Journal of Bioenergetics and Biomembranes, Vol. 20, No. 4, 1988

MINI-REVIEW

ATP Synthases—Structure of the F_1-Moiety and Its Relationship to Function and Mechanism

Xavier Ysern,[1] L. Mario Amzel,[1] and Peter L. Pedersen[2]

Received October 29, 1987

Abstract

A great deal of progress has been made in understanding both the structure and the mechanism of F_1-ATPase. The primary structure is now fully known for at least five species. Sequence comparison between chloroplast, photobacteria, aerobic bacteria, and mitochondrial representatives allow us to infer more general functional relationships and evolutionary trends. Although the F_1 moiety is the most studied segment of the H^+-ATPase complex, there is not a full understanding of the mechanism and regulation of its hydrolytic activity. The β subunit is now known to contain one and probably two nucleotide binding domains, one of which is believed to be a catalytic site. Recently, two similar models have been proposed to attempt to describe the "active" part of the β subunits. These models are mainly an attempt to use the structure of adenylate kinase to represent a more general working model for nucleotide binding phosphotransferases. Labelling experiments seem to indicate that several critical residues outside the region described by the "adenylate kinase" part of this model are also actively involved in the ATPase activity. New models will have to be introduced to include these regions. Finally, it seems that a consensus has been reached with regard to a broad acceptance of the asymmetric structure of the F_1-moiety. In addition, recent experimental evidence points toward the presence of nonequivalent subunits to describe the functional activity of the F_1-ATPase. A summary diagram of the conformational and binding states of the enzyme including the nonequivalent β subunit is presented. Additional research is essential to establish the role of the minor subunits—and of the asymmetry they introduce in F_1—on the physiological function of the enzyme.

Key Words: ATP synthase; F_1-ATPase; ATPase; oxidative phosphorylation; mitochondria; bioenergetics.

[1] Laboratories for Molecular and Cellular Bioenergetics, Department of Biophysics, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.
[2] Laboratories for Molecular and Cellular Bioenergetics, Department of Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

0145-479X/88/0800-0423$06.00/0

Introduction

The ATP synthases of the F_0F_1 type (H^+-ATPase) are large and complex systems which, in conjunction with the proteins of the electron transport system, are responsible for the oxidative phosphorylation and photophosphorylation reactions. The two systems appear to be coupled (Mitchell, 1966) and are found associated with energy-transducing membranes of mitochondria, chloroplasts, and bacteria. The functions of the H^+-ATPase, reviewed by Amzel and Pedersen (1983), include the vectorial proton crossing through F_0 in the membrane (translocation), the directed interaction of the protons with F_1 (coupling), and finally the synthesis of ATP from ADP and P_i (catalysis). The F_0F_1 complexes isolated in submitochondrial particles retain these functions. Proper solubilization conditions allow separation and isolation of the F_1-moiety from the water-insoluble F_0-sector. The F_0-sector spans the membrane, allows the passage of protons through the membrane, and is able to direct F_1 for proper complex formation. The isolated F_1-moiety is only capable of net hydrolysis of ATP, although near stoichiometric levels of ATP can be produced on the surface of F_1 provided the P_i concentration is high and a solvent like DMSO is present (Sakamoto and Tonomura, 1983).

The F_0F_1 ATPases are assembled with at least eight subunits. Thirteen different subunits have been reported for the bovine mitochondrial ATP synthase (Walker *et al.*, 1987). In *E. coli* the stoichiometry seems to be $\alpha_3\beta_3\gamma_1\delta_1\varepsilon_1a_1b_2c_{10-12}$ (Fillingame, 1981). The F_1-sector stoichiometries reported for other systems (Senior and Brooks, 1971; Catterall and Pedersen, 1971; Catterall *et al.*, 1973) are also $\alpha_3\beta_3\gamma\delta\varepsilon$, indicating that in all systems the F_1 molecule has intrinsic chemical asymmetry. The γ, δ, and ε chains do not have internal repetitive sequences to interact in an equivalent fashion with α and β chains, and consequently there is no way in which to isolate γ, σ, and ε chains such that all α and β subunits have identical surroundings. This asymmetry, although clearly suggested from earlier subunit stoichiometry data, was not appreciated by workers in the field until much later. Amzel *et al.* (1982) from X-ray diffraction low-resolution structural studies and from the earlier $\alpha_3\beta_3\gamma\delta\varepsilon$ stoichiometry first proposed a mode involving subunit asymmetry. Significantly, this model was able to explain the existing binding and labelling experimental data of F_1-ATPase. Thus, it was suggested that this intrinsic asymmetry found at the subunit level might be responsible for many of the unusual nucleotide and chemical modification properties of F_1, and may have profound implications for the function and mechanism of ATP synthases in general.

This mini-review attempts to describe the recent contributions to the knowledge of the primary, secondary, tertiary, and quaternary structures of the F_1-sector, to establish the continuity with previous work, and, where

possible, to emphasize relationships of structure and function. Where possible, also an attempt will be made to discuss the relationship of the function of F_1 to its asymmetric structure.

Structure of the F_1-Moiety

The F_1-moiety is an oligomeric protein of five different subunits with a stoichiometry of $\alpha_3\beta_3\gamma\delta\varepsilon$. Reconstitution experiments with purified F_1 subunits from the thermophilic bacterium PS3 were tested for all 31 possible subunit compositions (Kagawa *et al.*, 1979). Successful reconstitutions, judged by functional and structural considerations, were found only for $\alpha + \beta + \gamma$ combinations. The $\alpha_3\beta_3\gamma$ oligomer has size and specific activity similar to the native enzyme, indicating that these three subunits are essential for function in the ATP hydrolytic direction. Excellent reconstitution of $\alpha + \beta + \gamma$ subunits into a functional ATPase has been carried out also using *E. Coli* subunits (Dunn and Futai, 1980).

In *E. coli* the whole F_0F_1 ATPase is coded by the *atp* or *unc* operon that contains the eight structural genes and one 14 K Dalton open reading frame of unknown function located next to the promoter region. The phenomenon of uncoordinancy, production of different amounts of proteins from different cistrons with the same transcript, is still not satisfactorily understood. The genome organization for eukaryotic ATPases is more complex and its organization is beginning to be unveiled. In chloroplasts the genes appear to be segregated into different clusters and an intron has been found for the *b* subunit gene of the F_0-sector (Bird *et al.*, 1985). Recently, the presence of two different copies of the *b* subunit has been reported by Cozenz and Walker (1987). A summary of the genome organization for the ATP synthase is presented in Fig. 1.

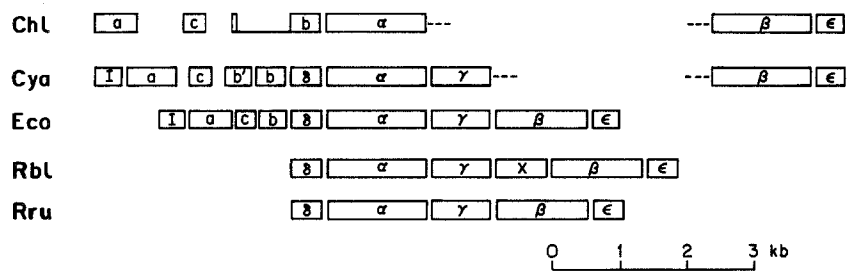


Fig. 1. Relative arrangement of the genes encoding for the H^+-ATP synthase. Modified from Cozens and Walker (1987). Abbreviations: Chl, chloroplasts; Cya, *Cyanobacterium synechococcus* 6301; Eco, *Escherichia coli*; Rbl, *Rhdopseudomonas blastica*; Rru, *Rhodospirillum rubrum*. The letters α, β, γ, δ, ε, *a*, *b*, and *c*, indicate the subunit encoded. I and X encoded for proteins of unknown function.

Primary Structure

A great deal of information about the amino acid sequences of the subunits of the F_1-moiety (Table I) has been accumulated in recent years as a result of the efforts of several laboratories. The complete sequences of the five subunits of *Rhodopseudomonas blastica* (Tybulewicz *et al.*, 1984), *Rhodospirillum rubrum* (Falk *et al.*, 1985), *Escherichia coli* (Kanazawa and Futai, 1982; Walker *et al.*, 1984a,b), bovine (Walker *et al.*, 1985), and *Cyanobacterium synechococcus* 6301 (Cozens and Walker, 1987) are now known mainly by DNA sequencing. The beef heart sequences were determined by direct protein sequence analysis. Sequence comparison between different species has shown that the α and β subunits are well conserved, in contrast to the γ, δ, and ε subunits. The degree of homology between species found for the α subunit is greater than 60%; for the β subunit the homology is even higher. The α and β subunits of the same species show similarities (Table II) through the whole polypeptide chain consistent with the suggestions of their evolutionary relatedness. In the γ subunit the low homologies found appear to be segregated mainly into two segments near the N- and C-terminus. The ε subunit has a relative high proportion of tryptophan residues; the only stretch of sequence identity found is histidine, alanine, and proline (residues 37 to 39 in the spinach numeration), which is generally preceded by either a glutamine or a glycine, and another proline residue. Bovine and *Neurospora crassa* δ chains seem to be the counterpart of chloroplast and bacterial ε subunits, while the bovine oligomycin sensitivity conferring protein (OSCP) appears to be the counterpart of the chloroplast and bacterial ε subunit.

Local homologies to proteins with different activities have been reported in several regions of the β chain (Table III). The first homology region, of about 30 amino acids, is located around β residue 173 for spinach (or 159 in human, etc.) and has similarities to the oncogene p. 21 and rec A (Walker *et al.*, 1984c; Futai and Kanazaga, 1983; Ohta and Kagawa, 1986). A second region around β 264 of spinach (256 for human, etc.) contains homologies with the ADP/ATP antiporter and with phosphofructokinase. Adenylate kinase (Fry *et al.*, 1986) and elongation factor Tu have homologies to both of those regions. A third short region of eight amino acids (serine and threonine rich, near lysine 317 in the spinach numeration for position reference) has homology to the sequence SDKTGTLT found in Na^+, K^+ ATPase (Walker *et al.*, 1984c) and Ca^{2+} ATPase (Shull *et al.*, 1985). In both cases the aspartic acid residue becomes phosphorylated during catalysis (MacLennan *et al.*, 1985). All $F_1\beta$ subunits have an identical threonine in this position.

