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

ATP Synthases in the Year 2000: Evolving Views about the Structures of These Remarkable Enzyme Complexes

Peter L. Pedersen,[1,2] Young Hee Ko,[1] and Sangjin Hong[1]

This introductory article briefly summarizes how our views about the structural features of ATP synthases (F_0F_1) have evolved over the past 30 years and also reviews some of our current views in the year 2000 about the structures of these remarkably unique enzyme complexes. Suffice it to say that as we approach the end of the first year of this new millinium, we can be conservatively confident that we have a reasonably good grasp of the overall "low-resolution" structural features of ATP synthases. Electron microscopy techniques, combined with the tools of biochemistry, molecular biology, and immunology, have played the leading role here by identifying the headpiece, basepiece, central stalk, side stalk, cap, and in the mitochondrial enzyme, the collar around the central stalk. We can be reasonably confident also that we have a fairly good grasp of much of the "high-resolution" structural features of both the F_1 moiety comprised of fives subunit types (α, β, γ, δ, and ε) and parts of the F_0 moiety comprised of either three (*E. coli*) or at least ten (mitochondria) subunit types. This information acquired in several different laboratories, either by X-ray crystallography or NMR spectroscopy, includes details about the active site and subunit relationships. Moreover, it is consistent with recently reported data that the F_1 moiety may be an ATP driven motor, which, during ATP synthesis, is driven in reverse by the electrochemical proton gradient generated by the electron transport chain. The real structural challenges of the future are to acquire at high resolution "complete" ATP synthase complexes representative of different stages of the catalytic cycle during ATP synthesis and representative also of key regulatory states.

KEY WORDS: ATP synthase; F_0F_1-ATP synthase/ATPase; F_1-ATPase; ATP synthesis; oxidative phosphorylation; molecular motors.

INTRODUCTION

The basic premise of Peter Mitchell's chemiosmotic hypothesis, i.e., that an electrochemical gradient of protons across the mitochondrial inner membrane drives the synthesis of ATP at the level of the ATP synthase (F_0F_1), gained general acceptance in the late 1960s (reviewed in Mitchell, 1979). Subsequently, attention in a number of laboratories turned to isolating, characterizing, and obtaining subunit information about ATP synthases (Fig. 1A). Structural studies commenced in the early 1970s using electron microscopy, and combined with biochemical, molecular biological, and immunological tools, have continued to this day. Significantly, these studies were not complemented with high-resolution X-ray crystallographic data until the mid- to late 1990s when three different groups solved the structures, in part, of F_1 preparations from three different sources (Abrahams *et al.*, 1994; Shirakihara *et al*, 1997; Bianchet *et al.*, 1998). Still lacking are high-resolution structural data of the F_0 moiety

[1] Department of Biological Chemistry, Johns Hopkins University, School of Medicine, 725 North Wolfe Street, Baltimore, Maryland 21205-2185.

[2] To whom correspondence should be addressed: e-mail: ppederse@welchlink.welch.jhu.edu

