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The detailed molecular mechanism of ATP synthesis in the F_0 portion of ATP synthase reveals a non-chemiosmotic mode of energy coupling

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Abstract

Based on our torsional mechanism of ion translocation, energy transduction and energy storage in ATP synthase [Curr. Sci. 75 (1998) 716]; [Curr. Sci. 77 (1999) 167]; [Curr. Sci. 78 (2000) 23]; [Biochem. Biophys. Res. Commun. 272 (2000) 629]; [FEBS Lett. 476 (2000) 113]; [Thermochim. Acta 378 (2001) 35], the molecular mechanism for rotation of the c-rotor and the subsequent rotation of the γ-subunit and the ε-subunit has been proposed. The details of the elementary steps involved in ion translocation and energy conversion in the F_0 portion of ATP synthase have been provided. Electrostatic effects drive the rotation of the c-subunits in steps of 15° each during proton binding as well as unbinding. During the rotation of the c-rotor, the energy of the ion gradients is transiently stored as twist in the c-subunits, and finally as torsional strain in the γ-subunit. The mechanism has been shown to be consistent with a general kinetic analysis of ATP synthesis by ATP synthase. The detailed molecular mechanism compels *a paradigm shift* from chemiosmotic dogma (where the membrane simply acts as an insulator and only energized aqueous media are permissible) towards a view where molecular interactions between ion and protein-in-the-membrane are critical for elementary steps involving transduction, storage and utilization of the energy of the ion gradients.

Keywords: ATP synthase; Oxidative phosphorylation; F_0; Torsional mechanism; Ion translocation; Energy transduction; Energy storage; c-Subunit; γ-Subunit; Twist; Rotation-twist-tilt energy storage mechanism; Chemiosmotic theory

1. Introduction

ATP is synthesized by the enzyme ATP synthase (or F_1F_0 ATP synthase). This enzyme transforms energy from transmembrane ion gradients into the chemical energy of ATP. It consists of two major parts: a membrane-extrinsic, hydrophilic F_1 containing three α, three β, and one copy each of γ, δ, and ε-subunits, and a membrane-embedded, hydrophobic F_0 composed of one a, two b, and 12 (and not 10, 11, 13 or 14) c-subunits. The F_0 and F_1 domains are linked by two slender stalks. The central stalk is formed by the ϵ-subunit and part of the γ-subunit, while the peripheral stalk is constituted by the hydrophilic portions of the two b-subunits of F_0 and the δ-subunit of F_1. The ion channel is formed by the interacting regions of a- and c-subunits in F_0. The catalytic binding sites of ATP synthase are predominantly located in the β-subunits of F_1 at the α–β interface [1].

More than 35 years ago, in classical experiments using the acid bath procedure on chloroplast ATP

