Hypothesis

ATP synthase: a tentative structural model

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Abstract Adenosine triphosphate (ATP) synthase produces ATP from ADP and inorganic phosphate at the expense of proton- or sodium-motive force across the respective coupling membrane in Archaea, Bacteria and Eucarya. Cation flow through the intrinsic membrane portion of this enzyme (F_O, subunits ab_2c_{9-12}) and substrate turnover in the headpiece (F_1, subunits $\alpha_3\beta_3\gamma\delta\varepsilon$) are mechanically coupled by the rotation of subunit γ in the center of the catalytic hexagon of subunits $(\alpha\beta)_3$ in F_1. ATP synthase is the smallest rotatory engine in nature. With respect to the headpiece alone, it probably operates with three steps. Partial structures of six out of its at least eight different subunits have been published and a 3-dimensional structure is available for the assembly $(\alpha\beta)_3\gamma$. In this article, we review the available structural data and build a tentative topological model of the holoenzyme. The rotor portion is proposed to consist of a wheel of at least nine copies of subunits c, ε and a portion of γ as a spoke, and another portion of γ as a crankshaft. The stator is made up from a, the transmembrane portion of b_2, δ and the catalytic hexagon of $(\alpha\beta)_3$. As an educated guess, the model may be of heuristic value for ongoing studies on this fascinating electrochemical-to-mechanical-to-chemical transducer.

Key words: Bioenergetics; F_OF_1-ATPase; Biomechanics; Molecular model; Stepper motor

1. Introduction

Adenosine triphosphate (ATP) serves as the general energy currency of the cell. The standard free energy of ATP synthesis from adenosine diphosphate (ADP) and inorganic phosphate (P_i) is about +30.5 kJ/mol. In photosynthetic and respiratory organisms, ATP is produced by ATP synthase [1–12]. ATP synthase utilizes protonmotive force [1] or, in some organisms, sodium-motive force [13,14] to drive this endergonic reaction. At least for the chloroplast enzyme, there is agreement that the enzyme conducts four protons across the coupling membrane for each molecule of ATP that it produces [15,16]. Whether the same stoichiometric ratio holds for this enzyme in bacteria, mitochondria, and chloroplasts is under contention. The elements for proton transport and for the ATP-synthesis/hydrolysis reaction are separated in the bipartite structure of this enzyme. The membrane-intrinsic portion (F_O) is electrically conducting and carries the selectivity filter for ions [17,18]. The peripheral portion (F_1) carries three catalytic nucleotide-binding sites. Three further sites are present but apparently without function since their abolition by site-directed mutagenesis does not inhibit the activity [19]. When F_1 is detached from F_O, and thereby decoupled from protonmotive driving force, it catalyzes the hydrolysis of ATP. Depending on the organism, ATP synthases are composed of at least eight different and a total of at least 20 polypeptide chains. The ATP synthase from *Escherichia coli* serves as a prototype with a subunit composition of $(\alpha\beta)_3\gamma\delta\varepsilon$ for F_1 and ab_2c_{9-12} for F_O [3,8,9,11,12].

Despite their complex structure and long period of evolutionary divergence, ATP synthases from bacteria, chloroplasts and mitochondria are remarkably conserved, not so much at the level of primary protein structure but rather at the level of tertiary and quarternary structure. Some chimeric constructs composed of subunits from different sources are functional. This holds for constructs merging subunits from chloroplasts or cyanobacteria with those from *E. coli* [20] and *Rhodospirillum rubrum* [21], or even for the sodium conducting F_O portion from *Propionigenium modestum* with the F_1 portion from *E. coli* [22]. The functionality of chimeric constructs justifies to merge data that have been obtained with ATP synthase from various organisms in order to construct a unified model of its function.

A wealth of biochemical evidence supports the concept of a rotating or alternating binding change mechanism, which involves at least two if not all three highly cooperative binding sites [23–26]. It envisages that the conversion of ADP+P_i into ATP occurs spontaneously on the enzyme without major input of free energy. However, ATP remains tightly bound. Substrate binding at another site coupled to product release from the currently active site is the major energy requiring step [24]. They are driven by a conformational change that is caused by ion flux through F_O. A mechanical coupling between F_O and F_1 has rather early been suggested based on experiments showing that a chemical modification within F_O causes affinity changes of the nucleotide binding sites in F_1 [27,28]. Obviously, events in the channel portion are 'conformationally communicated' over a long distance ($\sim$12 nm) into the catalytic portion.

