the cryo-em model of the F-ATPase. In this way, it should be possible to build up a detailed model of the entire stator complex, including the membrane domain which contains the essential a-subunit, as well as the minor subunits e, f, g and A6L.

4. The function of the peripheral stalk

Each 360° rotation of the central stalk in a clockwise manner (as viewed from the membrane) inside the F_1 domain generates

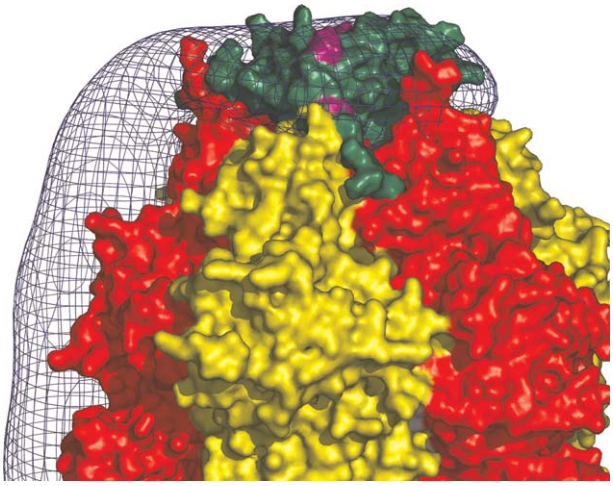

Fig. 11. Position of the N-terminal domain of the bovine OSCP in an electron density map of the F-ATPase. The structure of the N-terminal domain of OSCP was solved by solution NMR (green) [53] and docked manually into the stator domain of the electron density map of bovine F-ATPase [48].

three ATP molecules, and the rotation of the central stalk proceeds, to a first approximation, in 120° steps. The foot of the central stalk is intimately associated with the ring of c-subunits in the membrane, and the rotation of the ring is driven by the passage of protons inwards into the mitochondrial matrix. In the enzyme from yeast mitochondria, at least, the c-ring has ten c-subunits [60], and current models require each 360° rotation of the ring to be accompanied by the translocation of ten protons. This mis-match between the ten-fold symmetry of the ring and the approximate three-fold symmetry of the F_1 domain requires that during the stepping rotation of the central stalk, energy is stored transiently, presumably in an elastic element, and then released in a quantum to drive each 120° step. Where is this elastic energy storage element in the F-ATPase complex? The two most obvious candidates are the α-helical region of the γ-subunit in the central stalk and the peripheral stalk. Molecular dynamics simulations of the 120° rotary step suggested that the rotation of the central stalk is accompanied by a series of structural transitions in the γ-subunit that could be part of the energy storage mechanism [63], but this suggestion awaits experimental evidence.

It has also been suggested many times that the peripheral stalk is a flexible tether [43,48,64–71] that could also store elastic energy [72]. The ability to delete up to seven residues of the b-subunit in the *E. coli* enzyme or to introduce up to 14 additional residues to the same protein without impairing its function [64,65] has been interpreted as support for flexibility of the bacterial peripheral stalk. However, the impact of these changes on the structure of the peripheral stalk is not known, and necessarily the properties of the mutant forms rather of than those of the wild-type enzyme are being investigated in these experiments. Whilst the experiments demonstrate that the peripheral stalk can be elongated or shortened without deleterious effect, and that these changes are accommodated by the enzyme, they do not necessarily provide information about the structure and function of the peripheral stalk in the wild-type enzyme. One puzzling aspect of some of these experiments is that mutations were introduced in a region (residues 53–122) of subunit b that is required for its dimerisation and that cannot be disrupted without affecting function [73].

5. Perspectives

Recently, considerable progress has been made towards establishing high resolution structures of the peripheral stalk regions of both the bacterial and mitochondrial F-ATPases. The peripheral stalks clearly have common features, notably the structural homology between bacterial δ-subunit and the mitochondrial OSCP, and the similarity in their mode of binding of their N-terminal domains to the F_1 domain via the N-terminal region of a single α-subunit. The relationship between the sequences of the bacterial δ-subunits and the OSCP persists into their C-terminal domains, implying that they interact in a similar way with the bacterial b dimer and mitochondrial b (and possibly d and F_6) in a related way. However, the emerging structures of the bacterial b-dimer and the mitochondrial b·d·F_6 sub-complex appear to have rather different features, and their biophysical properties seem to differ. Some of these apparent differences may be resolved once their structures are completed and a more detailed examination of their biophysical properties has been carried out.

A number of issues concerning the peripheral stalk need to be resolved. First, how does the C-terminal region of the OSCP and the bacterial δ-subunit interact with other subunits of the peripheral stalk? Second, the regions of specific interaction of the peripheral stalk with other components in the F-ATPase complex need to be established. Are there regions of specific interaction on the surface of F_1-ATPase, and how does the membrane domain of subunit b interact with subunit a and other components in the membrane sector of the mitochondrial enzyme? Third, is the single interaction between the N-terminal region of the α-subunit with the N-terminal domain of the OSCP sufficient to resist the torque generation by the rotary mechanism of the mitochondrial enzyme? Fourth, is the peripheral stalk elastic or is it a rigid element? Once these and other questions have been answered the role of the peripheral stalk and the mechanism of ATP synthesis by the F-ATPase will be clearer.

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