The Energy Transmission in ATP Synthase: From the γ-c Rotor to the α3β3 Oligomer Fixed by OSCP-b Stator via the βDELSEED Sequence

Yasuo Kagawa[1] and Toshiro Hamamoto[1]

ATP synthase (F_0F_1) is driven by an electrochemical potential of H^+ ($\Delta\mu H^+$). F_0F_1 is composed of an ion-conducting portion (F_0) and a catalytic portion (F_1). The subunit composition of F_1 is $\alpha_3\beta_3\gamma\delta\epsilon$. The active $\alpha_3\beta_3$ oligomer, characterized by X-ray crystallography, has been obtained only from thermophilic F_1 (TF_1). We proposed in 1984 that ATP is released from the catalytic site (C site) by a conformational change induced by the βDELSEED sequence via $\gamma\delta\epsilon$-F_0. In fact, cross-linking of βDELSEED to γ stopped the ATP-driven rotation of γ in the center of $\alpha_3\beta_3$. The torque of the rotation is estimated to be 420 pN·Å from the $\Delta\mu H^+$ and H^+-current through F_0F_1. The angular velocity (ω) of γ is the rate-limiting step, because $\Delta\mu H^+$ increased the V_{max} of H^+ current through F_0, but not the $K_{m(ATP)}$. The rotational unit of F_0 (= ab_2c_{10}) is π/5, while that in $\alpha_3\beta_3$ is 2π/3. This difference is overcome by an analog-digital conversion via elasticity around βDELSEED with a threshold to release ATP. The αβ distance at the C site is about 9.6 Å (2,8-diN_3-ATP), and tight Mg-ATP binding in $\alpha_3\beta_3\gamma$ was shown by ESR. The rotational relaxation of TF_1 is too rapid (φ = 100 nsec), but the rate of AT(D)P-induced conformational change of $\alpha_3\beta_3$ measured with a synchrotron is close to ω. The ATP bound between the P-loop and βE188 is released by the shift of βDELSEED from γRGL. Considering the viscosity resistance and inertia of the free rotor (γ-c), there may be a stator containing OSCP (=δ of TF_1) and F_0-d to hold free rotation of $\alpha_3\beta_3$.

KEY WORDS: ATP synthase; biomotor; $\alpha_3\beta_3$ oligomer, DELSEED sequence; torque; rotor; stator; elasticity of protein; analog-to-digital conversion; OSCP.

INTRODUCTION

ATP synthase (F_0F_1)[2] is an ion-motive membrane ATPase that catalyzes oxidative phosphorylation (Mitchell, 1979; Kagawa, 1972). F_0F_1 is a multi-subunit complex composed of F_1 and F_0 (Fig. 1). By electron microscopy, F_0F_1 was seen as a sphere (diameter 90 Å, $\alpha_3\beta_3$ of F_1) connected by a stalk (length 45 Å) to a basal piece (Kagawa and Racker, 1966; Kagawa, 1972, 1978; Capaldi, 1994). F_0F_1 uses the electrochemical potential difference of protons ($\Delta\mu H^+$) across the membrane generated by the redox chain to

synthesize ATP (Mitchell, 1979; Skulachev, 1988). In fact, a proton current mediated by F_0F_1 generating the $\Delta\mu H^+$ has been demonstrated in both liposomes (Kagawa, 1972) and planar lipid bilayers (Muneyuki