0145-479X/00/0800-0325$18.00/0

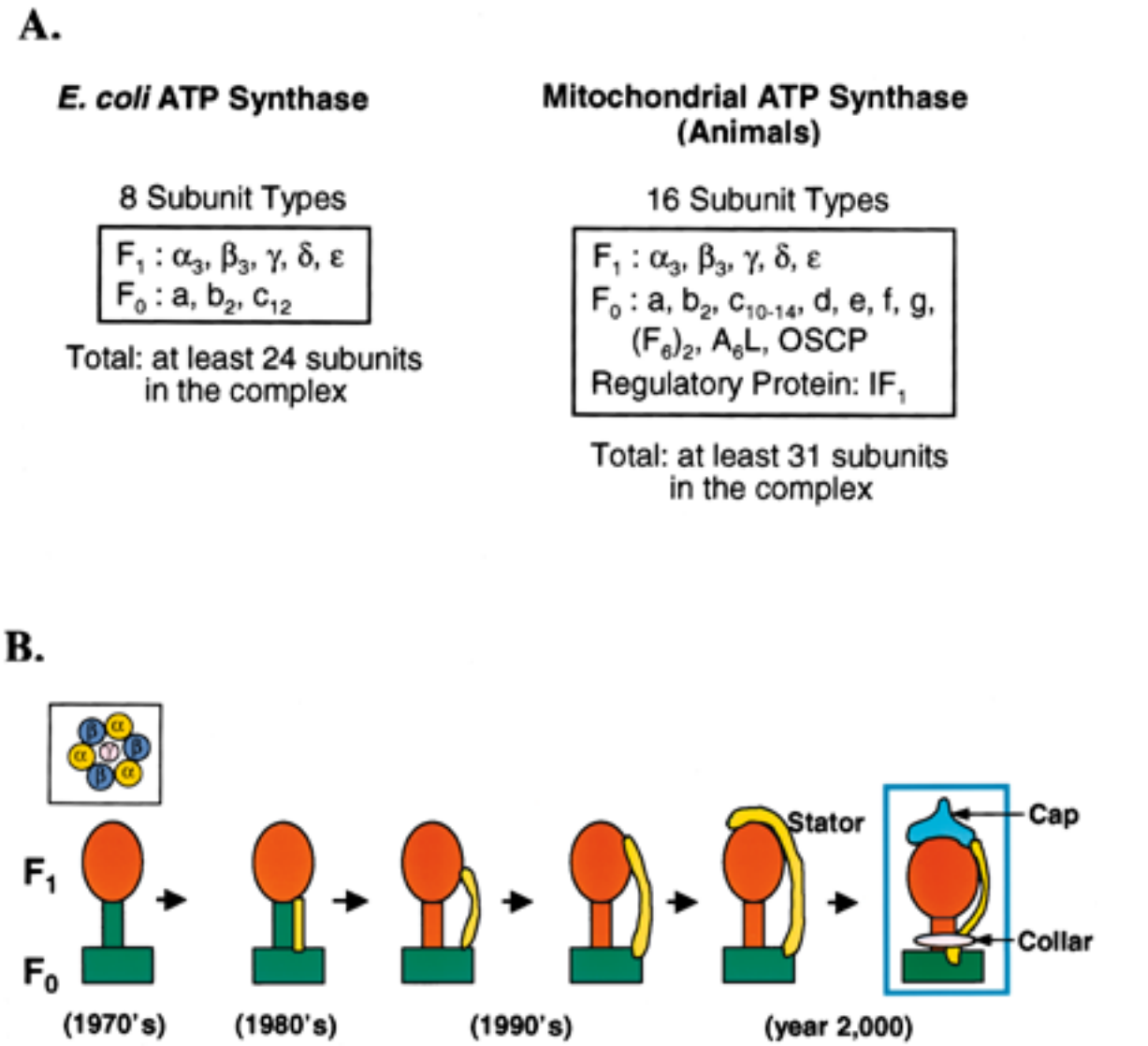

Fig. 1. (A) Comparison of the subunit composition of ATP synthase isolated from *E. coli* with that of the ATP synthase isolated from animal mitochondria. The ATP synthase from *E. coli* has a total of eight subunit types, of which five types are contained within the F_1 moiety and the remaining three within the F_0 moiety. In contrast, the ATP synthase from animal mitochondria has a total of sixteen known subunit types, five of which are contained within the F_1 moiety and ten within the F_0 moiety. A regulatory protein referred to as IF_1, depending on conditions, may or may not be bound to the F_1 moiety. The reason for the increased complexity of the substructure of the animal ATP synthase is completely unknown and remains a challenging problem for the future. Interestingly, the yeast ATP synthase has an even more complex substructure than the animal enzyme, with current evidence indicating a total of 20 subunit types. (B) Evolving views of the structural features of ATP synthases derived from electron microscopy and biochemical studies. The figure shows how most EM studies conducted from the early 1970s through the 1980s were able to visualize only the classical tripartite features (headpiece, basepiece, stalk) of ATP synthases. However, by the 1990s, with improved EM technologies, other features of ATP synthases became evident. These included, a second or side stalk, a cap at the top of the F_1 moiety, and, in the case of the animal ATP synthase, a collar around the central stalk. The function of these additional components is not entirely clear, although the side stalk is believed to be a stator that stabilizes the F_1 motor while ATP synthesis is taking place. The inset at the far left (top) is a model proposed for the F_1 moiety of the ATP synthase by Catterall and Pedersen in 1974 on the basis of EM and biochemical data on the rat liver enzyme. The simple model was shown to be correct upon completion of the X-ray structures of the rat liver and bovine heart F_1 moieties in the 1990s. [Note: since this paper was submitted, a reevaluation of the subunit c. stoichiometry of *E. coli* has resulted in a preferred value of 10 rather than the value of 12 originally proposed (Fillingame, R. H., Jiang, W., and Dimitriev, O. Y. (2000) J. Bioenerg. Biomemb, In Press.) Therefore, the total number of subunits in *E. coli* ATP synthase may be 22 rather than the 24 indicated above.]

within a complete ATP synthase complex, although such information has been obtained for some of the isolated F_0 subunits. In addition, a relatively low-resolution structure has been obtained for an F_1-(subunit $c)_{10}$ complex from yeast (Stock *et al.*, 1999), where the *c* subunit represents only one of the more than ten different types of subunits known to comprise the F_0 moiety in yeast (Velours and Arselin, 2000) and higher eukaryotes (reviewed in Pedersen, 1996).