This concept has been greatly strengthened by the crystal structure of a major part of the mitochondrial F_1 [29]. It shows that the three catalytic sites are non-equivalent at any given moment, a finding backed up by titration with nucleotides [30]. Although the enzyme is functionally asymmetrical at any instant, it probably carries functional C_3 symmetry on the time average. The crystal structure with subunit γ in the center of the $(\alpha\beta)_3$ hexagon has been interpreted as a snapshot of the crankshaft-like rotation of γ relative to $(\alpha\beta)_3$ [29].

Three lines of specific experiments that were stimulated by the crystal structure have increased confidence in the rotatory motion of γ.

(1) The group of Richard Cross has relied on cleavable

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(disulfide) cross-links between engineered subunits β and γ, showing that the association of γ with a particular β is replaced by its association with another β only if $(F_O)F_1$ hydrolyzes ATP, but not if the enzyme is inactive [31,32].

(2) Our group has presented the first recording in real time of the rotation of γ relative to $(\alpha\beta)_3$. **P**olarized **a**bsorption **r**ecovery **a**fter **p**hotobleaching (PARAP), a photoselection technique, was applied to the isolated spinach CF_1 that was immobilized via $(\alpha\beta)_3$ on an anion exchange resin [33]. The photobleachable dye eosin was linked through a maleimide function with subunit γ. We observed the rotation of γ over at least 280° [34]. The rotation occurred in about 100 ms, which conformed with the turnover-time of the enzyme under these conditions. There was no rotation in the presence of the non-hydrolyzable ATP analogue AMP–PNP.

(3) The groups of M. Yoshida and K. Kinosita have directly recorded the unidirectional rotation of a macroscopic fluorescent label which was attached to γ [35]. F_1 from a thermophilic bacterium was immobilized via engineered His-tags to a Ni-coated support. A fluorophore-labelled actin filament served as reporter. Filaments were about ~200 times longer than the diameter of F_1. In the presence of Mg^{2+}–ATP the clockwise rotation of γ was observed (viewed from top).

At this stage it is very likely that the hydrolysis of ATP by the three catalytic sites on $(\alpha\beta)_3$ drives γ around in three steps. It is also probable that the rotatory motion persists in the holoenzyme (see [32]), so that it may drive proton transport through the channel portion of the enzyme. The pivotal role of a rigid crankshaft in coupling ATP extrusion with proton flow is indirectly corroborated by the observation that proteolysed γ, cut in several positions, still allows for ATP hydrolysis, even at a higher rate, although it does not work in ATP synthesis [36].

ATP synthesis is conceived as the reversal of this process. Proton flow generates a rotation within F_O which is relayed through γ into F_1 where it promotes the liberation of ATP from the catalytic sites. In hindsight, it is astounding to what extent two basic concepts in bioenergetics, Mitchell's chemiosmotic theory [1] and Boyer's binding change mechanism [26] have been proven to be correct, despite the lack of detailed *structural* knowledge at the time when they were first published.

2. Known structural elements and a hypothetical structure of F

The crystal structure of a major portion of F_1 from bovine heart mitochondria has been determined at 2.8 Å by the groups of J.E. Walker and A.G.W. Leslie in Cambridge, UK [29]. Crystal growth was critically dependent on the concentrations of ADP and AMP–PNP, a non-hydrolysable ATP analogue. The structure represents the *inhibited* enzyme. The three alternating α and β subunits are arranged in a hexagon with subunit γ in the center. Part of the latter was disordered in the crystals. The resolved portion of γ consists of three α helices. Two of these, comprising the N-terminal 44 and the C-terminal 63 residues, are arranged like a left-handed antiparallel coiled-coil, and the remaining 11 resolved residues form a third α-helical stretch located roughly at a right angle underneath the $(\alpha\beta)_3$ hexagon. The α and β subunits are rather similar. They consist of a N-terminal β-barrel domain, an α/β nucleotide-binding domain and a C-terminal α-helical domain. The N-terminal β barrel in the holoenzyme comprises all six α and β subunits and presumably serves as a clamp. The C-terminal α-helical domain of the β subunits moves in order to open or close the active site.