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synthase, it was reported that ATP synthesis is driven entirely by ΔpH [2]. It is generally believed that these experiments support the chemiosmotic theory [3,4] which considers the protonmotive force created solely by protons, Δp (defined as a linear addition of chemical and electrical driving forces by the relationship $\Delta p = \Delta\psi - RT\Delta\text{pH/F}$) as the driving force for ATP synthesis. This need not necessarily be the case because in the acid bath procedure [2] no protonmotive force was imposed; hence the experiments cannot be taken as evidence for support of the chemiosmotic dogma, as pointed out later in an incisive critique [5]. Further, at the molecular mechanistic level, it is not at all clear how a pH gradient and a delocalized membrane potential can be added together and how they can exert equivalent molecular forces. Moreover, it is very difficult to conceive how the uncompensated, electrogenic transport of thousands of protons required to raise the bulk electrical potential of one aqueous phase in an organelle (vis-a-vis the other aqueous phase) and create a delocalized $\Delta\psi$ across the membrane of 120–180 mV envisaged by chemiosmosis can take place. (This delocalized electrical potential created by proton translocation is essential to the chemiosmotic theory because the Δp due to protons constitutes the *sole* link between oxidation and phosphorylation and the driving force created by the redox complexes, for example, needs to be made available to the ATP synthase at a spatial location far away from the redox sites). Since ion translocation takes place discretely (one ion at a time) through aqueous channels in biomembranes, it is not clear how the translocation of protons can continue against the *restrictive diffusion potential* that each primary translocation event will generate. From fundamental physical principles, this diffusion potential will prevent further translocation of protons. Transport of a single proton against its concentration gradient will generate only a negligible delocalized $\Delta\psi$ in Mitchell's model. Finally, the operation of such a chemiosmotic process will lead to a substantial alteration of pH in both cellular compartments which would have extremely deleterious physiological consequences for the organism. Williams appears to have been the first to articulate other serious difficulties with chemiosmosis [6,7] (though not those pointed out by us later [8–10] or those discussed in this section). In any case, decades of research subsequent to the acid bath experiments have demonstrated that in the chloroplast ATP synthase, as well as in the mitochondrial and bacterial enzyme, $\Delta\psi$ is a mandatory driving force for ATP synthesis. Thus, both ΔpH and $\Delta\psi$ are required for ATP synthesis. It should be emphasized, however, that there is absolutely no logical reason to believe that both the ΔpH as well as the $\Delta\psi$ are created by the *same* agent or source (e.g. protons) although, this assumption lies at the heart of the central dogma of bioenergetics. In fact, the two driving forces can be created by two independent sources, in which case they will be kinetically inequivalent. By use of innovative approaches applied in novel ways, we had proposed the first molecular mechanism of torque generation in the F_0 portion of ATP synthase that considers the role of both ΔpH and $\Delta\psi$ and in particular addresses the indispensable requirement of the $\Delta\psi_{\text{intrinsic}}$, the electrical potential difference due to the charge geometry of the c-rotor-a-stator, as well as the diffusion potential due to anion transport across the membrane, for ATP synthesis [8–12]. More specifically, in our mechanism, the roles of both ΔpH and $\Delta\psi$ are emphasized. Torque generation in the F_0 portion of ATP synthase is a result of change in the electrostatic potential, $\Delta(\Delta\psi)$, brought about by the binding of protons flowing along their concentration gradient to their binding sites in the c-subunit within the electrostatic potential field. This field is due to the charge configuration and that created by the translocation of anions moving along their concentration gradient [8–13]. In our view, the coupling between oxidation and phosphorylation is not solely due to a proton current (and nor is it due to the so-called protonmotive force, because the H^+ does not create a steady state delocalized $\Delta\psi$); rather, the energy for ATP synthesis is supplied by two independent sources: membrane-permeable (organic) anions as well as protons. Moreover, in our original proposals, only the proton (but not the anion) binds to and interacts with the protein-in-the-membrane.

In this work, we propose a detailed molecular mechanism for the rotation of the c-rotor and the conformational changes in the c-subunits accompanying this rotation. We have also discussed how the rotation of the c-rotor causes rotation of the ϵ-subunit and the bottom of the γ-subunit. The major biological implications of our proposed molecular mechanism have also been spelled out.

2. Overall torsional mechanism for the F_0 portion of ATP synthase: the $\Delta pH–\Delta\psi$ two mutually non-collinear half-channel model

The c-subunit, which functions as the rotor, has Asp-61 as the essential amino acid in each of its subunits. Arg-210 and His-245 are the key amino acids in the a subunit, which acts as the stator (the residue numbers for the F_0 domain refer to *Escherichia coli*). The exact spatial orientation of these charges is unknown and Fig. 1 represents a possible geometry that can lead to generation of a unidirectional torque. However, our molecular mechanism can readily accommodate other charge geometries. The geometry of the a- and c-subunits is such that while c is a complete cylinder, the a-subunit is part of a cylinder coaxial to c, covering two subunits of c [9–12].