The direct protein sequence analysis of the bovine mitochondrial F_1-moiety (Walker *et al.*, 1985) has now been completed after experimental difficulties were surmounted. The N- and C-terminal sequences of the α and

Table I. Amino Acid Sequences F_1-ATPase Subunits[a]

ALPHA SUBUNIT

```
                          10        20        30        40        50        60        70        80        90       100
Spi              MATIRADEISKIIRERIEGYNREVKVVNTGTVLQVGDGIARIHGLDEVMAGELVEFEEGTIGIALNLESNNVGVVLMGDGLMIQEGSSVKATGRIAQIPV
Pea              .V.S......Q...K...Q..T...I.................Y...D.........K...V........K.............................
Tob              .V........N.......Q......I.....................................................L.....................
Whe              ...F.V...H..V..L..Q...K.GIE.I.R.V.........I..G.I.S......A...R........K...I.............F.......P....
Cya              .VS..P....S...QQ..Q.SQD...E.V.............Y..QQ..S.......D..T.......ED...A....E.RN.....T.....K......
Eco               MQLNST...EL.KQ..AQF.VVSEAH.E..IVS.S..VI.....ADC.Q..MISLPGNRYA......RDS..A.V..PYADLA..MK..C....LEV..
Rba               MG.Q.A...A.LK.Q.KNFGKDAE.AEV.R..S.......VY...N.Q...M...PG.IR.M.....VD...I.IF..DRD.K..DT..R.KS.VDV.A
Rru               ME...A...A.LK.Q.ANFGT.AESAEV.Q..S.......VY...N.Q...M...AN.VK.M......D...I.IF...R..K..DT..R.QT.VDV..
Bov               EKTGTA.V.S.LE...L.ADTS.DLEE..R..SI......V...RN.Q.E.M...SS.LK.MS....PD.....VF.NDKL.L..DI..R..A.VDV..

          110       120       130       140       150       160       170       180       190       200
Spi   SEAYLGRVINALAKPIDGRGEITASESRLIESPAPGIMSRRSVYEPLQTGLIAIDAMIPVGRGQRELIIGDRQTGKTAVATDTILNQQGQNVI__________CVYVAIG
Pea   ..G.....V.............ST.............I.................S...I.................................LV__________.......
Tob   ......................S...F.....A....I.................S...I.....................................__________.......
Whe   ........V.........K...I............S.I.................S...I.....................K..G..._____.....__________.......
Cya   GD.LV...VSP.GA.L..K...A.T.N..........IA....H..M...IT.......I.....................I.I......K.ED.._____.....__________.......
Eco   GRGL....V.T.GA....K.PLDHDGFSAV.AI...VIE.Q..DQ.V...YK.V.S...I.................RL.I.A.I..RDSGIK__________.....I......
Rbl   GNGL....VD..GN....K.P.V...R.IADVK....IP.KG.H..M....K.V.....I.....................L......KVY.DAAGDDESKKLY.....
Rru   GKGL....VD..GN....K.DLVDV.RK.A.VK....IP.K..H..V...IK...SL..I.....................IL.....KAV.DKAKDDSEK_LF.....V.
Bov   G.EL....VD..GNA...K.P.GSKAR.RVGLK....IP.I..R..M...IK.V.SLV.I.................SI.I...I..KRF.DG__TDEKKKLY.I.....

                  210       220       230       240       250       260       270       280       290       300
Spi              QKASSVAQVVTNFQERGAMEYTIVVAETADSPATLQYLAPYTGAALAEYFMYRERHTLIIYDDLSKQAQAYRQMSLLLRRPPGREAYPGDVFYLHSRLLE
Pea              ...........TL.....................................................P..H.....................................
Tob              ...........TL.....................................................P.....................L.....................
Whe              .R.........T.H.E.........M.....................................................................
Cya              .......NIIEVLR....LD..V...AN.SE.........A...I......KGKA..V.....T.....................I.....................
Eco              ....TISN..RKLE.H..LAN....VA..SES.A.....RMPV.LMG...RD.GEDA...........V....I...........F.....................
Rbl              ..R.T...L.KKLE.T...A......A..SD..PM.F...FS.T.IG.F.RDNG..A.M.........V.....................................
Rru              ..R.T.....KVLADH..LD......A..SE...P..F......CTMG.F.RDNGM.AV.F....T...V.....................F.....................
Bov              ..R.T...L.KRLTDAD..K.....SA..SDA.P.......S.CSMG...RDNGK.A...........V.....................................

          310                 320       330       340       350       360       370       380       390       400
Spi   RAAKLSSLLGE__________GSMTALPIVETQAGDVSAYIPTNVISITDGQIFLSADLFNAGIRPAINVGISVSRVGSAAQIKAMKKVAGKLKLELAQFAELEAFAQFA
Pea   .V.....Q...__________.............S.....................................................Q.....................
Tob   .......S...__________.............S.....................S.....................Q.....................
Whe   .....N.....__________.............S.....................................L...........Q....S...........Q......
Cya   ......DA..G__________.......VI.....................S....S.L.................T..I..I..T........D..A......
Eco   ...RVNAEYV.AFTKGEVKGKT..L.....I.........FV..............ETN.........V.P........G...T.I...LS.GIRTA...YR..A..S...
Rbl   .S..MNGDF.A__________..L.....I......I..YI..............E.E..YQ.....V.T.L.......S..TS...S....V......YR.MA.....G
Rru   .....NDDN.A__________..L....VI.....N.....................ET...FK.....V...L.........S.......Q...SI......YR.MA......
Bov   ....MNDAF.G__________..L....VI.......................ETE..YK.........L..........TR...Q...TM......YR.VA.....G
```

Table I. Continued.

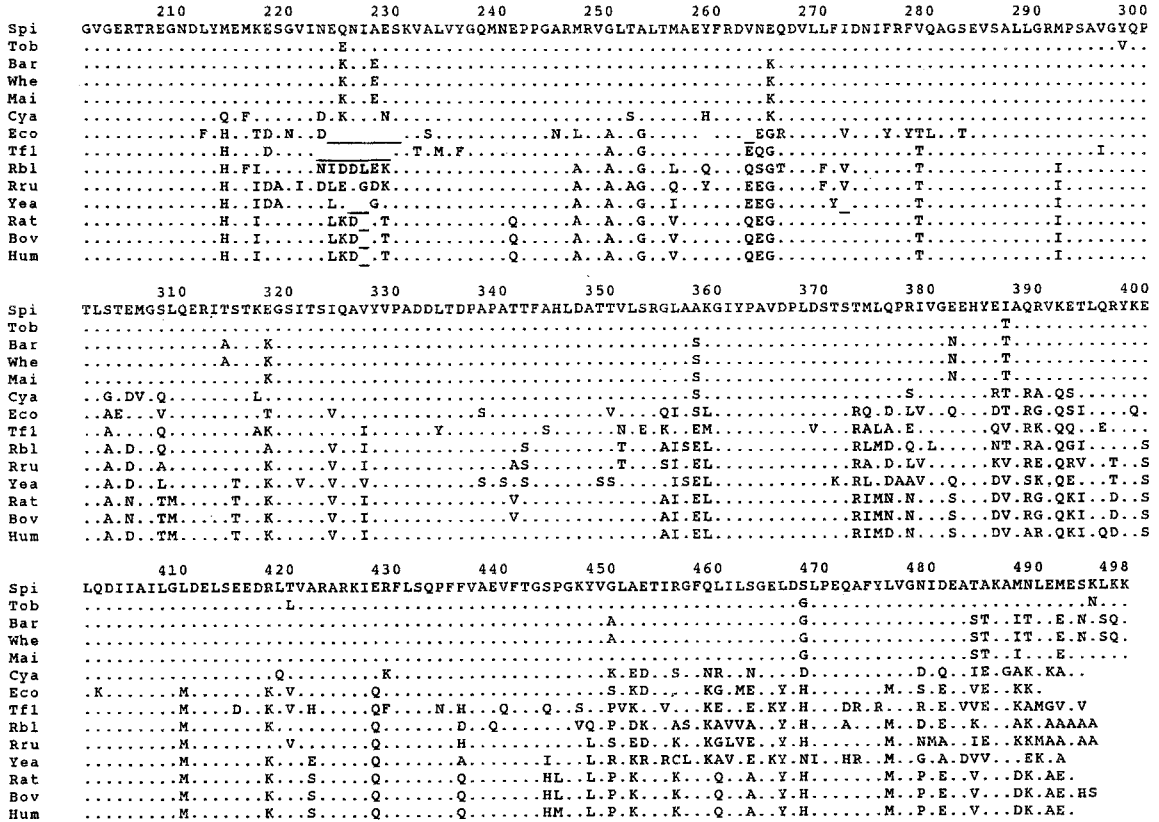
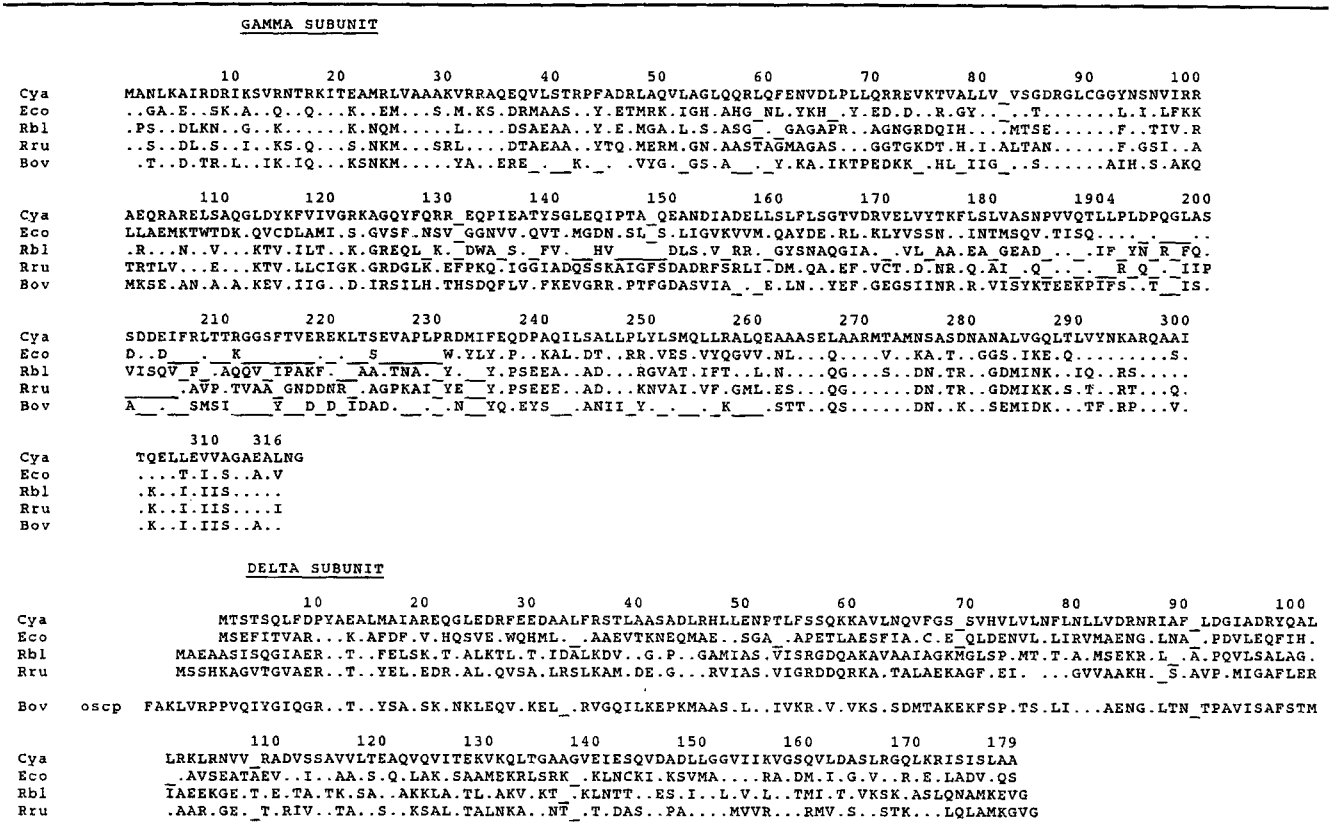
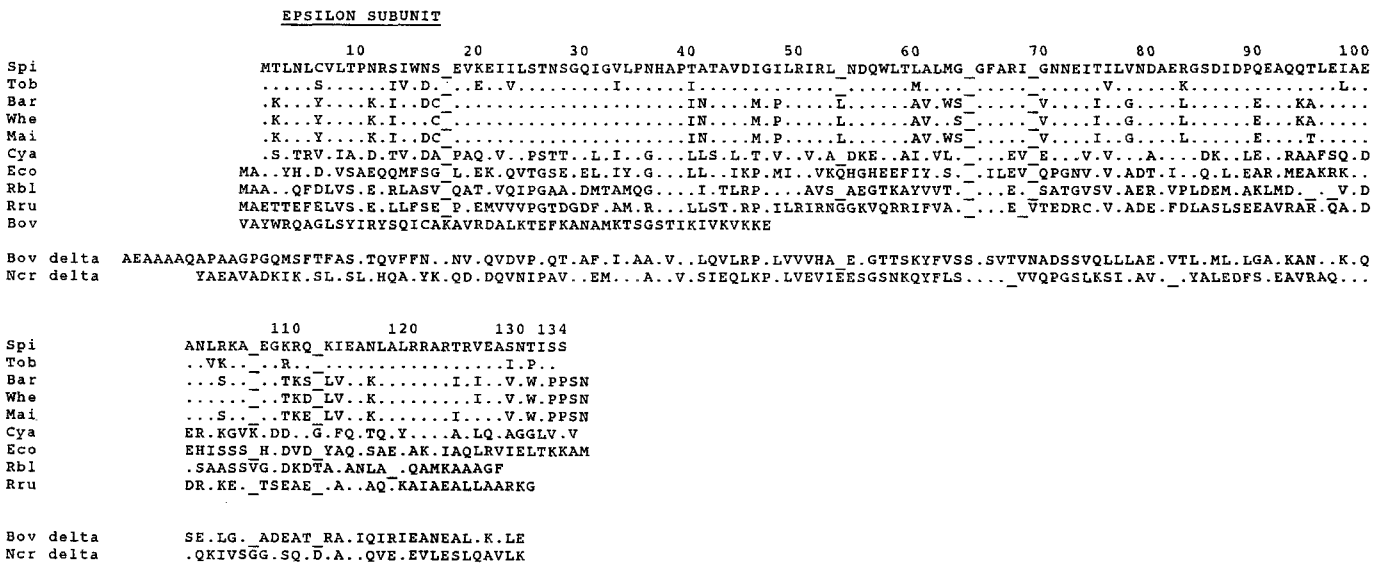