We cast the subunits of F_OF_1 into a tentative model by using the following strategy. Structural data obtained by direct and indirect techniques from various organisms were related to the enzyme from *E. coli*. The corresponding sequences of the *E. coli* enzyme were built into the crystal structure of the mitochondrial F_1 ([29], Brookhaven Protein Data Bank 1bmf) by homology modelling using the program WhatIf [37]. The solution structures obtained by NMR, of the *E. coli* subunits *c* ([38], PDB: 1aty), δ [39], and ε [40] were considered to represent the respective structures in the active enzyme. For those subunits where a partial structure was not available, namely EF_O-*a* and -*b*, and for the assembled oligomer of *c*, we were left with guesswork. For the assembly of all subunits and their docking to each other, we relied mainly on cross-linking data. The tentative model is illustrated in Fig. 1. As given in detail further down, it is compatible with all available data except few results from studies on resonance energy transfer with chloroplast F_1 (see further down). The major incompatibility is the position of the C-terminal end of γ. This discrepancy has not been resolved yet. The coordinate file for the model is available upon e-mail request to engel@uni-osnabrueck.de.

3. The rotation of γ and its contacts with α and β

Abrahams et al. have noted that the rotation of γ along its long axis might open and close the catalytic sites. It resembles the action of a crankshaft in combustion engines which is driven by the pistons. Aggeler and Capaldi [41] studied the formation of disulfide bridges in a EF_1 double mutant between residues $\alpha_{S411C}/\beta_{E381C}$ and (wild type) γ_{C87}. They observed that γ preferentially bound to β, although it could also be disulfide-bridged with α. The crystal structure of MF_1 has revealed catch contacts between γ and at least two β subunits [29]. Sequence comparison between β and γ from different organisms indicates conservation of ion pairs that may be important to transiently lock γ to β by electrostatic attraction. The respective pairs between the three β subunits (with ADP, AMP–PNP and empty) and γ are listed in Table 1. Their respective distances in the model shown in Fig. 1 were all about 0.3 nm. β_{E381} and β_{E384} belong into the so-called DELSEED sequence. It will be interesting to find out whether concerted mutations which reverse the polarity of these ion pairings leave the function unimpaired.

In principle, the three α subunits also could provide these contacts with γ since the listed residues are conserved also in α ($\beta_{D302} \approx \alpha_{D326}$, $\beta_{E381} \approx \alpha_{D412}$, $\beta_{E384} \approx \alpha_{D415}$). However, with a nucleotide load as pictured in the $(\alpha\beta)_3\gamma$ structure [29], docking γ onto α would result in a steric clash between γ and the DELSEED loop of either β(ADP) or β(ATP).

4. Subunits γ, δ, ε and *b* at the interface between F_O and F_1

In most views of the holoenzyme structure, based on electron microscopic data, subunits *b*, γ, δ and ε are displayed as elements of a single, rather narrow stalk between the two portions of the holoenzyme [8,12]. More recent cross-linking data indicate that there are *two separate* 'stalk' segments that belong to the rotor and the stator, respectively. The stalk as