Below are summarized some of the major contributions of electron microscopy, X-ray crystallography, and NMR spectroscopy to our understanding of the structures of ATP synthases. Other minireview articles in both this and the subsequent volume of this journal should reinforce what is presented here, describe it in more detail, and therefore provide the reader with an in-depth perspective about the current status of our knowledge of the structure and function of ATP synthases. The authors apologize in advance for not being able to acknowledge in this brief minireview all of the excellent work that has been done on ATP synthases.

ELECTRON MICROSCOPY

Contributions of electron microscopy techniques to our understanding of the structural features of ATP synthases have had a long and rich history, which dates back to the early 1960s when, via negative staining procedures, the tripartite nature (basepiece, stalk, and headpiece) of the ATP synthase was observed in submitochondrial particles (Ferandez-Moran, 1962). Subsequent to these findings the first detergent-solubilized ATP synthase molecules were isolated from bovine heart mitochondria and visualized under the electron microscope (Kagawa, 1972). Although these original pictures published in 1972 showed many clusters of ATP synthase molecules, they also showed several single molecules exhibiting the tripartite features (basepiece, stalk, headpiece) of the intact ATP synthase. About this time, "negative stain" electron microscopy techniques were also applied to isolated F_1 preparations. Significantly, rat liver F_1 revealed a hexagonal array of subunits some of which had a seventh mass in the center of the hexagon (Catterall and Pedersen, 1974). On the basis of these data, and data revealing the substructure of rat liver F_1 as $\alpha_3\beta_3\gamma\delta\varepsilon$ (Catterall *et al.*, 1973), it was proposed that F_1 contains a hexagonal array of alternating α and β subunits with the small subunits γ, δ, and ε being located in the center of the molecule (Fig. 1B, inset) (Catterall and Pedersen, 1974). Interestingly, this proposal did not gain general acceptance until it was verified by X-ray crystallography some two decades later (Abrahams *et al.*, 1994; Bianchet *et al.*, 1998).

Although electron microscopes and techniques for preparing samples for visualization continued to improve in the 1970s and 1980s, the general view of the tripartite (headpiece, stalk, basepiece) nature of ATP synthases still reigned. Much better pictures of single molecules appeared, first for the rat liver ATP synthase (Soper *et al.*, 1979) and then for other ATP synthases (Boekema *et al.*, 1986; Gogol *et al.*, 1987; Tsuprun *et al.*, 1989; Graber *et al.*, 1990; Oschida and Bowman, 1992). From the six different studies considered here, information was obtained about the overall dimensions of each of the units comprising the tripartite structure, which, on the average, gave a value of about 190 Å from the top of the headpiece to the bottom of the basepiece, with an average of 37 Å contributed to the central stalk. Notably, one of these studies that substituted cryotechniques for negative staining, confirmed that the central stalk was real and not a negative staining artifact (Gogel *et al.*, 1987). The latter cryoelectron microscopy study would usher in a new era in which Capaldi and his colleagues in the United States and several research groups in Europe, by combining electron microscopy with biochemical, molecular biological, and immunological techniques, would make several important discoveries about ATP synthases (reviewed in Capaldi *et al.* 2000). One of these discoveries provided some of the first direct evidence for rotational catalysis (Gogel *et al.*, 1990). A second was the visualization of a second or side stalk (Bottcher *et al.*, 1998; Wilkens and Capaldi, 1998a,b; Karrash and Walker, 1999), believed to be a "stator" to hold in place the F_1 molecule, while the centrally located γ subunit rotates (Junge *et al.*, 1997; Wilkens and Capaldi, 1998a). A third discovery was the demonstration that F_1 is capped by one or more proteins (Wilkens and Capaldi, 1998a,b; Karrash and Walker, 1999), while a fourth was the visualization of a collar surrounding the central stalk in the animal mitochondrial enzyme (Karrash and Walker, 1999).