210 220 230 240 250 260 270 280 290 300
Spi GVGERTREGNDLYMEMKESGVINEQNIAESKVALVYGQMNEPPGARMRVGLTALTMAEYFRDVNEQDVLLFIDNIFRFVQAGSEVSALLGRMPSAVGYQP
Tob
Bar
Whe
Mai
Cya
Eco
Tfl
Rbl
Rru
Yea
Rat
Bov
Hum
310 320 330 340 350 360 370 380 390 400
Spi TLSTEMGSLQERITSTKEGSITSIQAVYVPADDLTDPAPATTFAHLDATTVLSRGLAAKGIYPAVDPLDSTSTMLQPRIVGEEHYEIAQRVKETLQRYKE
Tob
Bar
Whe
Mai
Cya
Eco
Tfl
Rbl
Rru
Yea
Rat
Bov
Hum
410 420 430 440 450 460 470 480 490 498
Spi LQDIIAILGLDELSEEDRLTVARARKIERFLSQPFFVAEVFTGSPGKYVGLAETIRGFQLILSGELDSLPEQAFYLVGNIDEATAKAMNLEMESKLKK
Tob
Bar
Whe
Mai
Cya
Eco
Tfl
Rbl
Rru
Yea
Rat
Bov
Hum

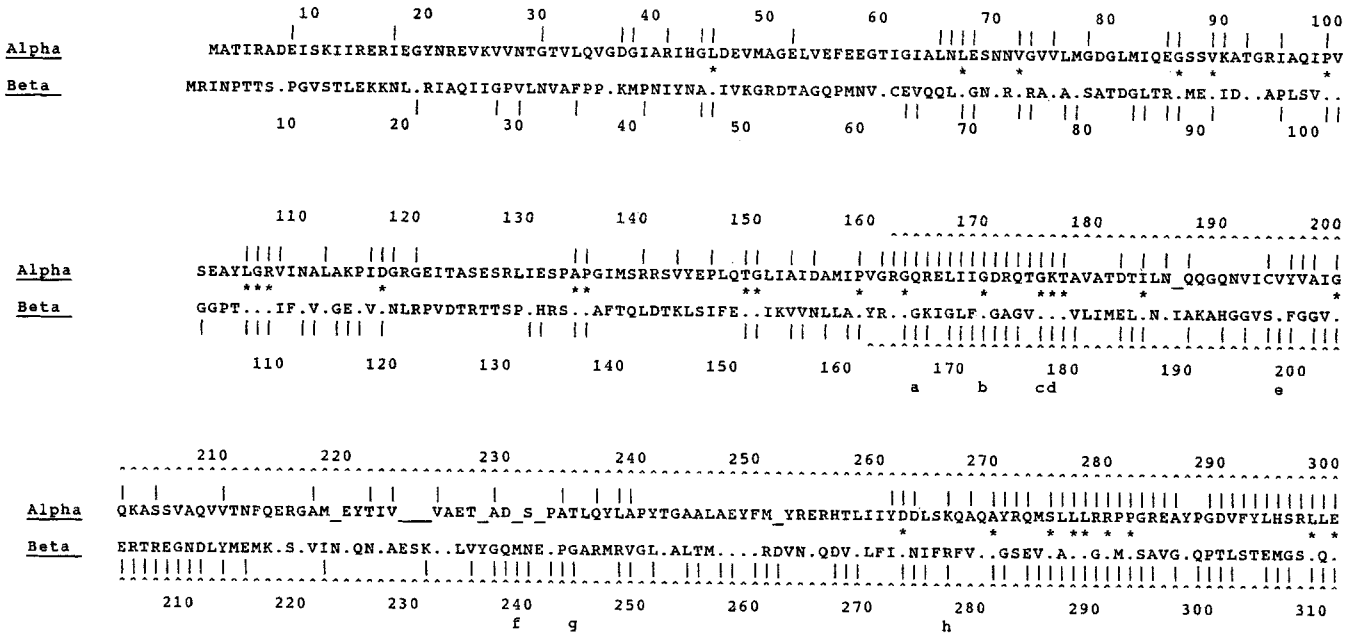
Table I. Continued.

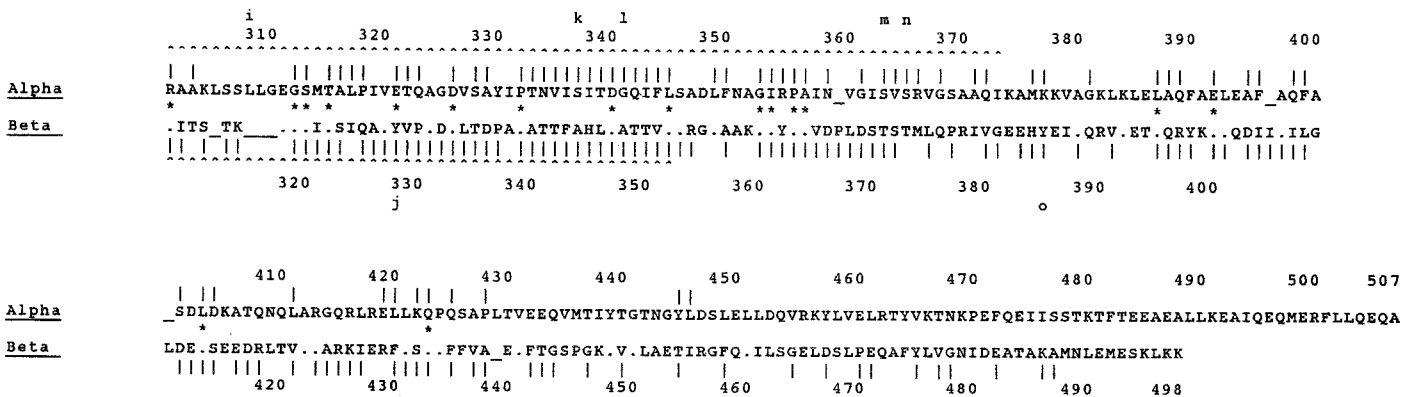




[a] Abbreviations used: Bar, barley chloroplast; Bov, bovine heart mitochondria; Cya, *Cyanobacterium synechococcus* 6301; Eco, *Escherichia coli*; Hum, human heart mitochondria; Mai, Maize chloroplast; Ncr, *Neurospora crassa*, Rat, rat liver mitochondria; Rbl, purple nonsulfur photosynthetic bacteria *Rhodopseudomonas blastica*; Rru, *Rhodospirillum rubrum*; Spi, spinach chloroplast; Tfl, thermopholic bacterium PS3; Tob, tobacco chloroplast; Whe, wheat chloroplast; Yea, yeast *Saccharomyces cerevisiae*. The numeration is based on the sequence for spinach chloroplast (γ and δ, based on *Cyanobacterium synechococcus* 6301), i.e., for the β subunit, spinach K178, Y328, Y362, Y385 would correspond to bovine K168, Y311, Y345, Y368, and to *E. coli* K155, Y297, Y298, Y321. Sequences adapted from Cozens and Walker (1987); Hudson *et al.* (1987); Garboczi *et al.* (1988b); Kagawa *et al.* (1986); Ohta and Kagawa (1986); Takeda *et al.* (1985); Walker *et al.* (1985), and references within. Dots represent identical residues to the reference subunit; lines indicate that residue is absent. Sequence alignment by the FASTP algorithm (Lipman and Pearson, 1985).