The negatively charged Asp-61 must be protonated when exposed to the membrane and unprotonated at the a–c interface. When both Asp-61 residues are unprotonated, the system is at equilibrium. The proton concentration at the inner membrane, H^+_{in}, is higher than the proton concentration, H^+_{in}, in the vicinity of the leading Asp-61 residue across the proton half-channel. This concentration gradient drives the proton through the half-channel, causing it to bind to the leading Asp-61 residue. Now the positively charged His-245 attracts the trailing unprotonated Asp-61, disturbing the equilibrium and causing the inner cylinder to rotate (Fig. 1). Thus, the leading Asp-61 moves into the membrane and a new protonated Asp-61 enters the interface. Rotation in the reverse direction is prevented by the large free energy barrier to transport an unprotonated Asp-61 from the interface into the hydrophobic membrane environment. When a new protonated Asp-61 residue enters the interface, it loses its proton to become unprotonated. The proton concentration in the vicinity of the trailing Asp-61 residue, H^+_b, is higher than the proton concentration in the matrix, H^+_{out}. As a result of this concentration gradient, the proton is driven out across the proton half-channel facing the matrix (Fig. 1). The ion gradients generate a $\Delta(\Delta\psi)$ of 50–60 mV each during proton binding to as well as unbinding from the c-subunit, which results in torque generation in the F_0 portion of ATP synthase. Thus, ΔpH and $\Delta\psi$ are the overall driving forces for ATP synthesis, involving the creation of $\Delta(\Delta\psi)$ through ion–protein interactions as an intermediate step for rotation of the c-rotor and subsequent storage of torsional energy in the γ-subunit to be used thereafter for synthesizing ATP [8–15].

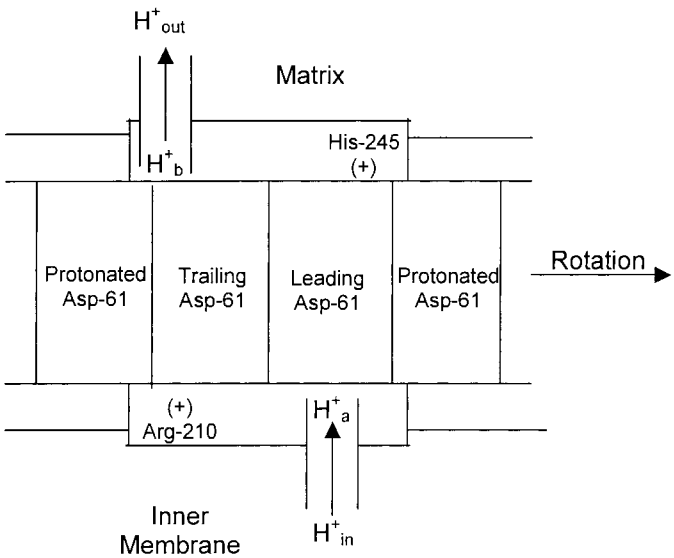

Fig. 1. Schematic diagram of the F_0 portion of ATP synthase based on the $\Delta pH–\Delta\psi$ two mutually non-collinear, half-channel model [9–12,16].

3. Details of the torsional mechanism in the F_0 portion of ATP synthase: How do the c, γ, and ϵ-subunits rotate?

The *E. coli* c-subunit is a hydrophobic protein that contains 79 residues and folds in a hairpin-like structure with two transmembrane α-helices and a polar loop region which interacts with the bottom of the ϵ-subunit and the bottom of the γ-subunit. The protonated Asp-61 residue lies in the interior of the F_0 portion of ATP synthase and at the center of the annular c-rotor; upon deprotonation, the c-subunit is visualized as adopting a twisted and bent conformation thereby bringing the Asp-61 residue to the periphery of the c-rotor [10,16]. According to the torsional mechanism of ATP synthesis, rotation of the c-rotor due to proton binding to the leading Asp-61 residue (Fig. 1) causes the Asp-61 residue of the new, incoming c-subunit to come to the a–c interface and deprotonate [9–12,16]. Since deprotonation of the Asp residue can only occur at the periphery of the c-rotor, we conclude that twisting and swiveling of the helices comprising the c-subunits is caused by the rotation of the c-rotor. Since all the protonated c-subunits are identical, we propose that all the untwisted (protonated) c-subunit helices should twist whenever the c-rotor rotates as a result of proton binding and unbinding during ATP synthesis. Taking these interpretations into consideration, a detailed molecular mechanism for inter-subunit and intra-subunit rotation in the F_0 portion of ATP synthase will now be proposed. The proposed molecular mechanism explains energy transduction and storage during both proton binding as well as proton unbinding. It uses asymmetry in the placement of the proton half-channels, elucidates each elementary step, and discusses all intermediate states for the leading and trailing Asp residues.