In summary, low-resolution structural studies using electron microscopy suggest that the animal ATP synthase can be depicted in cartoon fashion, as shown in Fig. 1B (far right), while the *E. coli* ATP synthase may be very similar but lacking the collar around the central stalk, and containing a somewhat smaller cap. Significantly, recent biochemical evidence supports the view that the animal ATP synthase (rat liver) does

Table I. Summary of Structures of ATP Synthase Components Determined to Date by X-Ray Crystallography, NMR Spectroscopy, or Theoretical Modeling

	PDB code	Source	Method	Resolution (Å)	Note	Date deposited	Authors (published paper)
F_1							
β	1e16	Sorghum	Theoretical modeling	None given	β Subunit from sorghum line cs3541	4/23/00	Jaiswal, to be published
β	1e1i	Sorghum	Theoretical modeling	None given	β Subunit from sorghum line 2077a	5/9/00	Jaiswal, to be published
α, β,	1sky	Thermophilic *Bacillus* PS3	X-ray diffraction	3.20	Nucleotide free, $\alpha_3\beta_3$ subcomplex; symmetric trimer of αβ pair	2/26/97	Shirakihara *et al.*, 1997
α, β, γ	1bmf	Bovine	X-ray diffraction	2.85	Asymmetric trimer of αβ pair; inhibitor azide present during crystallization	3/13/96	Abrahams *et al.*, 1994
	1cow	Bovine	X-ray diffraction	3.10	Complexed with aurovertin b	5/8/96	van Raaij *et al.*, 1996
	1d8s	*E. coli*	X-ray diffraction	4.40	7 Helixes of γ subunit; backbone atoms only	10/25/99	Hausrath *et al.*, 1999
	1e1q	Bovine	X-ray diffraction	2.61	$\alpha_3\beta_3\gamma$ complex at 100° K	5/10/00	Braig *et al.*, 2000
	1e1r	Bovine	X-ray diffraction	2.50	Inhibited by Mg^{2+} ADP and aluminium fluoride	5/10/00	Braig *et al.*, 2000
	1efr	Bovine	X-ray diffraction	3.10	Complexed with efrapeptin	5/24/96	Abrahams *et al.*, 1996
	1mab	Rat	X-ray diffraction	2.80	Symmetric trimer of αβ pair; redissolved crystals have full catalytic activity; no inhibitor present during crystallization	8/6/98	Bianchet *et al.*, 1998
	1nbm	Bovine	X-ray diffraction	3.00	Complexed with 4-chloro-7-nitrobenzofurazan	4/30/98	Orriss *et al.*, 1998
δ	1abv	*E. coli*	NMR	None given	N-terminal domain; minimized average structure	1/29/97	Wilkens *et al.*, 1997
ε	1aqt	*E. coli*	X-ray diffraction	2.30		7/31/97	Uhlin *et al.*, 1998
	1bsh	*E. coli*	NMR	None given	30 Structures	8/27/98	Wilkens and Capaldi, 1998
	1bsn	*E. coli*	NMR	None given	Minimized average structure	8/28/98	Wilkens and Capaldi, 1998
F_0							
Subunit *b*	1b9u	*E. coli*	NMR	None given	N-terminal membrane domain	2/15/99	Dmitriev *et al.*, 1999
Subunit *c*	1a91	*E. coli*	NMR	None given	10 Structures	4/15/98	Girvin *et al.*, 1998
	1aty	*E. coli*	NMR	None given	Ala67Cys mutant; 9 structures	10/5/94	Girvin and Fillingame, 1995
	1c0v	*E. coli*	NMR	None given	10 Structures	7/22/99	Girvin *et al.*, 1998
	1c17	*E. coli*	NMR	None given	a_1c_{12} Subcomplex	7/20/99	Rastogi and Girvin, 1999
	1c99	*E. coli*	NMR	None given	Asp61 deprotonated form of subunit *c*; 9 structures	4/30/99	Rastogi and Girvin, 1999

Table I.—*Continued*

	PDB code	Source	Method	Resolution (Å)	Note	Date deposited	Authors (published paper)
F_1F_0 α, β, γ, δ, subunit *c*	1qo1	Baker's yeast	X-ray diffraction	3.90	The coordinates are based on coordinates of models of subunits or domains derived from X-ray diffraction (1bmf, 1aqt) or NMR (1a91); a ring of 10 *c* subunits; backbone atoms only	11/1/99	Stock *et al.*, 1999

contain a cap derived in part from one or more F_0 subunits not present in *E. coli*, and that a collar, also derived from one or more F_0 subunits, surrounds the central stalk (Ko *et al.*, 2000).