Table II. Homologies between F_1-ATPase α and β subunits[a]





"Amino acid sequence for chloroplast spinach, used as a reference for numeration. α-Subunit sequence from Deno *et al.* (1983); β-subunit, Zurawski *et al.* (1982). "∧ ∧ ∧ ∧ ∧ ∧," Proposed nucleotide binding domain. α *E. coli*, Maggio *et al.* (1987); β *E. coli*, Duncan *et al.* (1986). "|||," identical residues for all species within a subunit. ". . . ," identical residues between spinach α and β subunits. "***," identical residues between α and β subunits, independent of the species. Small letters refer to the reported mutants for *E. coli*, α mutants on top of the numeration, β mutants below numeration: a = G142S, b = G149I, c = G154I, d = K155E and K155Q, e = S174F, f = M209I, g = G214R, h = R246C, i = E299K, j = Y297F, k = S347F, l = G351D, m = S373F, h = S375F, and o = Y354F. Mutant designated by the wild type one-letter code amino acid, the residue position, and the mutant amino acid.

Table III. Homology regions between β F_1 and Related Proteins[a]

	First region (A)	
	170 180	
Spi-β	LAPYRRGGKIGLF_GGAGVGKTVLIMELIN	
recA	GAGGLPMGRIVEIYGPESSGKTTLTLQVIA	(52- 84)
myosin	MLTDRENQSILIT_GESGAGKKVNTKRVIQ	(165-193)
EFTu	FGRTKPHVNVGTI_GHVDHGKTTLTAAITT	(6- 34)
AK	MEEKLKKSKIIFVVGGPGSGKGTQCEKIVQ	(1- 30)
	Second region (B)	
	260 270 280	
Spi-β	MAEYFRDVNEQDVLLFIDNIFRFVQA	
PFK	GIEQLKK_HGIQGLVVIGGDGSYQGA	(85-109)
A/At	SNVL_RGMGGAFULVLYDEIKKFV	(285-297)
EFTu	MITGAAQMDGAILVVAATDGPMPQTR	(91-116)
AK	GEEFERK_IGQPTLLLYVDAGPETMT	(102-126)
	Third region	
	320	
Spi-β	STKEGSIT	
N/K ATPase	SDKTGTLT	(368-375)
Ca ATPase	SDKTGTLT	(350-357)

[a]Spi, spinach; recA, recA-protein; myosin, rabbit myosin; PFK, phosphofructokinase; A/At, ATP/ADP translocase, N/K ATPase, $(Na^+ + K^+)$-dependent ATPase; Ca ATPase, $(Ca^{+2} + Mg^{+2})$-dependent ATPase; AK, adenylate kinase; EFTu, elongation Factor Tu.

β subunits are "ragged"; 65% of the α chain is blocked as pyrrolidone carboxylic acid, and the remainder starts at the second residue lysine. The heterogeneity found in the β chain is more complex with 45% commencing at the third residue, a serine. The δ chain is also heterogeneous; half of the N-terminal alanines within the population are absent. The N-termini of both α and β chains are solvent accessible and mild trypsinolysis removes fourteen to fifteen residues of the α-chain and between five to seven of the β-chain. Similar findings were reported for yeast ATPase (Todd and Douglas, 1981) and for the *E. coli* enzyme (Dunn *et al.*, 1980). Interestingly, trypsin-modified F_1-moieties preserve their hydrolytic activity and when added to F_1-depleted inner membrane vesicles, the bound complexes become oligomycin-sensitive, but the F_1-dependent energy-linked properties of the H^+ ATPase are lost (Pedersen *et al.*, 1981). The fact that trypsin-modified F_1 cannot bind the F_0 protein called "OSCP" supports functional similarity of OSCP to the δ chain of *E. coli* (Hundal *et al.*, 1983), already suggested by sequence similarities.

The work of Cozens and Walker (1987) on the complete primary structure of *Cyanobacterium synechococcus* 6301 by *c*DNA sequence determination

showed that these sequences are more homologous to the chloroplast sequences than to the equivalent chains in bacteria or in mammalian and plant mitochondria. The α subunit has a 71% homology to maize chloroplast compared to 53% to *E coli*, 61% to bovine, and 59% to maize mitochondria. In the β subunit the homologies are higher, 81% sequence homology to wheat chloroplast versus 67, 66, and 68% found in *E. coli*, bovine, and tabacco plant mitochondria, respectively. This trend is also found for the F_0 subunits. The chloroplast enzyme is regulated by a light-dependent thioredoxin system that uses the conserved cysteine (position 90) of F_1 γ subunits (Moroney *et al.*, 1984) which is oxidized to form a disulfide bond with a second cysteine found only in chloroplast γ chains. The second cysteine in spinach is found located in the rather unique central region of the γ chains, within the sequence CVDAAEDELFR that is homologous to GLASSDDEIFR (positions 197 to 207) of cyanobacterium which lacks this regulatory mechanism. The similarities found between *Synechococcus*, bacterial, and chloroplasts, at the level of genome organization and in the encoded sequences, constitute a strong support for the endosymbiotic origin for the chloroplast organelle (Cozens and Walker, 1987).

Secondary Structure

Secondary structure prediction based on the Chou and Fasman algorithms (Chou and Fasman, 1978) have been carried out for all the ATPase subunits of *E. coli* (Kanazawa and Futai, 1982), for the β subunits of bovine (Walker *et al.*, 1984c; Duncan *et al.*, 1986), thermophilic bacterium PS3 (Kagawa *et al.*, 1985), and rat mitochondria (Garboczi *et al.*, 1988b). Although these predictions should be taken cautiously, in all the β subunits at least three alternating strands of α-helices and β-sheets are predicted after residue 230, reminiscent of the topology associated with domains that bind nucleotides (Rossmann and Argos, 1981). It is also striking that an α-helical stretch 50 residues long (75 Å length) is predicted for the δ subunit. Circular dichroism measurements on the δ subunit (Sternweis and Smith, 1977) are consistent with a 60% α-helical content. Small-angle X-ray diffraction of the δ subunit of chloroplast F_1 (Schmidt *et al.*, 1977) were interpreted as a 25×90 Å ellipsoid. The possibility of a highly elongated structure suggested to several authors an important structural role for the δ subunit involved in attachment of F_1 to the F_0-sector.

The F_1-moiety of the thermophilic bacterium PS3 (TFl) is stable to a wide range of temperature and ionic strengths; it can be reconstituted after urea or guanidinium chloride denaturation of its individual subunits and is able to form hybrid F_1-moieties with *E. coli* α and γ subunits. The predicted content of secondary structure of TF_1 (30.1% α-helix and 22.3% β-sheet), calculated by the Chou and Fasman procedure, is higher than that calculated

for *E. coli* (27.3% and 13.75%, respectively) and other mesophilic β subunits. To gain an insight into the contribution of different residues to the protein thermal stability, Kagawa *et al.* (1986) aligned the β sequence of TF_1 to the analogous sequences of chloroplast (spinach CF_1), human (HF_1) and *E. coli* (EF_1), and analyzed the differences with the mesophiles in terms of secondary structure, external polarity (residues at reverse turns), and the potential benefit from internal hydrophobicity and packing. An increase in the predicted content of secondary structure in TF_1 versus F_{1s} from mesophiles parallels thermal stability. In the whole sequence the residues that can form reverse turns were well conserved (gly = 92.3%, pro = 82.0%, tyr = 75.0%). The tendency found in the analysis of the amino acid changes for several species of dehydrogenases (Argos *et al.*, 1979) where glycine, serine, lysine, and aspartic residues in mesophiles are generally substituted for alanine, threonine, arginine, and glutamic, respectively, in thermophiles, does not seem to be present in ATPases. Furthermore, the few changes found in these amino acids were observed to the same extent in both directions (Kagawa *et al.*, 1986). Almost 25% of the valine residues in the mesophiles F_1-β were found as isoleucines in TF_1. These changes are assumed to contribute to increased internal packing and hydrophobicity. There is an insert of about eight extra amino acids in TF_1, half of these being acidic, in a loop-forming region which contributes to increase in the hydrophilicity of the external surface. A detailed enumeration of individual changes of amino acid substitutions by residues with better potential of forming secondary structure found in β TF_1 is presented in Table 3 of Kagawa *et al.* (1986). The contribution to the thermal stability of several neutral-to-neutral amino acid substitutions do not seem to have an obvious interpretation.

In summary, it seems clear that despite many common features among secondary structures of the subunits of F_1-ATPases, there are some notable differences, with TF_1 representing the extreme example of an F_1 moiety capable of forming a very stable complex. Even with TF_1, however, the thermal stability of the enzyme seems to be due to three factors: (1) the additive contribution of several amino acid substitutions instead of a few critical ones; (2) the higher content of secondary structure-forming residues than the mesophiles; and (3) the presence of extra negatively charged residues around a reverse turn.

Tertiary and Quaternary Structure

Studies using electron microscopy and X-ray diffraction techniques have resulted in several models depicting the spatial organization of the F_1-moiety. Although the folding of the polypeptide chains for the F_1-moiety or for any of the individual subunits is still unknown, several laboratories have reported models for the three-dimensional structure of F_1 at low resolution.

Several studies on single F_1 particles using electron microscopy have been reported (for a review see Amzel and Pedersen, 1983). Recently, somewhat different models have been proposed by (Tiedge *et al.*, 1983; Tsuprun *et al.*, 1984; Boekema *et al.*, 1986). Tiedge *et al.* (1983) described their EM tilted micrographs as consisting of six similar spherical masses of radius about 30 to 35 Å. They interpreted the micrographs as showing alternating sequences of α and β subunits, arranged in the periphery of the molecule in similar fashion to the carbon atoms of the chair conformation of cyclohexane, with a three-fold axis perpendicular to the ring. Similar to the earlier observation by Catterall and Pedersen (1974), a central mass is observed. It is described by Tiedge *et al.* (1983) as a three-arm structure binding alternate spheres of the periphery. The six exterior masses are visualized as being located at the vertices of two equilateral triangles which are aligned in parallel at a distance of about 20 Å and rotated by 60° with respect to each other. The center of the central mass (assigned to correspond to the γ subunit) is displaced from the center 20 to 30 Å along the three-fold axis toward one of the triangles. This feature is the source of asymmetry for this model. Due to the deep stain conditions found for the central region, this region may need further substantiation (Tiedge *et al.*, 1983), and even the possibility of a six-arm structure for that region is not excluded.