As a starting point, we consider the initial state of the c-rotor with both leading as well as trailing Asp residues unprotonated [9–12,16] (Fig. 1), as discussed in the previous section. The c-subunits facing the a-subunit are twisted and bent. The remaining 10 c-subunits inside the membranous region being protonated, are untwisted, i.e. in their normal/resting state for the protonated Asp residues which lie in the interior of the c-rotor (Fig. 2). In this state, the exposed Asp residues interact with the charges on the a-stator, and the system is in a local equilibrium (Fig. 1). When the

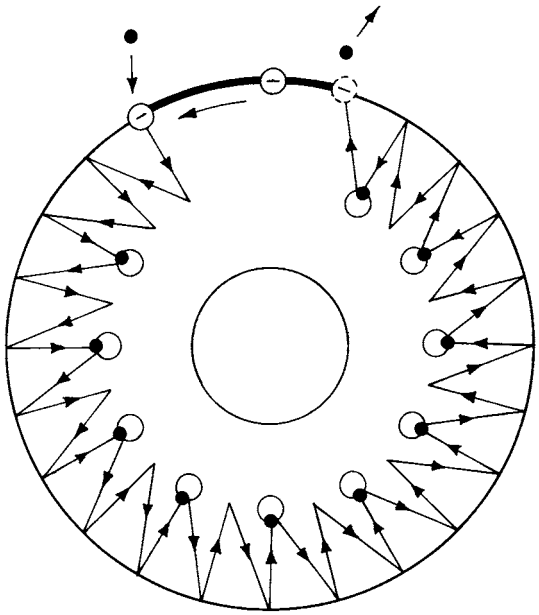

Fig. 2. The overall torsional mechanism of ion translocation and energy transduction in the F_0 portion of ATP synthase. Overall movement of the Asp-61 residues of the c-subunits in steps of 15° each. The outward movement of the Asp-61 residues depicts the twisting, tilting and bending of the c-subunit helices while inward movement represents the untwisting and straightening of the c-subunits. Proton entry and exit through the respective access half-channels following proton binding to and unbinding from the corresponding Asp-61 residues facing the a-stator are also shown.

incoming proton, which is transported along its concentration gradient through the proton half-channel facing the inner membrane (or outside) binds to the leading Asp-61 residue, the leading c-subunit immediately attains an untwisted conformation characteristic of a protonated c-subunit, and the now-protonated Asp-61 residue moves towards the center of the c-rotor. This alters the electrostatic interactions in the system and in order to attain a new local equilibrium state, the c-rotor rotates by 15°. As a result of this 15° rotation of the c-rotor, all the protonated c-subunits get twisted and bent, keeping the polar residues of the c-subunits, which are in contact with the bottom of the γ-subunit, stationary while the bottom of the c-subunits move by a full 15° displacement. This brings all the Asp-61 residues to the periphery of the c-rotor (Figs. 2 and 3). However, since this state is a metastable high-energy state for all the eleven