[1] Department of Biochemistry, Jichi Medical School, Tochigi-ken, 329-04, Japan.
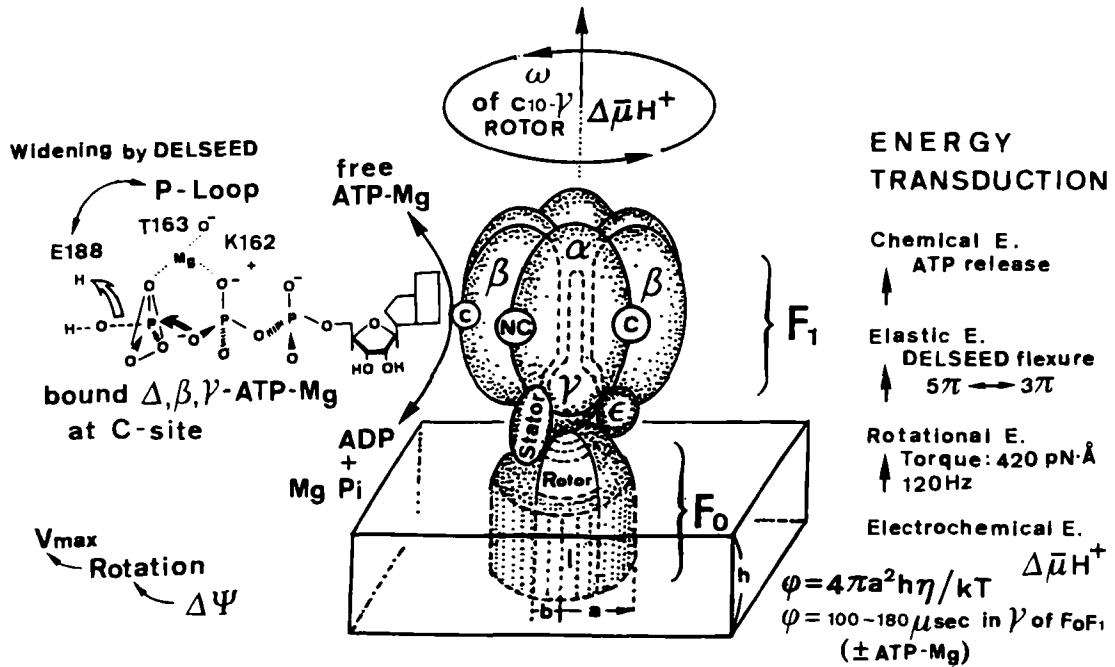
Fig. 1. A hypothetical model of physico-chemical energy conversion in ATP synthase. Center: Imaginary structure of F_0F_1 based on electronmicrography. C: catalytic site, NC: noncatalytic site, I: ion-binding site in the c subunit of F_0. Rotor ($=\gamma$-c_{10}): a wheel-shaped decamer of the c subunit which rotates with γ. Stator: a structure that fixes $\alpha_3\beta_3$ to F_0-a which is composed of F_0-b, OSCP (δ of TF_1), and F_6. Left: chemical aspects of the formation of free ATP-Mg. The in-line nucleophilic attack of ADP-oxygen on the pentavalent phosphate intermediate forming the Δ,β,γ-bidentate ATP-Mg complex has been proposed (Kagawa, 1984). The release of ATP from the C-site is the energy-requiring step in the ATP synthesis, because of the tight binding of Δ,β,γ ATP-Mg at the P-loop (T163, K162, etc.) and E188. ATP-Mg is released when the distance between E188 and the P-loop is widened by DELSEED. $\Delta\psi$: the external voltage applied to F_0F_1, which controls the V_{max} (rotation) but not the K_m for ATP. Right: physical aspects of the energy-transduction steps. The rotation of the axis of γ is 120 Hz with torque of 420 pN. The rotational energy (torque $\times$ rotation) generated by the proton current driven by $\Delta\mu H^+$ is transiently stored as an elastic energy of conformation around βDELSEED. When the threshold of the flexibility is reached at about $10\pi/3$ rotations, the distance of P-loop and E188 is widened via the conformation change to release ATP-Mg. ϕ: the rotational relaxation time of F_0 (radius $= a$) in a membrane (thickness $= h = 45$ Å, viscosity $= \eta = 0.2$ Pa sec). k: Botlzman constant (1.38×10^{-23} J·K^{-1}), T: absolute temperature (K), b: radius of a hypothetical rotor of c_{10} containing 20 α-helices.