X-RAY CRYSTALLOGRAPHY AND NMR SPECTROSCOPY

To date, a high-resolution structure of a complete, intact ATP synthase (F_0F_1) has not been obtained. Nevertheless, as shown in Table I, much has been accomplished toward this goal as it relates to working with different parts of this complex. Such efforts have also had a rather long history commencing with the preparation of diffraction quality crystals of the F_1 moiety of the rat liver ATP synthase in the late 1970s (Amzel and Pedersen, 1978). Surprisingly, the very first crystallization trial on only a few milligrams of F_1 provided large diffraction-quality crystals. Subsequent X-ray crystallographic studies on these crystals led, first, to a 9 Å map in 1982 depicting three major masses (Amzel *et al.*, 1982), and then to a 3.6 Å map in 1991 (identifying alternating α and β subunits, together with data implicating a nucleotide-binding site at the interface between each of the three αβ heterodimers (Bianchet *et al.*, 1991). In the meantime, work which had been initiated later by John Walker and his colleagues on the F_1 moiety of the bovine heart ATP synthase succeeded, in 1994, in obtaining the first high-resolution structure (2.8 Å) of an F_1 preparation (Abrahams *et al.*, 1994), a major accomplishment achieved in 1998 also for rat liver F_1 (Bianchet *et al.*, 1998).

Although the rat liver and bovine heart F_1 structures are highly similar in most respects by showing alternating α and β subunits and a centrally located γ subunit, which extends from the top of F_1 to below the bottom of its headpiece, they do differ in the occupancy of catalytic sites by nucleotide. All catalytic sites in rat liver F_1 are filled with nucleotide, whereas one of the β subunits remains empty in bovine heart F_1 with its active site, assuming a position twisted away from the central axis. Significantly, the two enzymes were crystallized under entirely different condition, with rat liver F_1 forming crystals in a medium containing initially ATP and KP_i, but during crystallization ATP, ADP, and P_i (Amzel and Pedersen, 1978), while bovine heart F_1 formed crystals in a medium containing AMP–PNP, limiting ADP, $MgCl_2$, deuterium oxide, and the ATPase inhibitor sodium azide (Lutter *et al.*, 1993). Crystals of rat liver F_1 can be redissolved with full retention of catalytic activity (Pedersen *et al.*, 1995; Bianchet *et al.*, 1998), while such data for bovine heart F_1 has not been reported. Not surprisingly, both research groups have suggested that their structures may be representative of configurations of F_1 on the main pathway for ATP synthesis, suggestions that may prove true. However, it is possible also that structures of the most representative physiological F_1 configurations remain to be obtained. This possibility cannot be excluded, as rat liver F_1 was crystallized in the absence of the physiological cation Mg^{2+}, while the bovine heart enzyme was crystallized under inhibited conditions with one of the two substrates for ATP synthesis totally absent (i.e., P_i) and the other substrate (ADP) in substoichiometric amounts. If the objective of future structural studies on F_1 are to understand the mechanism of ATP synthesis, it may be most profitable to obtain crystals, which contain all of the physiological substrates for ATP synthesis