Tsuprun *et al.* (1984) suggested a model for the F_1-sector consisting of six masses arranged in two layers and located approximately at the vertices of a triangular antiprism. Each of the six masses is connected to the two nearest neighbors leaving an empty central region. Recently, a 20 Å resolution model for beef heart F_1 has been proposed by Boekema *et al.* (1986). They developed a highly automated procedure that allows extraction and selection of digitized single-particle images from electron micrographs which are subjected to a multivariate statistical classification after being aligned relative to several different reference images. The main hexagonal view (40% of the population of images) is chosen for the discussion of their model. The F_1 is described to approximate an hexagonal arrangement of six densities for the outer ring and a seventh asymmetric mass close to the center of the image. The relative positions of the outer densities are suggested to be in a staggered disposition rather than an eclipse of the two layers of subunits. The central density, stain-excluding region, has a V-shaped form. The density of the tip of the "V" is comparable to the other six outer masses, and the extremes are connected to two neighboring masses of the external ring. The γ, δ, and ε subunits are thought to be contained in that "V" region supporting the evidence that the γ subunit interacts with both α and β subunits (Williams *et al.*, 1984).

Electron microscopy studies were carried out not only in single-particle preparations but also in crystalline preparations. Akey *et al.* (1983) reported

the study of two different forms of microcrystals of beef heart F_1. The resolution of the data is similar to or lower than the resolution of the previous electron microscopy of single-particle studies of F_1. Interpretation of the data is complicated due to the presence of twinning of the microcrystal and, in some cases, the two forms can be observed in the same section of the microcrystal. The model obtained from these studies for the F_1-moiety consists of a pseudohexagonal molecule of dimensions 115 × 115 × 70 Å.

The reported model with the most detail is still the 9 Å model obtained from X-ray diffraction studies (Amzel *et al.*, 1982) from single crystals of rat liver F_1-ATPase (Amzel and Pedersen, 1978). The F_1-moiety was found to have dimensions of 120 × 110 × 80 Å. It is formed by six globules, where each globule has dimensions of about 60 × 50 × 40 Å. The spatial arrangement of the six globules can be described as a distorted hexagonal or a collapsed octahedron. Each globule contains enough density to accommodate at least one α or one β subunit. The low-resolution model does not allow a direct assignment of individual F_1 subunits to each globule. The different classes of subunit arrangements compatible with the $\alpha_3\beta_3\gamma\delta\varepsilon$ stoichiometry (Fig. 2) have been presented in the original work and recently discussed by Amzel *et al.* (1984) and by Pedersen and Amzel (1985). The densities associated with the γ, δ, and ε subunits are probably not seen in the X-ray model because the single-copy subunits cannot obey the crystallographic symmetry R32 that is found in the diffraction pattern to 3.5 Å resolution (Amzel, 1981). The γ, δ, and ε subunits could either interact with α and β (globules filled with slanted lines in Fig. 2) in a region slightly shifted from the center in both the partially alternating or the contiguous model, or can somehow be located in the center for the side-by-side model.

The major difference between the X-ray model and the one obtained by EM appears to be the presence of a low density in the central region of the model derived by X-ray diffraction studies (Amzel *et al.*, 1982) and a corresponding strain-excluding region in the electron microscopy model presented by Tiedge *et al.* (1983). The explanation of this apparent discrepancy is probably trivial. In the X-ray model, since the minor subunits cannot conform to the crystal symmetry, their images are blurred so that even if they do reside at the center of the F_1 molecule their density would not appear much higher than background. In the EM, on the other hand, due to the lack of detail the center is seen as a stain-excluding region. In addition, the authors probably selected micrographs that had a central region, further accentuating the density of the central mass.

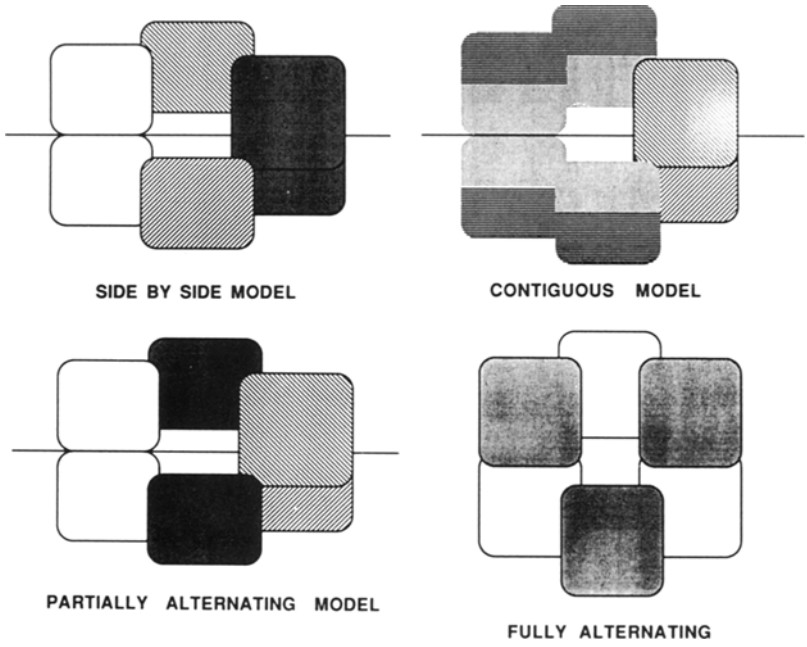


Fig. 2. Schematic representation of the F_1 subunit arrangements that are compatible with $\alpha_3\beta_3\gamma\delta\varepsilon$ stoichiometry. A particular shade could correspond to either α or β in an exclusive way. The diagonal striped regions would correspond to either α or β and some portion of minor subunits γ, δ, and ε.

Relationship to Function

Is There a Catalytic Domain on F_1 Similar to That Found on Adenylate Kinase?

Two similar models were proposed recently to describe a single nucleotide-binding region within the β subunit (Fry *et al.*, 1986; Duncan *et al.*, 1986). Based on NMR measurements of the MgATP binding site of rabbit muscle adenylate kinase (Fry *et al.*, 1985) and three-dimensional information from the X-ray determined structure of pig muscle adenylate kinase (Schulz *et al.*, 1974), a general model for the structure of the ATP-binding sites of homologous nucleotide-binding phosphotransferases was proposed (Fry *et al.*, 1986). This binding site is the locus of three segments whose sequence is homologous to regions of the F_1-β subunits and to the ras-encoded p21, among others. The model assumes that the mechanistic roles proposed for these segments in adenylate kinase can be extended to F_1-ATPase.

Crystals of porcine adenylate kinase (ADK) undergo a pH-induced transition among several forms that are interconvertible. Two conformations of crystalline ADK have been successfully studied by X-ray diffraction (Sachsenheimer and Schulz, 1977). Crystal form A has an ATP-free site and a closed adenosine pocket at the AMP site, whereas crystal form B has an ATP-free site and an open AMP site (Pai *et al.*, 1977). The two conformations differ in the spatial arrangement of four regions, the main and most relevant difference being the displacement of a glycine-rich loop analogous to the first region of homology of the F_1-β subunits. Using a different localization of the ATP-bound molecule (derived from their NMR data), Fry *et al.* (1985) propose a model for the ATPase mechanism that incorporates the idea of an induced conformational change similar to that reported for the X-ray structure of ADK in the glycine-rich loop. A similar conformational change is mimiced in the F_1-β subunit as a consequence of substrate binding or subunit interaction and would control the accessibility of ATP to the enzyme.

Another hypothetical model of the β subunit of *E. coli* F_1, also based on ADK comparisons, has been proposed by Duncan *et al.* (1986). This model is based also on the secondary structure predictions of Walker *et al.* (1984c) and of Kanazawa and Futai (1982), and has residues 141 to 321 of the *E. coli* β-subunit in the topology $-2X, 1X, 2X, 1X$ (Richardson, 1981) in a manner similar to the three-dimensional arrangements of the β-twist and the relative orientations of the α-helices and loops found in the crystal B form of the X-ray-determined structure of adenylate kinase by Sachsenheimer and Schultz (1977). The fact that there is only one α-helix predicted between the second and third β-strands, instead of three found in adenylate kinase, is taken into account in the model without any major disturbance in the pattern of folding. The ATP molecule is placed in the position suggested by Fry *et al.* (1985) with the constant lysine, part of the GXXXXGK sequence in the glycine-rich loop, stabilizing the α-phosphate of the ATP molecule. The conserved hydrophobic residues belonging to the end of the first homology region of the β-subunits are described as part of an α-helix and are assumed to provide a stabilizing environment for the adenine ring.

The second homology region (B region) contains the aspartate residue that could anchor the Mg^{2+} of bound MgATP in a similar way reported in phosphofructokinase (Evans *et al.*, 1981) and ADK (Fry *et al.*, 1985). Labelling experiments with 8-azido ATP (Hollemans *et al.*, 1983) and with 7-chloro-4-nitrobenzofurazan (NBF) (Andrews *et al.*, 1984a,b; Sutton and Ferguson, 1985b) to the bovine F_1-moiety are rationalized with this model of the β subunit by Duncan *et al.* (1986) using analogous target residues in ADK. They also provide (not surprisingly) an explanation for the protective roles of P_i, Mg^{2+}, and Mg^{2+} bound to P_i to the inactivation by NBF, and the different degrees of protection provided by ATP and ADP. The pH-induced

conformational change described in the X-ray diffraction studies of ADK are also extended to provide functional roles for the model. The conformational change is suggested to provide the molecular mechanism that achieves the extremely high affinity of the putative "first" catalytic site of F_1-ATPase (Duncan *et al.*, 1986).

Although β subunit models based on structural comparisons with the enzyme ADK are not incompatible with some of the available data, they have the limitation of not being able to take into account the role of residues after amino acid 341 such as tyr 345, his 427, and tyr 368 (Bullough and Allison, 1986a,b; Cross *et al.*, 1987), recently reported to be involved in catayltic and regulatory sites for the bovine enzyme. In addition, it should be noted that the position of ATP was calculated from the NMR data in an indirect way. That is, the position of the Cr^{3+} in Cr-ATP bound to ADK was determined by several distances to amino acid protons on the enzyme; the ATP was located based on the relative position of the adenine-ribose moeity with respect to Cr^{3+}; and finally, the phosphate chains were fitted for a minimum disturbance. It is not clear from an inspection of the model that there is a path for the ATP molecules to diffuse into the proposed site. Most importantly, the NMR structure of ATP binding to ADK, which is the basis for these models, is not in agreement with the position of ATP suggested by the recently determined structure of a complex of yeast ADK with the inhibitor P^1, P^5-di(adenosine-5′) pentaphosphate, assumed to be in part equivalent to ATP. This report (Egner *et al.*, 1987) is based on X-ray diffraction data and tends to corroborate the early ATP site assignment in the porcine ADK (Pai *et al.*, 1977). For comparison between the two, Fig. 3A shows the NMR model (Fry *et al.*, 1986) (coordinates provided generously by Dr. A. S. Mildvan) in a similar orientation as the X-ray model of ADK with inhibitor as in Fig. 5 of Egner *et al.* (1987). Obviously, neither model can provide information about whether the site(s) involved are catalytic or regulatory.