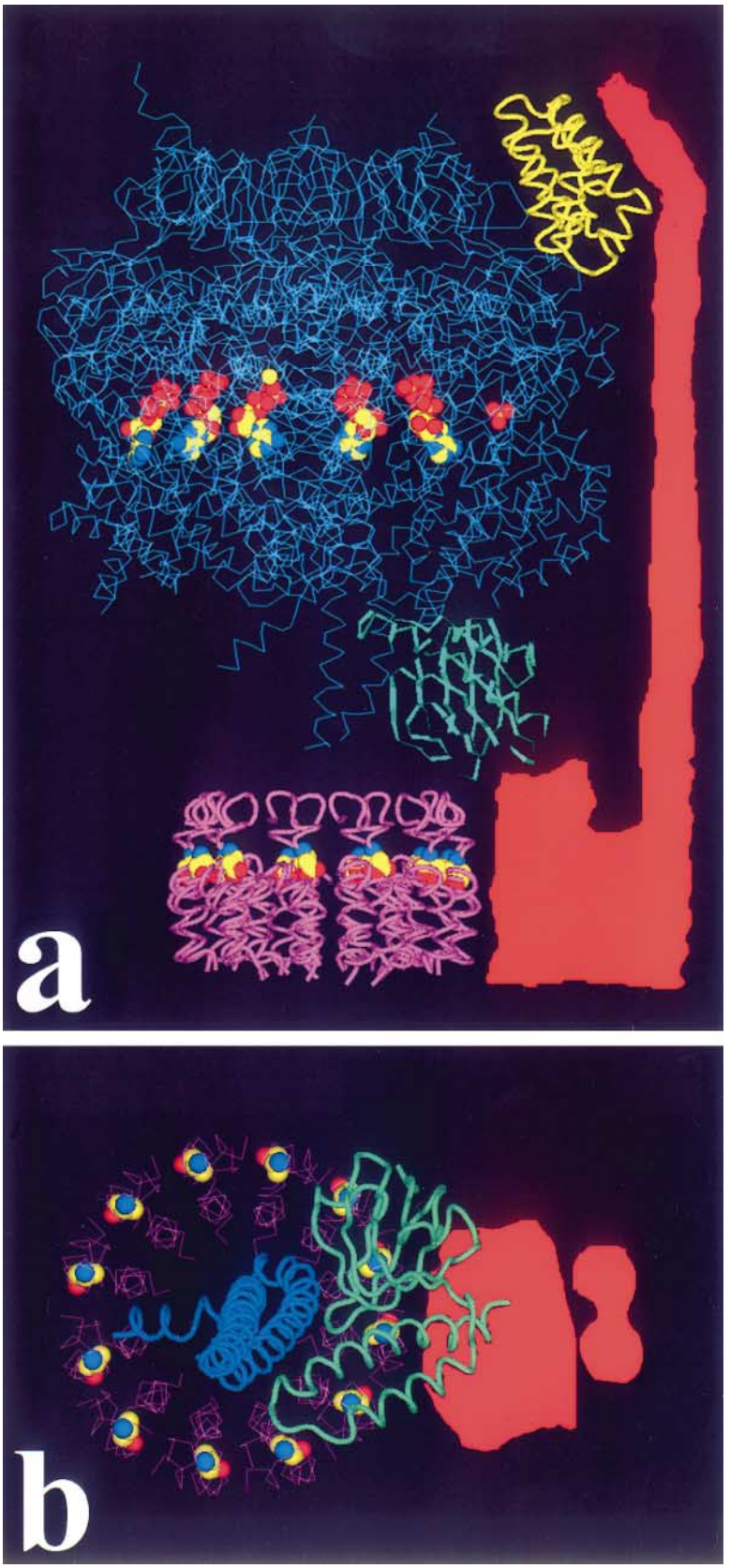

Fig. 1. a: Front view of the EF_OEF_1 *hypothetical* molecular model. C_α traces are shown. Subunits are coded from blueish (α, β, γ) to yellow (δ), green (ε), and magenta (*c*) in order to remind the reader of the relative 'reliability' of the model. In place of subunits *a* and *b* a red area is shown just in order to illustrate the approximate sizes of these two subunits. b: Top view (i.e. viewed from the F_1 side) of the C_α traces of subunits *c* (magenta) with residues D_{61} highlighted as Corey-Pauling-Koltun models. C_α traces of subunits γ (blue) and ε (green) along with the approximate areas taken by subunits *a* and *b* (red) are also shown. See text for further details.

seen previously is composed of those parts of subunit γ which are not resolved in the X-ray analysis, and of subunit ε. The second stalk comprises subunits *b* and δ. It connects $(\alpha\beta)_3$ of F_1 with subunit *a* of F_O at the outer surface.

The structure of ε consists of two domains, a 10-stranded β barrel joined to an α-helix–turn–α-helix motif [40]. Whether this structure represents ε within assembled F_1 is unknown. Cross-linking studies on both the native [42] and the mutated protein in *E. coli* [41,43–47] and in chloroplasts have revealed four interfaces of subunit ε with other subunits. Relevant contact residues are listed in Table 1.

The two antiparallel C-terminal helices of ε in connection with the plane resulting from the three helices of subunit γ sticking out at the bottom of the $(\alpha\beta)_3\gamma$ structure only allow for two positions of ε relative to the other three subunits. They are related by C_2 symmetry. In the model shown in Fig. 1 ε is oriented such as to appose residue ε_{S108} to residues α_{S411} and β_{E381}. Considering the broken symmetry in $(\alpha\beta)_3\gamma$, the respective regions of the pair α_{empty} (chain A) and β_{ADP} (chain D) are the prime candidates. Rotation of ε by 180° would have resulted in ε_{S108} being close only to α_{ADP} (chain C). The chosen positioning of ε takes into account that ε cross-links in high yield with β if F_1 is loaded with ADP [43].