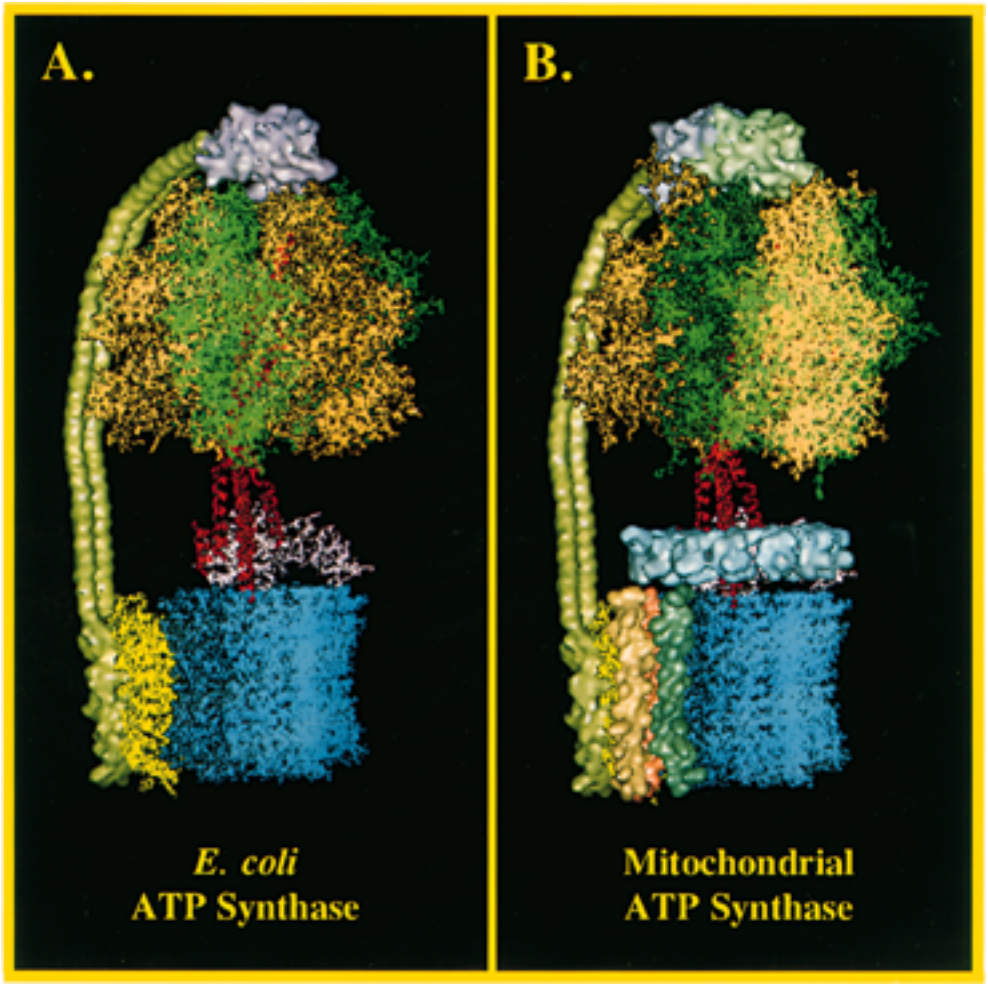

Fig. 2. Structural models proposed by the authors for ATP synthases from *E.coli* and animal mitochondria. For the construction of the models, yeast F_0F_1-ATP synthase (1qo1) was used as a template for the spatial arrangement of each subunit. In the structures, the coordinates of each subunit (except γ) of the yeast F_0F_1-ATP synthase (1qo1) were replaced by superimpositon with the coordinates from high-resolution structures [1aqt for the δ (mitochondria) or ε (*E. coli*) subunit, 1b9u for the transmembrane part of the *b* subunit, 1bmf for the α and β units of *E. coli*, 1mab for mitochondrial α and β units, and 1c17 for the *a* and *c* subunits]. The γ subunit of 1qo1 was substituted by the γ subunit of 1d8s (*E. coli*). The structures of the collar, of the transmembrane parts of other subunits of mitochondrial ATP synthase (*f, g,* and A6L), and of the hydrophilic part of the *b* subunit were generated manually using Quanta. In the structures, the following six subunit types are shown in wire representation: α (orange), β (green), γ (red), δ in mitochondria or ε in *E. coli* (pink), a (yellow), and *c* (blue). Shown in surface representation are subunits *b* (light green) and δ in *E.coli* or OSCP in mitochondria (purple). The mitochondrial enzyme also shows in surface representation a second mass (unidentified) at the top of F_1 (light green), and at the bottom the transmembrane segments of three other subunit types, *f, g,* and A6L. The relative positions of these latter subunits colored in off white, orange, and green are not known. No positions are assigned to the mitochondrial subunits *d, e,* and IF1 (regulatory protein), one or more of which is a candidate for the collar (off-white) surrounding the stalk, and represented here in surface representation. References to most of the information described here are provided in Table I.

(i.e., ADP, P_i, and Mg^{2+}) and to demonstrate that the crystals being used will provide, upon redissolving, an active enzyme capable of rebinding to F_1-depleted membrane vesicles and catalyzing ATP synthesis.

It should be noted that in the two F_1 structures described above, the two smallest subunits δ and ε and a significant portion of the γ subunit were not located. Therefore, both research groups suggested that these subunits were not ordered in the structure. However, in a more recent structure obtained at a resolution of 2.4 Å with crystals of bovine heart F_1 inhibited with dicyclohexylcarbodiimide (DCCD) (Gibbons *et al.*, 2000), these previously "invisible" subunits have been located. These studies, when taken together with the

recently reported crystal structure (3.9 Å) of a yeast F_1-(subunit *c*)$_{10}$ complex (Stock *et al.*, 1999), confirm that the γ subunit extends about 50 Å below the F_1 core to interact with the *c* subunit ring comprising part of the proton channel within the F_0 moiety of the ATP synthase complex. The new 2.4 Å structure of inhibited bovine heart F_1 also shows that the ε subunit interacts intimately with both the γ and δ subunits.