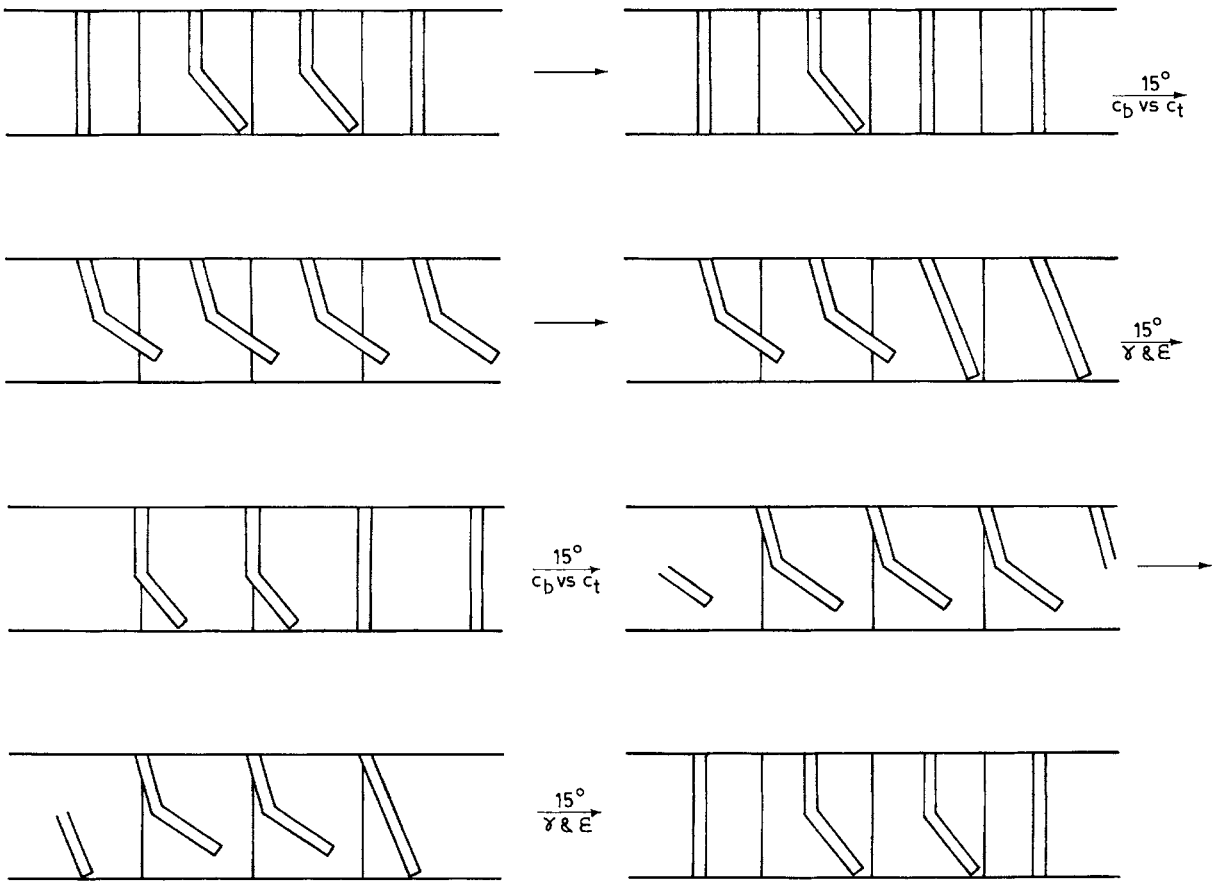

Fig. 3. The detailed torsional mechanism of ion translocation and energy transduction in the F_0 portion of ATP synthase. Details of the conformational changes in the c-subunits facing the a-stator as well as in the adjoining c-subunits for each proton translocation across the F_0 portion of ATP synthase are schematically depicted and discussed in the text. The bent c-subunits in the diagram also adopt a twisted conformation. The diagram is drawn so as to be almost true to c-subunit relative positions as well as to the position of each c-subunit as a whole with respect to the a–c interface and to changes in angles (e.g. to 15° rotations), however, helix lengths, bend lengths and intrasubunit angles are not designed to scale. The subscripts b and t refer to the bottom and top, respectively, of the c- or γ-subunit.

protonated c-subunits, all these c-subunits except the new, incoming c-subunit (discussed later) untwist simultaneously carrying all the Asp-61 residues of the 10 protonated c-subunits inside. The impulse thus created moves the polar residues of these 10 protonated and untwisted c-subunits by 15°, thereby causing these c-subunits to straighten (untilt) (Fig. 3). The electrostatic interactions between the polar residues of these 10 c-subunits and the γ-subunit cause the bottom of the γ-subunit to rotate by 15°. Hence, the energy released during the proton binding step is transiently stored as strain energy in the c-subunits as twist and is later stored as strain energy in the γ-subunit as torsion. The final state of the F_0 portion attained after the 15° rotation has the c-subunit immediately before the trailing c-subunit at the interface of the membranous and the non-membranous region, and the leading c-subunit at the other interface. The *E. coli* c-subunit contains a Pro-64 and a series of hydrophobic residues toward the end of the C-terminal helix. The portion of the C-terminal helix beyond Pro-64 bends such that the new, incoming trailing c-subunit has part of the c-subunit protruding out of the membranous region, with the rest of the helix still inside the membranous