et al., 1989; Hirata et al., 1986). The F_1 of Na$^+$-translocating F_0F_1 of *Propionigenium modestum* (Amann et al., 1988) can be reconstituted with H$^+$-translocating *E. coli* F_0 (EF$_0$) into H$^+$-translocating F_0F_1 and vice versa. Thus, ion selectivity depends on F_0, and F_1 and F_0 are functionally independent. F_1 (ATP $\leftrightarrow$ displacement) is analogous to myosin (a linear motor), while F_0 ($\Delta\mu H^+ \leftrightarrow$ rotation) is analogous to flagellar motor. In fact, the catalytic site (C site) in the $\alpha_3\beta_3$ oligomer has a structure (P-loop) common to myosin (Rayment et al., 1993), and the force (2 pN) of the single linear motor molecule is directly measured (Yanagida and Ishijima, 1995). The ion binding site (I site) of F_0 is the c subunit (D61 in EF$_0$), while that of flagellar motor has also been studied in detail (Berg, 1995).

The coupling device between the C and I sites was proposed to be a conformational change of the C site induced by the βDELSEED (single-letter code for amino acids) sequence via F_0-$\gamma\delta\epsilon$ (Kagawa, 1984). On the other hand, the binding change mechanism proposed by Boyer's group (Kayalar et al., 1977; see the review by Boyer, 1993) led to hypotheses for rotation of γ during ATP synthesis (Kandpal and Boyer, 1987; Oosawa and Hayashi, 1986; and several others) or F_0-b (Cox et al., 1984). Data on the structure of F_1 (Bianchet et al., 1991; Kagawa et al., 1992) and F_0F_1 (Capaldi, 1994) have been too preliminary to test these hypotheses. But with more detailed X-ray crystallographic data on mitochondrial F_1 (MF$_1$) (Abrahams et al., 1994) and TF$_1$ (Shirakihara et al.,

1995) together with those on the cross-linking of γ-βDELSEED in *E. coli* F_1 (EF$_1$) (Duncan *et al.*, 1995; Aggeler *et al.*, 1995), the hypothesis has become more plausible.

SPECIAL COMMENTS ON F_0F_1's AND THEIR RESIDUE NUMBERS

Unless specified, the residue numbers used here are those of bovine MF$_1$ (Abrahams *et al.*, 1994), which are identical to those of human MF$_1$ (Kagawa *et al.*, 1992; Akiyama *et al.*, 1995). They are expressed with single-letter code for amino acids, like Y345, and its localization in the β subunit is expressed as βY345. However, neither mutagenesis of MF$_0$F$_1$ or reconstitution from isolated subunits of MF$_1$ is possible. The active $\alpha_3\beta_3$ oligomer and the $\alpha_1\beta_1$ protomer are the fundamental structures of F_1 ($=\alpha_3\beta_3\gamma\delta\epsilon$), but they have been obtained only from TF$_1$ (Kagawa *et al.*, 1989, 1992). The mutagenesis data are mainly based on EF$_1$ and EF$_0$ (Futai *et al.*, 1995; Senior, 1990). Many physical measurements (X-ray crystallography, ESR, synchrotron radiation, etc.) of isolated α and β, including those on nucleotide binding, are possible only in TF$_1$. Thus, in deducing the common mechanism of ATP synthesis in F_0F_1, the data in Table I are combined.

In MF$_1$, δ and ε of prokaryotic F_1's, such as TF$_1$ and EF$_1$ correspond to oligomycin sensitivity-conferring protein (OSCP) and δ, respectively. Prokaryotic F_0 contains three basic subunits (= ab$_2$c$_{10}$), but mitochondrial F_0 (MF$_0$) contains d, e, f, g, OSCP, F$_6$, and A6L in addition to subunits to a, b, and c (Collinson *et al.*, 1994a,b). The stalk contains minor subunits γδεb$_2$ in TF$_0$F$_1$, and *E. coli* F$_0$F$_1$ (EF$_0$F$_1$). But in MF$_0$F$_1$, the stalk contains OSCP, b, γδε, F$_6$, and d, and the locations of e, f, and g are not clear (Collinson *et al.*,

1994a,b). There have been many reports on MF$_0$F$_1$, but very few reports on its reconstitution with pure proteins, such as OSCP and F$_6$ to functional liposomes capable of ATP synthesis (Kagawa and Racker, 1971; Kagawa, 1972).