Recent NMR studies, which are restricted to relatively small proteins or protein complexes, have nevertheless been valuable in providing structures of the *E. coli* F_1 δ and ε subunits (Wilkens *et al.*, 1997; Wilkens and Capaldi, 1998c), the *E. coli* F_0 *b* and *c* subunits (Girvin *et al.*, 1998; Dmitriev *et al.*, 1999), and, finally, to a complex between the *E. coli a* and *c* subunits (Rastogi and Girvin, 1999). When taken together with biochemical studies, NMR studies have also led to a model for that part of the *E. coli* proton channel formed by the *c*-subunit ring (Dmitriev *et al.*,1999).

Currently, there appears to be considerable differences in the number of *c* subunits reported to form the *c*-subunit ring found within the F_0 moiety of ATP synthases, the values being 10 (yeast) (Stock *et al.*, 1999), 12 (*E. coli*) (Dmitriev *et al.*, 1999), and 14 (chloroplasts) (Seelert *et al.*, 2000). Interestingly, the three different values have been obtained by using not only a different source, but different techniques. Therefore, although it is possible that ATP synthases from different sources may have a different number of subunit *c* molecules (variable stoichiometry), this argument seems difficult to rationalize in the face of the fact that the catalytic F_1 moieties of the same complexes exhibit the same stoichiometry ($\alpha_3\beta_3\gamma\delta\varepsilon$) in almost every species examined to date.

OUTLOOK AND PROPOSED STRUCTURES FOR *E.coli* AND ANIMAL ATP SYNTHASES

As indicated previously in this short report, the real structural challenges of the future are to acquire at high-resolution "complete" ATP synthase complexes representative of different stages of the catalytic cycle during ATP synthesis and representative of key regulatory states. A second challenge is to use this structural knowledge to determine why the F_0 moieties of ATP synthases from animal and yeast cells are much more complex than those from bacteria and plant chloroplasts. Finally, on the basis of (1) current views about the overall structural features of ATP synthases derived from electron microscopy and biochemical studies, (2) the large body of data that is currently available in the protein data bank (PDB) as it concerns F_1 structures and the structures of some of the individual F_0 subunits (Table 1), and (3) some molecular modeling and speculation, we have included in Fig. 2 the authors' current views of the structures of the *E. coli* and animal mitochondrial ATP synthases. These views are very similar to those being arrived at by other investigators.

ACKNOWLEDGMENT

This article was written while the authors were supported by NIH grant CA 10951to PLP.

REFERENCES

Abrahams, J. B., Leslie, A. G. W., Lutter, R., and Walker, J. E. (1994). *Nature (London)* **370**, 621–628.
Abrahams, J. G., Buchanan, S. K., van Raaij, M. J., Fearnley, I. M., Leslie, A. G., and Walker, J. E. (1996). *Proc. Natl. Acad. Sci. USA* **93**, 9420–9424.
Amzel, L. M. and Pedersen, P. L. (1978). *J. Biol. Chem.* **253**, 2067–2069.
Amzel, L. M., McKinney, M., Narayanan, P., and Pedersen, P. L. (1982). *Proc. Natl. Acad. Sci. USA* **79**, 5852–5856.
Bianchet, M. A., Ysern, S., Hullihen, J., Pedersen, P. L., and Amzel, L. M. (1991). *J. Biol. Chem.* **266**, 21197–21201.
Bianchet, M. A., Hullihen, J., Pedersen, P. L., and Amzel, L. M. (1998). *Proc. Natl. Acad. Sci. USA* **95**, 11065–11070.
Boekema, E. J., Berden, J. A., and Van Heel, M. G. (1986). *Biochem. Biophys. Acta* **851**, 353–360.
Bottcher, B., Schwarz, L., and Graber, P. (1998). *J. Mol. Biol.* **281**,757–762.
Braig, K., Menz, R. I., Montgomery, M. G., Leslie, A. G., and Walker, J. E. (2000). *Structure Fold Des.* **8**, 567–573.
Capaldi, R. A., Schulenberg, B., Murray, J., and Aggeler, R. (2000). *J. Exp. Biol.* **203**, 29–33.
Catterall, W. A. and Pedersen, P. L. (1974). *Biochem. Soc. Spec. Publ.* **4**, 63–88.
Catterall, W. A., Coty, W. A., and Pedersen, P. L. (1973). *J. Biol. Chem.* **248**, 7427–7431.
Dmitriev, O., Jones, P. C., Jiang, W., and Fillingame, R. H. (1999). *J. Biol.Chem.* **274**, 15598–15604.
Ferandez-Moran, H. (1962). *Circulation* **26**, 1039–1065.
Gibbons, C., Montgomery, M. G., Leslie, A. G. W., and Walker, J. E. (2000). *Biochem. Biophys. Acta*, EBEC Short Reports, **11**, 212, Nature Struc. Biol. **7**, 1055–1061.
Girvin, M. E. and Fillingame, R. H. (1995). *Biochemistry* **34**, 1635–1645.
Girvin, M. E., Rastogi, V. V., Abildgaard, F., Markley, J. L., and Fillingame, R. H. (1998). *Biochemistry* **37**, 8817–8824.
Gogel, E. P., Lucken, U., and Capaldi, R. A. (1987). *FEBS Lett.* **219**, 274–278.
Gogel, E. P., Johnson, E., Aggeler, R., and Capaldi, R. A. (1990). *Proc. Natl. Acad. Sci. USA* **87**, 9585–9589.
Graber, P., Bottcher, B., and Boekema, E. J. (1990). *Bioelectro-*