region. The twist causes the hydrophobic residues to face the N-terminal helix and keeps them away from the aqueous medium. When the remaining 10 protonated c-subunits untwist to release the strain, this c-subunit is unable to do so, as this would require the hydrophobic residues to be in direct contact with the aqueous medium, which is energetically highly unfavorable. The untwisting of the 10 c-subunits drives the rotation of the bottom of the γ-subunit by 15°. Rotation of the bottom of the γ-subunit forces this new, trailing c-subunit to straighten. The straightening of this new, trailing c-subunit brings its Asp-61 residue to the a–c interface. This Asp-61 residue then loses its proton to the matrix (or inside) through the exit half-channel to become unprotonated. The deprotonation of this Asp-61 residue keeps the already twisted c-subunit in the same conformation characteristic of the unprotonated c-subunit. The change in the electrostatics of the system again results in a rotation of the c-rotor by 15° in order to attain a new local equilibrium position. As a result of this 15° rotation, all the protonated and, therefore, untwisted c-subunits get twisted and tilted (keeping the polar residues and the γ-subunit stationary), thereby bringing their Asp-61 residues to the periphery of the c-rotor (Fig. 3). As this is a high-energy state for all the 10 protonated c-subunits, they untwist, and the impulse created causes these protonated c-subunits to untilt by moving their polar residues by 15° (Fig. 3). The movement of the polar residues causes the bottom of the γ-subunit to rotate by an equal 15° angular displacement. It should be noted that the ϵ-subunit interacts with the bottom of the γ-subunit and the polar residues of the c-subunits, and therefore, the ϵ-subunit rotates together with the bottom of the γ-subunit [14,15]. In this way, each proton translocation drives the rotation of the ϵ-subunit and the bottom of the γ-subunit by 30° (in two steps of 15° each) and the consequent storage of energy as torsional strain in the γ-subunit. The final state of the system after these elementary steps is the same as the initial state, i.e. with both the Asp-61 residues facing the a-stator unprotonated, except that the ϵ-subunit and the bottom of the γ-shaft have rotated by 30° relative to the initial state. The movement of the ϵ-subunit and torsion in the γ-subunit cause conformational changes at the catalytic sites in the F_1 portion of ATP synthase [10,14,15], and four such proton translocations lead to the synthesis of one molecule of ATP [10,13–15,17–19]. During the synthesis of ATP, the torsional strain in the γ-subunit is released, and the process of ion translocation, energy transduction, and energy storage again follows the above-described sequence of events.

Amino acid sequences from a variety of sources, e.g. *Escherichia coli*, *Saccharomyces cerevisiae*, *Rattus norvegicus* and *Homo sapiens* were examined for the presence of Asp (or Glu), Pro, and a series of hydrophobic amino acid residues towards the C-terminus of the c-subunits. As can be seen from Fig. 4, all the studied sequences of the c-subunits contain Asp, Pro after the Asp residue on the C-terminal helix, and several hydrophobic residues towards the end of the same helix. The Asp (or Glu) is essential for proton binding/unbinding, the Pro for helix bending, and together with the hydrophobic residues for ensuring that the new incoming c-subunit does not untwist and untilt along with the remaining 10 protonated c-subunits as discussed above, otherwise proton unbinding and ion translocation will not occur. This strengthens the proposed role assigned to these residues.

Kinetic analysis based on a general kinetic scheme for ATP synthesis [9,12] considering all the elementary steps shows competitive inhibition by the proton bound to the trailing Asp-61 residue to the binding of proton to the leading Asp-61 residue. Hence, unless proton unbinding occurs from the trailing Asp-61 residue, proton binding to the leading Asp-61 residue cannot take place in the steady state physiological mode of functioning. The asymmetry of placement of the proton half-channels (the exit half-channel at the a–c interface, the entry half-channel at the center of the leading c-subunit) (Fig. 1) explains that proton unbinding from and proton translocation through the exit half-channel precedes proton binding through the entry half-channel.

A mechanism such as that presented in detail in this section (which we refer to as the rotation-twist-tilt energy storage mechanism in F_0 within the torsional mechanism of ATP synthesis) *within* the F_0 portion of ATP synthase has not been proposed earlier. Even the overall torsional mechanism for the F_0 portion of ATP synthase (the $\Delta pH-\Delta\psi$ two mutually non-collinear half-channel model) delineated in Section 2 differs in principle from some half-a-dozen other models for the F_0 portion [20–24], as described earlier [12]. Our overall mechanism is in broad agreement with, in our