SYNTHESIS OF ATP TIGHTLY BOUND TO THE P-LOOP WITHOUT A $\Delta\mu H^+$

Isolated α and β subunits both bind AT(D)P (Kagawa, 1978, 1984). Thus, X-ray crystallography confirmed the six AT(D)P binding sites in F_1 (Abrahams *et al.*, 1994) and the $\alpha_3\beta_3$ oligomer (Shirakihara *et al.*, 1995). The binding constant of ATP bound to MF$_1$ is 10^{12} M^{-1} (Penefsky, 1985), which is enough to synthesize ATP from ADP and Pi without external energy. However, the roles of the the noncatalytic (NC) sites are unknown. In contrast to MF$_1$ and EF$_1$, NC sites of purified TF$_1$ are vacant. When the ADP-TF$_1$ (1:1) complex is incubated with Pi and Mg, ATP in a TF$_1$-bound form (ATP:ADP:TF$_1$ = 0.55:0.45:1) is synthesized (Yohda *et al.*, 1986). The energy of $\Delta\mu H^+$ transformed at F$_0$ is used for the extrusion of ATP bound at the C-site (Fig. 1). The C and NC sites of ATP synthase are located at three αβ interfaces, because α and β can both be cross-linked with 2,8-diN$_3$-ATP (α and β, 9.6 Å apart), and both αβ dimer and trimers are formed (Schäfer *et al.*, 1995). Both sites contain nucleotide binding motif A called the P-loop (-GXXXXGKT/S-) and motif B (-LVVID-). The P-loop is located at β156–163 and α169–176 as shown by X-ray crystallography (Abrahams *et al.*, 1994). K in the P-loop bins to the γP of ATP, and its site-directed mutagenesis into I resulted in the loss of both nucleotide binding and catalysts (Yohda *et al.*, 1988). T in the P-loop binds Mg^{++} (Fig. 1, left), and the spin label (SL) ESR spectra of both MF$_1$ and TF$_1$ revealed

Table I. Available Data on F_1's from Different Sources

X-ray crystallographic data	Active component	Site-directed mutagenesis	Electrophysiology	Minor subunits Rotor:Stator
MF$_1$ $\alpha_3\beta_3\gamma^a$	None	None	Some	γδF$_0$-c:OSCP, F$_0$-b,d,F$_6$
TF$_1$ $\alpha_3\beta_3{}^b$	α,β, $\alpha_3\beta_3$, etc.	Some	Many	γε:δ, F$_0$-b (dimer)
EF$_1$ None	Only $\alpha_3\beta_3\gamma$	Many	None	γε:δ, F$_0$-b (dimer)

^{a} The model of 2.8 Å resolution contains 2,983 amino acids in the following sequences: $\alpha_{DP19–510}$, $\alpha_{TP25–510}$, $\alpha_{E24–510}$; 9–474 in β_{DP}, β_{TP} and β_E; $\gamma_{1–45}$, $\gamma_{73–90}$, and $\gamma_{209–272}$ (Abrahams *et al.*, 1994).
^{b} Shirakihara *et al.*, 1995.

the role of Mg in tightening at the P-loop (Burgard et al., 1994). βY345 is the binding site for 2-N_3-ATP, 2-N_3-ADP, and Bz-ATP. The SL ESR spectra of 2-N_3-SL-AT(D)P-TF$_1$ and 2-N_3-SL-AMPPNP-TF$_1$ also show two different environments of the spin labels [2Azz: 68G = NC site, and 55G = C site]. The isolated β-subunit-SL-ATP gave only a C site signal, which was depressed by the addition of GTP. In fact, the C site has a wide hydrophobic base (A,G,I, etc.)-binding environment, whereas the NC site has several hydrogen bonds (αQ432, etc.), which makes it specific for AT(D)P binding (Abrahams et al., 1994). The 2Azz-value of the C site increases from 49G in $\alpha_3\beta_3$ to 53G in $\alpha_3\beta_3\gamma$, and 55G in TF$_1$ due to higher immobilization of SL (Burgard et al., 1995). This clearly indicates the tight binding of ATP at the C site when γ is added to the $\alpha_3\beta_3$.