chemistry III (Milazzo, G. and Blank, M., eds.), Plenum Press, New York, pp 247–276.
Hausrath, A. C., Gruber, G., Matthews, B. W., and Capaldi, R. A. (1999). *Proc. Natl. Acad. Sci. USA* **96**, 13697–13702.
Junge, W., Lill, H., and Engelbrecht, S. (1997). *Trends Biochem. Sci.* **22**, 420–423.
Kagawa, Y. (1972). *Biochem. Biophys. Acta* **265**, 297–338.
Karrash, S. and Walker, J. E. (1999). *J. Mol. Biol.* **290**, 379–384.
Ko, Y. H., Hullihen, J., Hong, S., and Pedersen, P. L. (2000). *J. Biol. Chem.*, **276**, 32931–32939.
Lutter, R., Abrahams, J. B., van Raaij, M. J., Todd, R. J., Lundquist, T., Buchanan, S. K., Leslie, A. G. W., and Walker, J. E. (1993). *J. Mol. Biol.* **229**, 787–790.
Mitchell, P. (1979). *Science* **206**, 1148–1159.
Orriss, G. L., Leslie, A. G., Braig, K., and Walker, J. E. (1998). *Structure* **6**, 831–837.
Oschida, W. J. and Bowman, B. J. (1992). *J. Biol. Chem.* **267**, 18783–18789.
Pedersen, P. L. (1996). *J. Bioenerg. Biomembr.* **38**, 389–395.
Pedersen, P. L., Hullihen, J., Bianchet, M., Amzel, L. M., and Lebowitz, M. S. (1995). *J. Biol. Chem.* **270**, 1775–1784.
Rastogi, V. K. and Girvin, M. E. (1999). *Nature (London)* **402**, 263–268.
Seelert, H., Poetsch, A., Dencher, N. A., Engel, A., Stahlberg, H., and Muller, D. J. (2000). *Nature (London)* **405**, 418–419.
Shirakihara, Y., Leslie, A. G. W., Abrahams, J. P., Walker, J. E., Udea, T., Sekimato, Y., Kambara, M., Saika, K., Kagawa, Y., and Yoshida, M. (1997). *Structure* **5**, 825–836.
Soper, J. W., Decker, G. L., and Pedersen, P. L. (1979). *J. Biol. Chem.* **254**, 11170–11176.
Stock, D., Leslie, A. G. W., and Walker, J. E. (1999). *Science* **286**, 1700–1705.
Tsuprun, L., Orlova, E. V., and Mesyanzhinova, J. V. (1989). *FEBS Lett.* **244**, 279–282.
Uhlin, U., Cox, G. B., and Guss, J. M. (1998). *Structure* **5**, 1219–1230.
van Raaij, M. J., Abrahams, J. P., Leslie, A. G. W., and Walker, J. E. (1996). *Proc. Natl. Acad. Sci. USA* **93**, 6913–6917.
Velours, J. and Arselin, G. (2000). *J. Bioenerg. Biomemb.* **32**, 383–390.
Wilkens, S. and Capaldi, R. A. (1998a). *Nature (London)* **393**, 29.
Wilkens, S. and Capaldi, R. A. (1998b). *Biochim. Biophys. Acta* **1365**, 93–97.
Wilkens, S. and Capaldi, R. A. (1998c). *J. Biol. Chem.* **273**, 26645–26651.
Wilkens, S. Dunn, S. D., Chandler, J., Dahlquist, F. W., and Capaldi, R. A. (1997). *Natur. Struct. Biol.* **4**, 198–201.