Due to the high homologies of α and β subunits in the regions noted above (Table II), a similar binding site should be found in the α subunit.[3] However, most recent evidence points to the location of the nucleotide-binding sites in the β subunits (or to the possibility of binding at the α–β interface). The role of lys 155 in the interaction of phosphate groups at ATP has been challenged by the report of Parsonage *et al.* (1987) in that the mutation lysine 155-to-glutamine (K155Q) in *E. coli* has a greater detrimental effect in the impairment of catalysis than a lysine-to-glutamate mutation

[3] Although the isolated α subunit does bind nucleotide (Dunn and Futai, 1980) photoaffinity labelling of intact F_1 followed by dissociation reveals that the nucleotide is either bound to only β subunits (Kironde and Cross, 1987) or preferentially to β subunits with some label recovered in α subunits (Williams and Coleman, 1982). There remains controversy as to whether the β subunit binds 1 or 2 mol nucleotides per mol β (Rao *et al.*, 1988).

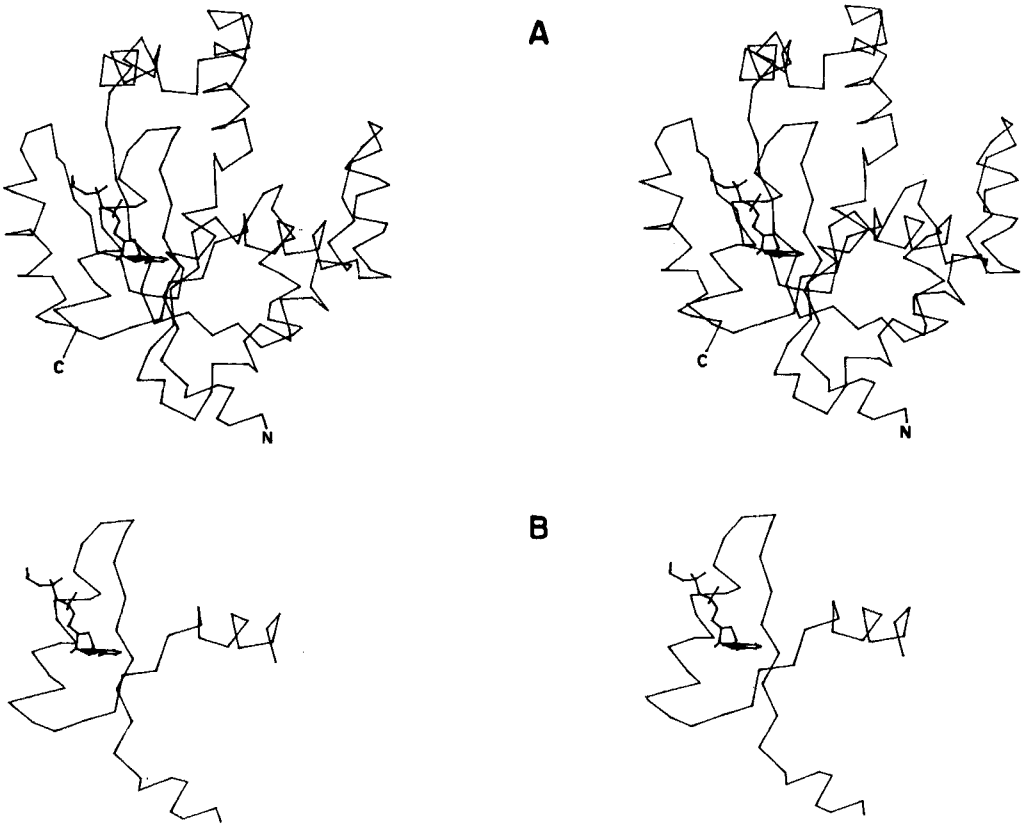


Fig. 3. Stereo drawing of the C-backbone of: (A) adenylate kinase with the ATP bound as described by Fry *et al.* (1986) from their MgATP binding site NMR data and (B) the fifty-residue N-terminus peptide, with the ATP molecule as found in the whole enzyme.

Table IV. Experimentally Modified Residues Affecting ATPase Activity

Labelling			
Reagent	Target	Condition	Label/F_1
Nbf-Cl[1]	βY311		One
FSBA[2,3]	βY368	pH 8	Three
	βH427	pH 6	Three
FSBI[3]	βY345		One
2-AziAde[4]	βY345	F_1[3, 0]	One
	βY368	F_1[0, 0]	Two

Mutants		
Mutation	Assembly	Catalysis
αE229K	abolished	[i]
αS347F	normal	impaired[k]
αG351D	normal	impaired[l]
αS373F	normal	impaired[m]
αS375F	normal	impaired[n]
βG142S	normal	(− −)[a]
βG1491	normal	(−)[b]
βG1541	normal	(− −)[c]
βG155E	normal	(− −)[d]
βK155Q	normal	(− − −)[d]
βS174F	normal	(−)[e]
βM2091	normal	(−)[f]
βG214R	abolished	[g]
βR246C	normal	(−)[h]
βY297F	normal	(−)[j]
βY354F	normal	normal[o]

Notes: Nbf-Cl, 7-chloro-4-nitrobenzofuran; FSBA, 5′-*p*-fluorosulfonylbenzoyl adenosine; FSBI, 5′-*p*-fluorosulfonylbenzoyl inosine; 2-AziAde, 2-azidoadenine. Amino acids are designated by consecutive labelling of the subunit, the one-letter amino acid code, and the position number. In the case of mutants, the last letter indicates the mutant amino acid. $F_1[x, y]$, F_1 containing x mol of adenine nucleotide at noncatalytic sites and y mol of adenine nucleotide at catalytic sites per mole of enzyme (Kironde and Cross, 1986). The labelling experiments (and their numeration) correspond to beef heart mitochondria, and the mutation, to *E. coli*. Letter superscripts indicate their position in Table II. The minus signs are an arbitrary scale to indicate the relative impairment of catalysis. Numeral superscripts are: 1, Andrews *et al.* (1984a); 2, 3, Bullough and Allison (1986a,b); 4, Cross *et al.* (1987); 5, results from Senior's laboratory (Parsonage *et al.*, 1987a,b and Maggio *et al.*, 1987).

(K155E). See Table IV for a summary of single residue mutations. The role of asp 127 as a putative anchor of the Mg^{+2} of bound MgATP, from previous analogy with phosphofructokinase, seems to be challenged also by the recent findings of Hellinga and Evans (1987). They found that the main effect of changing aspartic 127 to serine is to reduce the turnover number of *E. coli* phosphofructokinase and now interpret the role of asp 127 as a general base

catalyst that increases the nucleophilicity of the 1-OH of fructose-6-phosphate.

In order to gain a further insight into models of the β subunit based on ADK, Garboczi *et al.* (1988a) synthesized a fragment that contains the residues 141–190 of the rat liver β subunits (the first 50 amino acids of the model for the putative active site region of F_1-β). Figure 3B shows the backbone of this peptide as found in the analogous region of adenylate kinase with the ATP in the position reported by Fry *et al.* (1986). Significantly, Garboczi *et al.* (1988a) demonstrated that this peptide does interact strongly with ATP. Added Mg^{++} is not required just as Mg^{++} is not required for nucleotide binding to F_1. ATP, ITP, and GTP, all catalytic substrates for F_1, result in precipitation of the peptide. Addition of the peptide in solution to trinitrophenol-ATP, a strong competitive inhibitor of F_1, results in a 7-fold fluorescent enhancement of this analog. Although one might argue that the interactions observed are due to electrostatic interactions of a nonspecific nature, the finding that AMP, P_i, and Mg^{2+} do not interact with the peptide tend to negate this view.

In summary, the β subunit of F_1 does have three amino acid stretches that are homologous with a MgATP binding domain in adenylate kinase. Available data is consistent with the view that a similar, but certainly not identical, nucleotide-binding domain may exist within F_1-β. Moreover, assuming that the nucleotide-binding domain on F_1-β predicted by the work of Fry *et al.* (1985) and the model of Duncan *et al.* (1986) is involved in nucleotide binding, it should be noted that this domain is unlikely to form a complete catalytic site as one or more amino acid residues on the C-terminal β stretch extending beyond the adenylate kinase homology regions also appear to be essential for catalysis. Finally, it should be kept in mind that $F_1\beta$ subunits appear to contain two nucleotide binding domains. Therefore, even if partially correct, the domain predicted on the basis of homology arguments which adenylate kinase may only be "part of the story."

Are α–β Subunit Interactions Essential for "Promoted" Catalysis?

Strong evidence of α–β intersubunit interactions has been provided by a series of mutants of the α subunit of *E. coli* that are able to impair catalysis but do not cause structural perturbation (Maggio *et al.*, 1987). These mutants are S347F, S351D, S373F, and S357F, and they lie in a short stretch near the C-terminus. As the evidence accumulates in favor of adenine nucleotide sites for β subunits, the results obtained with these four mutants strongly suggest that β–α–β intersubunit interaction are essential for "promoted" catalysis in F_1-ATPase. Even single anions such as iodine at low concentration react preferentially with the β subunit and inactivate the catalytic capacity of the intact F_1 (Petrone *et al.*, 1987).

Since the N-terminal is accessible to mild proteolysis, the α subunits seem to be oriented in F_1 in such a way that the N-terminal region is in the external or solvent-accessible region and the C-terminus points toward the interior, interacting with neighbor β subunits. One of the α subunits appears to be bound to the γ subunit (Williams *et al.*, 1984). Interestingly, the single nonexchangeable Mg^{+2} site that remains associated with F_1 is recovered in the $\alpha\gamma$ fraction after cold denaturation (Williams *et al.*, 1987). The ε subunit has been shown previously to interact with the γ subunit. Experiments using monoclonal antibodies and cross-linking reagents indicate that the C-terminus of the β subunit is the locus of the interaction with the ε subunit (Tozer and Dunn, 1987).

Does F_1 Function in Catalysis as though the Three β Subunits Are Involved in a Sequential "Equivalent" Manner (Rotational Catalysis)?