An in-line mechanism of the pentavalent phosphate intermediate during hydrolysis of the Δ,β,γ ATP-Mg bidentate complex at the C site has been proposed (Kagawa, 1984) from studies using [$^{16}O,^{17}O,^{18}O,^{35}S$] thiophosphate and Cd-ATPγP (Senter et al., 1983) (Fig. 1, left). A water molecule near the γP of ATP is hydrogen-bonded to the carboxylate of βE188 in the C site, while in the NC site there is no water molecule in the spatial equivalent of αQ208 (Fig. 1, left). The structure of the P-loop is hardly changed by the addition of AT(D)P, but its distance from βE188 is widened when ATP is released. The P-loop is also conserved in the A subunit of V-type ATPases (Steinert et al., 1995) and many nucleotide binding proteins such as myosin (Rayment et al., 1993).

THE SYMMETRICAL $\alpha_3\beta_3$ OLIGOMER IS FUNCTIONALLY ASYMMETRIC

X-Ray crystallographic analysis of the $\alpha_3\beta_3$ oligomer revealed three equivalent C sites in the absence of nucleotide (Shirakihara et al., 1995: cubic, $a = b = c = 160$ Å, 3.2 Å resolution). However, in the presence of ATP, the C-sites become cooperative and nonequivalent. According to the binding change theory of Boyer (1993), owing to negative cooperativity between the C sites, the first ATP binds very tightly ($K_d = 10^{-12}$ M), and the last one hardly at all. The three conformationally different C sites are called tight, loose, and open. Moreover, owing to the positive catalytic cooperativity between the C-sites of the $\alpha_3\beta_3$ oligomer, the rate constant of ATPase activity of the oligomer is 10^6 times higher at high substrate concentration (multi-

site activity) than that at low (ATP/F$_1$ < 1) substrate concentration (uni-site activity). The activity of the isolated $\alpha_1\beta_1$ protomer is higher than the uni-site activity with a K_m value of 70 μM, and is kinetically not cooperative (Saika and Yoshida, 1995). Moreover, the $\alpha_3\beta_3$ oligomer was inhibited by only one mole of [^{3}H]-Bz-ADP per oligomer, like both MF$_1$ and TF$_1$ (Aloise et al., 1991; Kagawa et al. 1992). To inhibit the remaining protomer activity completely, one mole of inhibitor was needed per mole of $\alpha_1\beta_1$ protomer. The ATP-dependent reassociation of the $\alpha_3\beta_3$ oligomer and its ADP-dependent dissociation into the $\alpha_1\beta_1$ protomer have been analyzed by synchrotron radiation (Harada et al., 1991; Kagawa et al. 1992; Sato et al., 1995). There are many mutations in F$_0$F$_1$ which affect the conformational cross-talk betweeen liganded NC sites and C sites (Futai et al., 1995; Senior, 1990; Jault et al., 1995). These findings also indicate the conformational change induced by the nucleotide binding.

The inherent asymmetry of the F$_1$ structure is caused by the addition of γ to the $\alpha_3\beta_3$ oligomer (Kagawa, 1984; Abrahams et al., 1994). X-ray crystallography of MF$_1$ in the presence of ligands (AMP-PNP:ADP:Pi = 50:1:0) revealed that there are three different conformations of β's; AMPPNP-loaded β_{ATP}, ADP-loaded β_{ADP} and empty β_E, which may correspond to loose, tight, and open states, respectively. These βs have different modes of interaction with the central α-helical coiled-coil structure in the γ subunit (Abrahams et al., 1994). In fact, the asymmetry of the $\alpha_3\beta_3\gamma$ caused by the γ subunit generates a high-affinity C site (Kaibara et al., 1996). When structural asymmetries were introduced by adding the three minor subunits, the resulting $\alpha_3\beta_3\gamma$, $\alpha_3\beta_3\gamma\delta$, $\alpha_3\beta_3\gamma\epsilon$, $\alpha_3\beta_3\delta$, and $\alpha_3\beta_3\gamma\delta\epsilon$ complexes showed characteristic inhibitor sensitivity (Paik et al., 1993).