With the chloroplast enzyme the cross-link pattern was independent of activity and cross-linking was not inhibitory, at least not for a cross-link between two residues on ε (Ser_{28} and Ala_{41}) and subunit γ [67]. These observations support the notion that ε and γ are fixed relative to each other. They rotate together during turnover of the enzyme.

Six residues of subunit ε have been cross-linked with residues on subunit γ. The spatial positions of the latter are located on the particular portion of γ which is still unresolved by the X-ray analysis. Since the six residues on ε are spread out rather evenly throughout one surface of the β barrel, it is likely that the missing domain of γ also covers an extended area. This is no problem, as the size of the unresolved portion of γ even exceeds the size of ε (~170 vs. 138 residues). The asymmetrical position of ε within the stalk is inevitable, regardless of whether the two separate domains of ε also share identical positions in ε which is bound to F_1. We consider it unlikely that the β barrel changes its conformation drastically upon binding of ε to F_1. It may rather serve as a rigid joint between the *c*-subunit oligomer of F_O and γ.

The two roles of ε, inhibitory for ATP hydrolysis but essential for ATP synthesis, may be explained as follows. *Clockwise* rotation of γ and ε (viewed from top) in ATP hydrolysis drives the C-terminal α helices of ε towards the C-terminal α-helical domain of subunit β and causes a sterical clash with the 'open' β. *Counter-clockwise* rotation in ATP synthesis proceeds without a clash because the respective portion of β, driven by the helix pair of γ, has moved out of the way. The situation is reminiscent of that one causing the 'hysteretic' inhibition by aurovertin of the mitochondrial F_OF_1 [49]. The location of two aurovertin-binding sites within MF_1, one high-affinity site inhibiting ATP synthesis and one low-affinity site inhibiting ATP hydrolysis, is most elegantly explained by the inversion of the rotation of γ relative to $(\alpha\beta)_3$. Its direction switches from clockwise to counterclockwise upon switching from hydrolysis to synthesis.

Subunit *b* within EF_OF_1 forms an elongated homodimer [50]. This is probably required to stabilize a structure spanning more than 10 nm. Still, the size of *b* is sufficiently large not only to *contact subunit* δ, but to actually *shield* it. This would explain why polyclonal antibodies easily recognize δ in F_1, but not in F_OF_1 [51,52].

Subunit δ had been mostly viewed as an element of the narrow stalk at the inner interface of F_O and F_1. Recent cross-link data qualify this view. In the chloroplast enzyme

Table 1
Summary of contact residues within EF_OEF_1

Interacting subunits	Residues			Reference	Remarks
α–ε	$S_{411}C$	↔	$S_{108}C$	[41]	
β–γ	E_{381}	↔	R_{242}	[29]	a
β–γ	D_{302}	↔	R_{268}	[29]	b
β–γ	D_{305}	↔	R_{268}	[29]	b
β–γ	E_{384}	↔	K_{96}	[29]	c
β–ε	$E_{381}C$	↔	$S_{108}C$	[41]	
β–ε	?	↔	$M_{138}C$	[11]	d,e
γ–ε	?	↔	C_7	[67]	d,e,f
γ–ε	?	↔	$S_{10}C$	[46]	d,f
γ–ε	?	↔	$S_{29}C$	[67]	d,e,f
γ–ε	?	↔	$H_{38}C$	[46]	d,f
γ–ε	?	↔	$A_{42}C$	[67]	d,e,f
γ–ε	?	↔	$T_{43}C$	[46]	d,f
γ–*c*	Y_{205}	↔	$Q_{42}(C)$	[47,66]	e
γ–*c*	Y_{205}	↔	$P_{43}(C)$	[47,66]	e
γ–*c*	Y_{205}	↔	$D_{44}(C)$	[47,66]	e
ε–*c*	$S_{29}C$	↔	?	[67]	d,e,f
ε–*c*	$E_{31}C$	↔	$A_{40}C$	[48]	
ε–*c*	$E_{31}C$	↔	$Q_{42}C$	[48]	
ε–*c*	$E_{31}C$	↔	$P_{43}C$	[48]	
ε–*c*	H_{38}	↔	?	[47]	d,e
ε–'outside'	$S_{18}C$, $S_{65}C$, $S_{87}C$			[46,67]	e,f