Extending the earlier views of Repke and Schön (1974) and Adolfsen and Moudrianakis (1976), Gresser *et al.* (1982) proposed a model for F_1 which involves "rotational catalysis." Each β subunit at a given point in time is "tagged" by the smaller subunits ($\gamma\delta\varepsilon$) and is then proposed to make (or hydrolyze) ATP on its surface from ADP and P_i with an equilibrium constant near 1. The electrochemical gradient of protons releases the bound ATP on the first β subunit while ADP and P_i bind to a second β. Upon being tagged by the smaller subunits, ATP synthesis now occurs on the second β and is released again by the electrochemical proton gradient while ADP and P_i bind to the third β subunit. Finally, as the smaller subunits "tag" the third β subunit, ATP is made and then released by the electrochemical gradient and the cycle of rotation begins again. Presumably, the smaller subunits $\gamma\delta\varepsilon$ rotate, specifying each of the β subunits in turn for ATP synthesis (or conversely, the larger subunits rotate and become "tagged" by a stationary trio of the smaller subunits).

Although there is evidence from the work of Grubmeyer and Penefsky (1981a) that bovine heart F_1 is capable of hydrolyzing ATP (at a "single uni-site") to give ADP and P_i with an equilibrium constant near 1, there is no evidence that there is actual rotation in which three β subunits are "tagged" in turn by the smaller subunits $\gamma\delta\varepsilon$. Of particular interest are two recent reports (Bullough *et al.*, 1987; Yohda and Yoshida, 1987) which indicate that the high-affinity ATP site resulting from the above equilibrium may not be involved in "promoted" ATP hydrolysis when F_1 functions as an ATPase. That is, the evidence presented in these two reports indicates that one of the three β subunits does hydrolyze ATP at a low rate ("uni-site catalysis") but *is not* rapidly accelerated in rate by binding ATP to another β subunit. That is, "positive cooperativity" in the sense envisioned by Grubmeyer and Penefsky (1981b) may not be operative. Rather there may be one

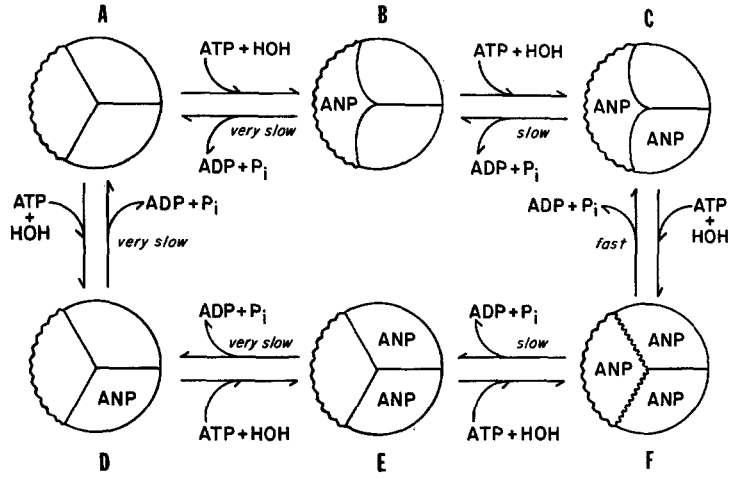


Figure 4. Summary diagram of the conformational and binding states of the F_1-moiety. The F_1 is represented by three $\alpha\beta$ pairs. One pair (drawn with ragged lines) is nonequivalent with the other two because of tighter interactions with the smaller subunits γ, δ, and ε. Very tight or nonexchangeable nucleotide sites are not shown explicitly, as they vary for different F_1's and probably play structural or regulatory roles. To consider how this scheme explains known data, the reader should start with form A, a totally inactive form with all exchangeable sites open, and then proceed to, form F. Form F is a fully active F_1-ATPase. To get to form F, the most likely route is going A → B → C → F. However, the route going A → D → E → F is also possible. The most critical point is that in this scheme it is only when form F has been attained (all three nucleotide binding sites occupied) that F_1 catalyzes "promoted" ATPase activity. In all other intermediate states low ATPase activity results. Consider the route going A → B → C → F. First, ATP binds to the nonequivalent β subunit and undergoes a very low rate of "uni-site" hydrolysis. This binding causes the conformation of the other two β subunits to change (cooperativity). A second nucleotide binds to one of these two β subunits and it could catalyze ATP hydrolysis at a slow rate. Finally, the third ATP molecule binds, causing both of the β subunits to catalyze "promoted" ATP hydrolysis. It is very important to note that the nonequivalent β remains asymmetric at all "promoted" ATP hydrolysis. ANP stands for the equilibrium ATP + H_2O = ADP + P_i, so each form represents a population distribution. For example, it is known that form B has half of the population containing ATP and the other half ADP and P_i (Grubmeyer *et al.*, 1982); the proportions of ATP and ADP + P_i in the other forms are not known. [This diagram is a modified version of that presented by Bullough *et al.* (1987) and represents a generalized description of all relevant "forms" (states) of the enzyme in the sense of King and Altman (1956).][4]

[4] As Fig. 4 is depicted, it is assumed that at step F only two of the β subunits catalyze "prompted catalysis" while the asymmetric subunit catalyzes rates less than V_{max} (Bullough *et al.*, 1987). More recently Penefsky (1988) has indicated that the site normally catalyzing unisite catalysis (assumed in Fig. 4 to be the asymmetric β) does catalyze V_{max} rates when the concentration of ATP is greater than the concentration of F_1. Significantly, Fig. 4 readily becomes compatible with the latter data if the "label" in the step F to E is changed from "slow" to "fast." It is important to note that in Fig. 4 there is no requirement for physical subunit rotation as envisioned by Gresser *et al.*, (1982) regardless of which set of data [i.e., Bullough *et al.* (1987) or Penefsky (1988)] proves to be correct.

β subunit which hydrolyzes ATP at a low rate, and this hydrolysis prepares the two other β subunits in "promoted" catalysis (i.e., multisite catalysis involving only *two* β subunits). This view depicted by the summary diagram shown in Fig. 4 contains all the highlights of the Bullough *et al.* (1987) model and involves cooperativity among β subunits but not as envisaged by Grubmeyer and Penefsky (1981a,b).

As F_1 exhibits asymmetry (i.e., one of the three $\alpha\beta$ pairs is complexed with the smaller subunit trio $\gamma\delta\varepsilon$), one could argue that uni-site catalysis may take place on the "asymmetric" β which may remain permanently "asymmetric" during the catalytic cycle, while either one of the other two β subunits catalyze promoted hydrolysis. The fact that several reagents such as Nbf-Cl, FSBI, 2-azidoadenosine, and benzophenone ATP (Wang, 1984, 1985, 1986; Bullough and Allison 1986a,b; Kironde and Cross, 1986, 1987; Ackerman *et al.*, 1987; Wu *et al.*, 1987) exhibit one-third site reactivity (i.e., inhibit nearly completely "promoted" ATP hydrolysis; see Table IV for pertinent labelling experiments) may be due to their binding at the "asymmetric" β or to binding to one of the two equivalent β's operating cooperatively. Of particular interest in future experiments will be to delineate agents which inhibit both uni-site catalysis and multisite catalysis from those that inhibit only multisite catalysis.

In summary, it will be very important to assess the critical role of the small subunits and of the asymmetry they introduce on the F_1-moiety. Experiments on function of these subunits will address the fundamental questions of whether all the β subunits within an F_1 do in turn adopt the same conformation during catalysis.

Acknowledgments

We are grateful to Dr. Albert Mildvan for making available to us the coordinates from the ATP-binding site to adenylate kinase from their NMR studies, and to David Garboczi for his critical reading of the manuscript during its preparation. We acknowledge the use of the Interactive Graphics Facility of the Department of Biophysics, Johns Hopkins University School of Medicine. This facility was established and maintained by NIH and NSF grants and by a gift from the Richard King Mellon Foundation. This work was supported by NIH Grant CA 10951 to Dr. Peter L. Pedersen and NIH Grant GM 25432 to Dr. L. Mario Amzel.

References

Ackerman, S. H., Grubmeyer, C., and Coleman, P. S. (1987). *J. Biol. Chem.* **262**, 13765–13772.
Adolfsen, R., and Moudrianakis, E. N. (1976). *Arch. Biochem. Biophys.* **172**, 425–433.
Akey, C. W., Spitsberg, V., and Edelstein, S. J. (1983). *J. Biol. Chem.* **258**, 3222–3229.