Escherichia coli

PHE PHE ILE VAL MET GLY LEU VAL **ASP** ALA ILE **PRO** *MET ILE ALA VAL GLY LEU GLY LEU TYR VAL MET PHE ALA VAL ALA*

Saccharomyces cerevisiae

PHE PHE ILE VAL MET GLY LEU VAL **ASP** ALA ILE **PRO** *MET ILE ALA VAL GLY LEU GLY LEU TYR VAL MET PHE ALA VAL ALA*

Rattus norvegicus

SER PRO LEU GLN VAL ALA ARG ARG GLU PHE GLN THR SER VAL ILE SER ARG ASP ILE **ASP** THR ALA ALA LYS PHE ILE GLY ALA GLY ALA ALA THR VAL GLY VAL ALA GLY SER GLY ALA GLY ILE GLY THR VAL PHE GLY SER LEU ILE ILE GLY TYR ALA ARG ASN **PRO** SER LEU LYS GLN GLN LEU PHE SER TYR ALA *ILE LEU GLY PHE ALA LEU SER GLU ALA MET GLY LEU PHE CYS LEU MET VAL ALA PHE LEU ILE LEU PHE ALA MET*

Homo sapiens

PHE PRO LEU GLN VAL ALA ARG ARG GLU PHE GLN THR SER VAL VAL SER ARG ASP ILE **ASP** THR ALA ALA LYS PHE ILE GLY ALA GLY ALA ALA THR VAL GLY VAL ALA GLY SER GLY ALA GLY ILE GLY THR VAL PHE GLY SER LEU ILE ILE GLY TYR ALA ARG ASN **PRO** SER LEU LYS GLN GLN LEU PHE SER TYR ALA *ILE LEU GLY PHE ALA LEU SER GLU ALA MET GLY LEU PHE CYS LEU MET VAL ALA PHE LEU ILE LEU PHE ALA MET*

Fig. 4. Amino acid sequences collected from the National Center for Biotechnology Information database for the c-subunits of (a) *E. coli*; (b) *S. cerevisiae*; (c) *R. norvegicus*, and (d) *H. sapiens*. Important amino acid residues as per the torsional mechanism (Asp, Pro, and the hydrophobic residues in the series of residues in the C-terminal helix) are shown in bold or in italics.

view, the pioneering experimental work of Fillingame et al. on the F_0 portion of ATP synthase, for instance on the occluded nature of the protonated Asp-61 residue (as opposed to its positioning on the c-surface and, therefore, to an exposed nature in other models), and the envisaged need for structural changes in the c-rotor [25–28]. However, the mechanical means by which "proton transport drives the stepwise rotation of the c-subunit ring" is left as a "crucial unanswered question" [26], and the "mechanism by which energy is stored during the stepwise transport of protons" is considered "problematic" [25], and subsequently, as a question that "needs further investigation" [26], and in a recent work as "difficult to envision at this time" [28]. Moreover, numerous differences between their overall scheme and the torsional mechanism exist, for example in the proposed number of c-subunits (which is crucial to mechanism) and in their conception of a c-oligomer that remains fixed with respect to the α- and β-subunits of F_1 and their proposal of a movement of the ϵ-subunit from one copy of c to another as ATP is synthesized [25,28], which we do not subscribe to at all. In summary, our proposals are novel, and we believe that our original approach and insights elucidate the molecular mechanism of ATP synthesis.

4. Biological implications

In Mitchell's chemiosmotic theory, energy flow is confined to concentration and electrical gradients associated with protons, and a *macroscopic*, *delocalized* driving force (the protonmotive force, $\Delta p = \Delta\psi - RT\,\Delta\mathrm{pH/F}$, conceived as a linear addition of the two gradients) between two energized aqueous media separated by an *inert, rigid and insulating* membrane is envisaged. In the chemiosmotic framework, no force acts on membrane constituents, and no energy is stored in the membrane. This is also the essence of Mitchell's protonmotive osmotic energy storage equation. Thus, in chemiosmosis, *two* protons flow from the aqueous medium through a channel to the ADP site, and ATP is synthesized directly *without any changes taking place in the membrane*. Our detailed molecular mechanism shows that the ion–protein interaction energy is transiently stored as twist in the α-helices of the c-subunits of F_0 and that membrane conformational changes are intimately connected to energy transduction. This emphasizes the dynamic cyclical changes in protein structure in the membrane-bound F_0 portion of ATP synthase [8–19]. Hence, there is an imperative need to understand not only what happens across the membrane but also what happens within it. Finally, there is nothing inherently *osmotic* about the mechanism of ATP synthesis, and osmotic energy is not directly converted to chemical energy. Our molecular mechanism implies that energy transduction and transient storage cannot be understood using osmotic principles alone. Energy can indeed be stored as ion gradients across a membrane in two bulk aqueous phases; however, the membrane is not just an insulator, and according to the torsional mechanism, *molecular interactions* between ion and protein-in-the-membrane are critical for elementary steps involving transduction, storage and utilization of the energy of the ion gradients. Thus, the fundamental process of energy coupling in ATP synthesis is not chemiosmotic, but *mechano(electro)chemical*.