[a]β containing ADP in the Leslie-Walker structure [29].
[b]The 'empty' β [29].
[c]β containing AMP–PNP [29].
[d]? = cross-linked residue unknown
[e]Cross-linking by a heterobifunctional, photo-activable cross-linker.
[f]Data obtained with spinach chloroplast ε renumbered like *E. coli*.

five cysteines that are distributed over the length of δ have produced cross-links with the distal outer (N-terminal) portion of α and of β [53]. Studies with *E. coli* corroborated this view [39]. In EF_1, δ can be disulfide-bridged via residue δ_{C140} with a Cys on subunit α [54–56]. According to the model of EF_1, α_{C90} is the most probable partner. δ contains a second Cys in position 64. δ_{C140} and δ_{C64} were shown to be rather close in *E. coli* δ [57]. With these data in mind and the structure of ~60% of *E. coli* δ available [39], δ can be tentatively docked to F_1 as shown in Fig. 1.

Another cross-link product between δ and *b* has been reported for the chloroplast enzyme [58] suggesting a tight association between δ and *b*. Functional chimeric enzymes from *P. modestum* and *E. coli* require that δ and *b* were derived from the same source [22]. The aggregate of δ and b_2, which is attached to the outer surface of $(\alpha\beta)_3$, is an excellent candidate for the stator function. It might be fixed via the transmembrane span of *b* to the largest subunit of F_O, *a*. The rhomboid construction of two subunits *b* clamped together by δ on one side and by subunit *a* on the other could serve as an elastic element which transiently stores the free energy gained from the subsequent translocation of protons until the reaction proceeds further to the liberation of sequestered ATP.

5. The proteolipid molecules are arranged as a ring

The 'proteolipid', subunit *c* in *E. coli*, is folded like a hairpin with two transmembrane helices connected by a short loop that faces the cytoplasmic side of the membrane [38]. Images of F_O by electron [60] and atomic force microscopy [61] have supported this view.

Whether the two branches of each proteolipid molecule are radially or tangentially oriented towards the ring is an open question. In favour of a radial placement the replacement of the essential acid residue in the middle of one branch, D_{61} in *E. coli*, by glycine kills the enzyme function, but it can be restored by a second-site revertant at position Ala_{24}, at the same depth in the membrane but on the other branch of the hairpin [59]. A ring structure that fulfills this requirement is shown in Fig. 1b. To maximize the contacts between helices, we have chosen a distance of 0.5 nm between their backbones. This conforms with the average distance for other structurally resolved membrane proteins of α-helical construction (bacteriorhodopsin, photosynthetic reaction center, cytochrome oxidase). The resulting outer diameter of our model, namely ~6 nm, is compatible with the diameter resulting from the above cited work by electron (7 nm [60]) and atomic force microscopy (6–7 nm [61]).

6. The proteolipid 'ring' generates torque at the expense of proton-motive force

A minimum model explaining how a ring of proteolipid molecules might be used to generate torque from proton flow has been proposed by one of us (W. Junge 1993, [62]). It is based on three elements that are common with models describing the generation of torque by the flagellar motor: (1) an *electrostatic constraint*, (2) an element endowing the ring with *chirality*, and (3) *Brownian motion*. The majority of residues D_{61} faces the hydrophobic core of the membrane. Their carboxyl group is always protonated because of the high Born self-energy of a charged residue in contact with a hydrophobic surrounding. Only a minority of residues, the ones facing subunit *a* may be deprotonated. As a consequence of the electrostatic constraint there are only Brownian fluctuations of very small angular amplitude unless a proton is bound to a charged residue. It is assumed that there are two channels leading into that portion of the ring which contacts subunit *a*. The channels are not colinear but shifted against each other by at least one angular step. They are responsible for the chirality of the device. When a proton enters one of the entrance channels its binding to the acidic residue of one proteolipid allows the ring to step further by one unit. It is obvious that this engine would also work with sodium ions instead of protons given that the specificity of the access channels and the binding pocket around the acidic residue on the proteolipid are modified accordingly.