Amzel, L. M. (1981). *J. Bioenerg. Biomembr.* **13**, 109–121.
Amzel, L. M., and Pedersen, P. L. (1978). *J. Biol. Chem.* **253**, 2067–2069.
Amzel, L. M., and Pedersen, P. L. (1983). *Annu. Rev. Biochem.* **52**, 801–824.
Amzel, L. M., McKinney, M., Narayanan, P., and Pedersen, P. L. (1982). *Proc. Natl. Acad. Sci. USA* **79**, 5582–5586.
Amzel, L. M., Narayanan, P., and Pedersen, P. L. (1984). In *H^+ ATPase (ATP Synthase): Structure, Function, and Biogenesis. The F_0F_1 Complex of Coupling Membranes. Quaternary Structure of F_1-ATPases* (Papa, S., *et al.*, eds.), Adriatica Editrice, Bari, Italy, pp. 125–134.
Andrews, W. W., Hill, F. C., and Allison, W. S. (1984a). *J. Biol. Chem.* **259**, 8219–8225.
Andrews, W. S., Hill, F. C., and Allison, W. S. (1984b). *J. Biol. Chem.* **259**, 14378–14382.
Bird, C. R., Koller, B., Auffret, A. D., Huttly, A. K., Howe, C. J., Dyer, T. A., and Gray, J. C. (1985). *EMBO J.* **4**, 1381–1388.
Boekema, E. J., Berden, J. A., and VanHeel, M. G. (1986). *Biochim. Biophys. Acta* **851**, 353–360.
Bullough, D. A., and Allison, W. S. (1986a). *J. Biol. Chem.* **261**, 5722–5730.
Bullough, D. A., and Alison, W. S. (1986b). *J. Biol. Chem.* **261**, 14171–14177.
Bullough, D. A., Verburg, J. G., Yoshida, M., and Allison, W. S. (1987). *J. Biol. Chem.* **262**, 11675–11683.
Catterall, W. A., and Pedersen, P. L. (1971). *J. Biol. Chem.* **246**, 4987–4994.
Catterall, W. A., and Pedersen, P. L. (1974). In *Membrane ATPases and Transport Processes* (Bronk, R. J., ed.), Biochem. Soc. Spec. Publ., Vol. 4, pp. 63–68.
Catterall, W. A., Coty, W. A., and Pedersen, P. L. (1973). *J. Biol. Chem.* **253**, 2067–2069.
Chou, P. Y., and Fasman, G. D. (1978). *Annu. Rev. Biochem.* **47**, 251–276.
Cozens, A. L., and Walker, J. E. (1987). *J. Mol. Biol.* **194**, 359–383.
Cozens, A. L., Walker, J. E., Phillips, A. L. Huttly, A. K., and Gray, J. C. (1986). *EMBO J.* **5**, 217–222.
Cross, R. L., Cunninghan, D., Miller, C. G., Xue, Z., Zhou, J-M., and Boyer, P. D. (1987). *Proc. Natl. Acad. Sci. USA* **84**, 5715–5719.
Deno, H., Shinozaki, K., and Sugiura, M. (1983). *Nucleic Acids Res.* **7**, 2185–2191.
Duncan, T. M., Parsonage, D., and Senior, A. E. (1986). *FEBS Lett.* **208**, 1–6.
Dunn, S. D., and Futai, M. (1980). *J. Biol. Chem.* **255**, 113–118.
Dunn, S. D., Heppel, L. A., and Fullmer, C. S. (1980). *J. Biol. Chem.* **255**, 6891–6896.
Egner, U., Tomasseli, A. G., and Schulz, G. E. (1987). *J. Mol. Biol.* **195**, 649–658.
Esch, F. S., and Allison, W. S. (1978). *J. Biol. Chem.* **253**, 6100–6106.
Evans, P. R., Farrants, G. W., and Hudson, P. J. (1981). *Philos. Trans. R. Soc. London B* **239**, 53–62.
Falk, G., Hampe, A., and Walker, J. E. (1985). *Biochem. J.* **228**, 391–407.
Fillingame, R. H. (1981). *Curr. Top. Bioenerg.* **11**, 35–106.
Fry, D. C., Kuby, S. A., and Mildvan, A. S. (1985). *Biochemistry* **24**, 4680–4694.
Fry, D. C., Kuby, S. A., and Mildvan, A. S. (1986). *Proc. Natl. Acad. Sci. USA* **83**, 907–911.
Futai, M., and Kanazawa, H. (1983). *Microb. Rev.* **47**, 258–312.
Garboczi, D. N., Shenbagamurthi, P., Hullihen, J., and Pedersen, P. L. (1988a). *J. Biol. Chem.* **263**, 812–816.
Garboczi, D. N., Fox, A. N., Gerring, S. L., and Pedersen, P. L. (1988b). *Biochemistry*, **27**, 553–560.
Gresser, M. A., Meyers, J. A., and Boyer, P. D. (1982). *J. Biol. Chem.* **257**, 12030–12038.
Grubmeyer, C., and Penefsky, H. S. (1981a). *J. Biol. Chem.* **256**, 3718–3727.
Grubmeyer, C., and Penefsky, H. S. (1981b). *J. Biol. Chem.* **256**, 3728–3734.
Grubmeyer, C., Cross, R. L., and Penefsky, H. S. (1982). *J. Biol. Chem.* **257**, 12092–12100.
Hellinga, H. W., and Evans, P. R. (1987). *Nature (London)* **327**, 437–439.
Hollemans, M., Runswick, M. J., Fearnley, I. M., and Walker, J. E. (1983). *J. Biol. Chem.* **258**, 9307–9313.
Hudson, G. S., Mason, J. G., Holton, T. A., Kolber, B., Cox, G. B., Whitfeld, P. R., and Bottomley, W. (1987). *J. Mol. Biol.* **196**, 283–298.
Hundall, T., Norling, B., and Ernster, L. (1983). *FEBS Lett.* **162**, 5–10.
Kagawa, Y., Sone, N., Hirata, and Okamoto, H. (1979). *J. Bioenerg. Biomembr.* **11**, 39–78.

Kagawa, Y., Ishizuka, M., Saishu, T., and Nakao, S. (1986). *J. Biochem.* **100**, 923–934.
Kanazawa, H., and Futai, M. (1982). *Annu. N.Y. Acad. Sci.* **402**, 45–63.
King, E. L., and Altman, C. (1956). *J. Phys. Chem.* **60**, 1375.
Kironde, F. A. S., and Cross, R. L. (1986). *J. Biol. Chem.* **261**, 12544–12549.
Kironde, F. A. S., and Cross, R. L. (1987). *J. Biol. Chem.* **262**, 3488–3495.
Lipman, D. J., and Pearson, W. R. (1985). *Science* **227**, 1435–1441.
MacLennan, D. H., Brandl, C. J., Korczak, B., and Green, N. M. (1985). *Nature* (*London*) **316**, 697–700.
Maggio, M. B., Pagan, J., Parsonage, D., Hatch, L., and Senior, A. E. (1987). *J. Biol. Chem.* **262**, 8981–8984.
Mitchell, P. (1966). *Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation*, Glyn Res. Lab., Bodmin, Cornwall, England.
Moroney, J. V., Fullmer, C. S., and McCarty, R. E. (1984). *J. Biol. Chem.* **259**, 7281–7285.
Ohta, S., and Kagawa, Y. (1986). *J. Biochem.* **99**, 135–141.
Pai, E. F., Sachsenheimer, W., Schirmer, R. H., and Schultz, G. E. (1977). *J. Mol. Biol.* **114**, 37–45.
Parsonage, D., Duncan, T. M., Milke-Mounts, S., Kironde, F. A. S., Hatch, L., and Senior, A. E. (1987a). *J. Biol. Chem.* **262**, 6301–6307.
Parsonage, D., Wilke-Mounts, S., and Senior, A. E. (1987b). *J. Biol. Chem.* **262**, 8022–8026.
Pedersen, P. L., and Amzel, L. M. (1985). In *Achievements and Perspectives of Mitochondrial Research.* Vol. I: *Bioenergetics. Structure of ATPases of the F_0F_1 Type: Chemical Asymmetry and Implications for Mechanism* (Quagliariello, E., *et al.*, eds.), Elsevier, Amsterdam, pp. 169–189.
Pedersen, P. L., Hullihen, J., and Wehrle, J. P. (1981). *J. Biol. Chem.* **256**, 1362–1369.
Penefsky, H. S. (1988). *J. Biol. Chem.* **263**, 6020–6022.
Petrone, G., Garboczi, D. N., and Pedersen, P. L. (1987). *Biochemistry* **26**, 4016–4021.
Rao, R., Al-Shawi, M. K., and Senior, A. E. (1988). *J. Biol. Chem.* **263**, 5569–5573.
Repke, K. R. H., and Schon, R. (1974). *Acta Biol. Med. Ger.* **33**, K27–K38.
Richardson, J. S. (1981). *Adv. Protein Chem.* **34**, 167–339.
Rossmann, M. G., and Argos, P. (1981). *Annu. Rev. Biochem.* **50**, 497–532.
Sachsenheimer, N., and Schulz, G. E. (1977). *J. Mol. Biol.* **114**, 23–36.
Sakamoto, J., and Tonomura, Y. (1983). *J. Biochem.* **93**, 1601–1614.
Salemme, F. R. (1983). *Prog. Biophys. Mol. Biol.* **42**, 95–133.
Schmidt, V. D., and Paradies, H. (1977). *Biochem. Biophys. Res. Commun.* **78**, 1043–1052.
Schulz, G. E., Elzinga, M., Marx, F., and Schirmer, R. H. (1974). *Nature* (*London*) **250**, 120–123.
Senior, A. E., and Brooks, J. C. (1971). *FEBS Lett.* **17**, 327–329.
Shull, G. E., Schartz, A., and Lingrel, J. B. (1985). *Nature* (*London*) **316**, 691–695.
Sternweis, P. C., and Smith, J. B. (1977). *Biochemistry* **16**, 4020–4025.
Sutton, R., and Ferguson, S. (1985a). *FEBS Lett.* **179**, 283–288.
Sutton, R., and Ferguson, S. (1985b). *Eur. J. Biochem.* **148**, 551–554.
Takeda, M., Vassarotti, A., and Douglas, M. G. (1985). *J. Biol. Chem.* **260**, 15458–15465.
Tiedge, H., Schafer, G., and Mayer, F. (1983). *Eur. J. Biochem.* **132**, 37–45.
Todd, R. D., and Douglas, M. G. (1981). *J. Biol. Chem.* **256**, 6990–6994.
Tozer, R. G., and Dunn, S. D. (1987). *J. Biol. Chem.* **262**, 10706–10711.
Tsuprun, V. L., Mesyanzhinova, I. V., Kozlov, I. A., and Orlova, E. V. (1984). *FEBS Lett.* **167**, 285–289.
Tybulewicz, V. L. J., Falk, G., and Walker, J. E. (1984). *J. Mol. Biol.* **179**, 185–214.
Walker, J. E., Runswick, M. J., and Saraste, M. (1984a). *Biochim. Biophys. Acta* **768**, 164–200.
Walker, J. E., Gay, N. J., Saraste, M., and Eberle, A. N. (1984b). *Biochem. J.* **224**, 799–815.
Walker, J. E., Saraste, M., and Gay, N. (1984c). *Biochim. Biophys. Acta* **768**, 164–200.
Walker, J. E., Fearnley, I. M., Gay, N. J., Gibson, B. W., Northrop, F. D., Powell, S. J., Runswick, M. J., Saraste, M., and Tybulewicz, V. L. J. (1985). *J. Mol. Biol.* **184**, 677–701.
Walker, J. E., Runswick, M. J., and Poulter, L. (1987). *J. Mol. Biol.* **197**, 89–100.
Wang, J. H. (1984). *Biochemistry* **23**, 6350–6354.
Wang, J. H. (1985). *J. Biol. Chem.* **260**, 1374–1377.

Wang, J. H. (1986). *J. Bioenerg. Biomembr.* **18**, 101–111.
Wang, J. H., Cesana, J., and Wu, J. C. (1987). *J. Biol. Chem.* **26**, 5527–5533.
Williams, N., and Coleman, P. (1982). *J. Biol. Chem.* **263**, 5569–5573.
Williams, N., Hullihen, J. M., and Pedersen, P. L. (1984). *Biochemistry* **23**, 780–785.
Williams, N., Hullihen, J. M., and Pedersen, P. L. (1987). *Biochemistry* **26**, 162–169.
Wu, J. C., Chuan, H., and Wang, J. H. (1987). *J. Biol. Chem.* **262**, 5145–5150.
Yohda, M., and Yoshida, M. (1987). *J. Biochem.* **102**, 875–883.
Yoshida, M., Sone, N., Hirata, H., and Kagawa, Y. (1977). *J. Biol. Chem.* **252**, 3480–3485.
Zurawski, G., Bottomley, W., and Whitfeld, P. R. (1982). *Proc. Natl. Acad. Sci. USA* **79**, 6260–6264.