THE V_{max} NOT THE K_m $_{ATP}$, OF H$^+$-CURRENT OF F$_0$F$_1$ IS AFFECTED BY $\Delta\mu$H$^+$

The study of H$^+$-translocation requires strict control of the components of $\Delta\mu$H$^+$, which are the pH gradient and the voltage ($\Delta\Psi$) across the membrane (Mitchell, 1979; Skulachev, 1988). In this respect, a planar phospholipid bilayer plugged through by F$_0$F$_1$ is the most suitable system, since both sides of the membrane are accessible and controllable (Muneyuki et al., 1989). Passive transport of H$^+$ was demonstrated when the F$_0$ portion alone was incorporated into lipid bilayers. Active transport of H$^+$ by F$_0$F$_1$ upon addition

of ATP-Mg resulted in a steady state H^+-current that showed simple Michaelis–Menten type kinetics, and a $K_{m(ATP)}$ of 140 μM. This value was close to the $K_{m(ATP)}$'s for the ATPases of TF_1 and TF_0F_1 in the steady-state multi-site catalytic cycle, indicating that proton translocation is coupled to the steady-state ATPase reaction. In contrast to the voltage-dependent V_{max} of the H^+-current, the K_m showed no apparent dependence on the membrane voltage. In the experimental conditions, an applied voltage of −30 mV should have increased the K_m value to 600 μM (Muneyuki et al., 1989). This indicates that the rate-limiting step of the ATP release from P-loop-$\beta E188$ is the angular velocity of the rotation (V_{max}) by $\Delta\mu H^+$, not the torque applied to reduce the affinity of the P-loop to bind ATP (K_d or K_m) (Fig. 1).

When $\Delta\mu H^+$ of −180 mV (negative on the F_1 side) was loaded, the current was entirely suppressed. Assuming that the standard free energy change of ATP hydrolysis is −30.5 kJ and [(ADP)(Pi)/(ATP)] is 10^{-4}, the free energy change (ΔG) of F_0F_1 was calculated to be −552 mV. Therefore the H^+/ATP stoichiometry is 3 (Hirata et al., 1986). Although the stoichiometry of 4 in photosynthetic F_0F_1 is also proposed by Van Walraven (1996), based on the established P/O ratio, the electron transport system should supply 25% more protons to F_0F_1. The torque of the rotation in F_0F_1 should be derived from this overall energetics.

Both the ATPase activity of F_0F_1 and the H^+-current through F_0 are inhibited by DCCD (dicyclohexylcarbodiimide) which binds to the I site (cE56 of TF_0, cD61 of EF_0). The conformational change of the inhibited F_0 decreased ATP binding to F_1 in the F_0F_1 (Penefsky, 1985).

THE DELSEED SEQUENCE IN β AS THE ENERGY TRANSMITTER

Conformational energy transmission from F_0 to F_1 was proposed to proceed from $\gamma\delta\epsilon$ to the $\alpha_3\beta_3$ oligomer via the βDELSEED sequence (Kagawa, 1984), because of the highly conserved sequence among F_1's, β-γ cross-linking, and a stalk containing $\gamma\delta\epsilon$ connecting the $\alpha_3\beta_3$ oligomer and F_0 (Kagawa, 1978). In fact, X-ray crystallographic analysis of MF_1 revealed a specific point of contact between γRGL and βDELSEED sequences of AMPPNP-loaded β_{ATP} (Abrahams et al., 1994) (Fig. 2). The sequence is conserved in the F_1s of 48 different species, and the very similar DELSDED sequence was found in β's from