As far as the necessity for experimental evidence (before any mechanism can ever be proposed) is concerned, we would like to point out that experiments on F_1-ATPase have been carried out in the hydrolysis mode; nevertheless, mechanisms of ATP synthesis by F_1F_0-ATP synthase have been proposed [29]. We have shown convincingly in our works that ATP synthesis is not a simple reversal of ATP hydrolysis. Hence, one cannot propose a mechanism for ATP hydrolysis, with the enzyme acting as a hydrolase, and simply reverse the arrows to obtain a mechanism of ATP synthesis by the ATP synthase. To date, *not a single experiment* that directly monitors molecular events during steady state ATP synthesis by ion gradients in F_1F_0–ATP synthase has been reported in the literature, chiefly due to technical problems [30]. Hence *all* proposed mechanisms of the catalytic cycle of ATP synthesis are, *hypotheses*: they have literally no *direct* experimental support. Therefore, imposition of a prior requirement of experimental support, especially on an extraordinarily complex system, is an untenable proposition, has little merit, and can be dismissed outright. Moreover, as we have shown, theoretical science, and, in particular, *engineering* methodologies, if imaginatively conceived and innovatively applied, can be successfully employed to reveal mechanisms of molecular machines, and to reach levels

that experiment has not yet attained. Engineers have tremendous experience working with machines, and this has led to the development of a new field: Molecular Engineering [10,31,32]. Such methodologies can be expected to play an even more important role in this century as biology becomes increasingly dominated by computation. Nevertheless, experimental verification of some of the key predictions of our mechanism has been successfully carried out recently [33,34].

5. Summary

The molecular mechanism for ion translocation through the F_0 portion of ATP synthase has been proposed based on the torsional mechanism of ATP synthesis by ATP synthase. Proton binding to the leading Asp-61 residue causes the rotation of the c-rotor by 15°. It is proposed here that this rotation results in twisting of the protonated c-subunits, and thus, the energy of the ion gradients is stored as strain energy in the c-subunits. The release of this energy due to untwisting of the protonated c-subunits causes untilting of these c-subunits, which makes the ϵ-subunit and the bottom of the γ-subunit rotate by 15°, as explained in detail in this paper. The structure of the c-subunits ensures that the new incoming trailing c-subunit does not untwist before the bottom of the γ-subunit rotates. Rotation of the bottom of the γ-subunit untilts the new incoming trailing c-subunit without causing it to untwist, thereby exposing the Asp-61 residue to the a–c interface resulting in its deprotonation and the transport of the proton through the exit half-channel. The deprotonation of the Asp-61 residue of the new incoming trailing c-subunit drives the rotation of the c-rotor by 15°. Rotation of the c-rotor again causes the protonated c-subunits to twist which, upon untwisting, results in rotation of the ϵ-subunit and the bottom of the γ-subunit by another 15°, as discussed. Thus, mechanoelectrochemical effects are key to ion translocation in the F_0 portion of ATP synthase and result in storage of the energy of the ion gradients as torsional energy in the γ-subunit [8–19,29,31,32]. The detailed formulation of the torsional mechanism of ion translocation, energy transduction, energy storage, and ATP synthesis enables us to look at the entire process of energy dynamics in the ATP synthase along with each elementary step of transport, conformational change and reaction. This explains how these elementary events take place at the molecular level, and provides us with a *unified* view of the mechanism of ATP synthesis. It also compels us to question existing dogma and opens our minds to a new way of looking at energy dynamics at the molecular level and designing macroscopic and nanomolecular machines for the new millennium [32].

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