The above model has gained experimental support by 'site-directed second-site suppressor mutations' which identified residues $a_{R210/E219/H245}$ of EF_O as essential for proton translocation [63]. Our model does not explain how the ab_2c_{9-12} hetero-oligomer is kept assembled during rotation of c_{12} relative to ab_2.

7. Subunits γ and ε interact with a part of the c-oligomer but not with all copies

Subunits ε and γ are asymmetrical constructs. Subunit *c*, the proteolipid, is present in 10 ± 1 copies [64]. In view of the C_3 symmetry of F_1 numbers like 9 or 12 are intuitively more appealing. In the light of the stoichiometry of protons translocated per ATP produced, namely 4, 12 subunits is the number of choice for chloroplasts. The established rotation of γ relative to $(\alpha\beta)_3$ and the observation that ε and γ can be cross-linked to each other without loss of enzyme activity suggest that the complex of ε and γ as a whole makes contacts with the rotating element in the F_O portion of ATP synthase. A symmetrical contact with all copies of *c* is then impossible, but it has to be asymmetrical at any moment. There are two types of an arrangement between ε and γ and the many copies of *c*, either permanently fixed and asymmetrical, or transiently fixed but symmetrical in the time average. In more pictorial words, ε and γ may be fixed to a few out of the 12 subunits *c*, like a wheel's spoke. As an alternative, they may be moving from one copy of *c* to the next, driven by a ratchet-like motion of the loop region of the proteolipid. The first version is in line with our hypothesis of a stochastic rotor wheel, whereas the latter is in line with R. Fillingame's ideas [65] on the interrelation of proton binding and conformational changes in the loop region of the proteolipid. Two types of dichotomizing experiments are obvious: (a) the assessment of effects of ε, γ-to-*c* cross-links on the activity and (b) measurements of the rotation of the proteolipid relative to $(\alpha\beta)_3$.

Until now, one cross-linked product between subunits γ and *c* has been reported. The evidence is based upon cross-linking after chemical modification [66] and upon disulfide-bridging the appropriate mutants [47]. Apposed residues are listed in Table 1. Disulfide-bridging the double mutant $\gamma_{Y205C}c_{Q42C}$ did not greatly reduce ATP hydrolysis activity. Proton pumping is inhibited by the reagents required to induce the covalent linkage between γ and *c*, but it is not known whether ATP synthesis is also affected [47].

By placing the assembled oligomer of 12 subunits *c* concen-

trically underneath the rotor axis of γ, an acidic residue within γ close to the structurally resolved C-terminal helix would be a prime candidate for an interaction with the strongly conserved Arg within the hairpin loop of subunit *c* (R_{41}). Such a strongly conserved residue is γ_{E208}, in full accordance with observed cross-links of mutants like c_{Q42C}, c_{P43C}, and c_{D44C} with γ_{Y205} [47,66]. Since a single electrostatic interaction between one Arg/Glu(Asp) couple is hardly sufficient to provide a tight connection between γ and *c*, it might serve as a guide during assembly.

Disulfide bridges between subunits *c* and ε (cf. Table 1) are other contacts which link the 'rotor' portion of F_O (the ring of subunits *c*) with the rotor portion of F_1 (γε). Subunit ε, along with the as yet unresolved portion of subunit γ, rotates along a radius which must under all circumstances be larger than the diameter of the *c* oligomer. It is therefore both necessary to place subunits *a* and *b outside* the ring of *c* subunits and to postulate the relevant region of subunit *a not* to rise significantly above the membrane surface in order to provide enough space for γε to freely rotate.

The subunit arrangement of ATP synthase in our model is an approximation which is based upon the crystal structure of $(\alpha\beta)_3\gamma$ and the solution structures of subunits δ, ε, and *c*. It is supported by cross-link data and the demands of a rotatory mechanism of catalysis.

It is a major challenge to unravel the details of cation conduction through F_O, the generation and transient storage of torque between rotor and stator and to finally establish both high-resolution snapshots of F_OF_1 and the movie of ATP synthase at work.

8. Note added in proof

Since submission of the manuscript a number of pertinent findings has been published. Groth and Walker presented a model of the *c* oligomer along with a validity check [68]. Capaldi's group presented evidence in favor of the 'spoke on the wheel' concept (cf. section 7) [69]. They also showed that *E. coli* δ is part of the stator linking F_1 and F_O [70] and ε part of the rotor [71].

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