TF_1 (Kagawa et al., 1986), Bacillus, and the Na^+-translocating F_0F_1 of Propionigenium modestum (Amann et al., 1988). Similar DE-rich sequences are also conserved in the A subunit of V-type ATPases (Steinert et al., 1995; Hamamoto and Kagawa, to be published). But neither types of sequences are found in any proteins other than the pumps. X-Ray crystallographic analysis of the $\alpha_3\beta_3$ oligomer without ATP revealed downward translocation of the DELSDED from the central axis (Shirakihara et al., 1995). The ATP-binding P-loop is located just above the DELSE(D)ED sequence, and the P-loop-$\beta E188$ distance is widened when the DELSE(D)ED is dislocated downwards from γ in β_E (Abrahams et al., 1994). The βD305 of DELSEED interacts with βG150 in the P-loop region which binds AT(D)P, because the frame shift mutation of the γ subunit was restored by the βG150D mutation (Jeanteur-De Beukelaer et al., 1995). It is also significant that X-ray crystallography of myosin revealed the 60 Å movement of the myosin head by the addition of ATP at the P-loop pocket (13 Å wide and 13 Å deep) and a base cluster interacts with acidic cluster like DELSEED (Rayment et al., 1993).

DELSEED is the site of binding for amphipathic cations, such as rhodamine 6G, which reversibly inhibit F_1. Additions of the minor subunits (γ, δ, and ε) of TF_1 to the $\alpha_3\beta_3$ oligomer revealed that the inhibitory effect of rhodamine 6G is affected by either γ or ε (Paik et al. 1993). This is in line with the results in the γε-rotation experiment of Aggeler et al. (1995). The SGSTGT sequence of ATP inhibitor is cross-linked to βD349 (D363 of yeast MF_1) which is close to βY345, the binding site for 2-N_3-AT(D)P, and DELSEED (Ichikawa et al., 1996). The bovine ATPase inhibitor contains a similar sequence, and the synthetic DELSEED-containing peptide also inhibited the ATPase activity of MF_1, perhaps by competing for the essential binding of βDELSEED to γ (Stout et al., 1993).

βDELSEED-γ CROSS-LINKING INHIBITED THE ROTATION OF $\alpha_3\beta_3$

There are several morphological (Capaldi, 1994) and chemical (Duncan et al., 1995; Aggeler et al., 1995) reports on the rotation of the $\alpha_3\beta_3$ oligomer around γε during ATP hydrolysis. These experiments are based on three different states of the β subunit (β_{ATP}, β_{ADP}, and β_E) and the position of γ at any given instant, and interconversion of the states is explained
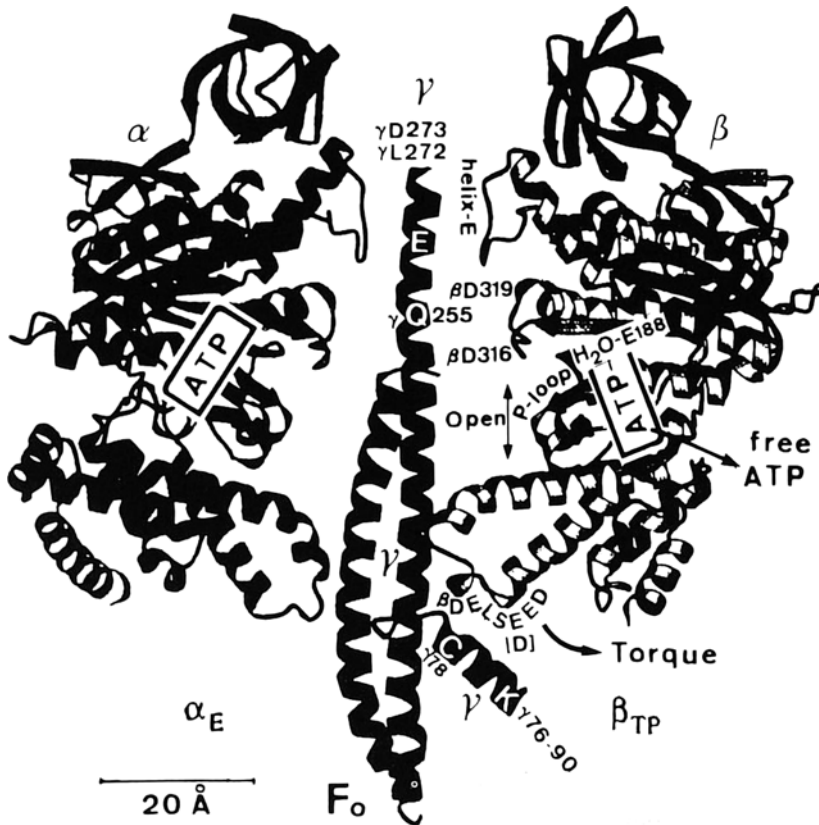
Fig. 2. The torque applied to the βDELSEED sequence increases the distance between P-loop and E188 to release ATP. The original data on X-ray crystallography of MF₁ (Abrahams *et al.*, 1995), and various physico-chemical data on TF₁. The ATP binding sites (P-loops in α and β) at the αβ interface are boxed. The C-site is mainly on β, and the NC-site is mainly on α. There are β-γ contacts between βD316–D319 (mainly in β_ADP) and γQ255, E262, and a loop next to helix-E, and the loop containing the βDELSEED (in βATP 394–400) sequence and RGLCG—K—K (γ 75–90). When the βDELSEED is moved downwards, the distance between P-loop and E188 is increased and ATP is released. The covalent radii in ATP and amino acid residues are of the order of Å (P=1.10 Å, O=0.60 Å, and H = 0.30 Å).

by rotation of the $\alpha_3\beta_3$ oligomer (Abrahams *et al.*, 1994). The following set of mutants of EF_0F_1 (residue numbers are for EF_0F_1) has been generated by site-directed mutagenesis in the βDELSEED (#380–386) sequence and ε: βE381C, βS383C, βE381/εS108C, and βS383C/εS108C (Aggeler *et al.*, 1995). Treatments of EF_1 and EF_0F_1 isolated from any of these mutants with $CuCl_2$ induces disulfide (S–S) bond formation and loss of ATPase activity. In the single mutants, βE381C and βS383C, a β-γ S–S bond is formed at γC87 of EF_1 (γC78 of MF₁). γC78 is not conserved in TF₁ (γA89) and not essential (Ohta *et al.*, 1988). In the double mutants, β-γC87 and β-εC108 S–S bonds are formed. The yield of β-γ S–S bonds is highest in ATP, that of β-ε S–S bonds, in ADP. A βD380C mutation also resulted in βC380-γC87 S–S

bond formation and inactivation of EF_1 (Duncan *et al.*, 1995) (Fig. 2). Using a dissociation/reassembly approach with cross-linked βC380-γC87, radiolabeled β was incorporated into the two β positions. After reduction of the initial nonradioactive β-γ S–S cross-link, only exposure to conditions for catalytic turnover of ATP resulted in similar reactivities of unlabeled and radiolabeled β with γC87 upon reoxidation. These findings demonstrate that γ rotates relative to β during the ATPase reaction (Duncan *et al.*, 1995).

TORQUE OF THE ROTOR: AN INTENSIVE VARIABLE OF ENERGY

As shown in Table II, energy is the product of an intensive variable and its extensive variable (electric

Table II. Torque Estimated from $\Delta\mu H^+$ and Overall Energy of ATP[a]

	c_{10}	γ-βDELSEED	$\alpha_3\beta_3$
Intensive variable	$\Delta\mu H^+$ = 180 mV	T = 420 pN·Å	ΔG = 550 mV
Extensive variable	Transported H^+ = 3	ωt = 3/2π	Moles ATP = 1

[a] ω is the angular velocity of the rotor; t is time (sec).

energy = voltage × quantity of electricity; electrochemical energy = $\Delta\mu H^+$ × g-ion H^+ transported, etc.). From the law of conservation of energy, and the loss of energy during the conversion, the energy of rotation in F_0F_1 can be estimated. The rotation energy of the rotor (γ-c_{10}) should be of the same order of magnitude as the free energy of ATP synthesis ($\Delta G^{o'}$ = −30.5 kJ) and proton movement (3H^+/ATP), because the efficiency of oxidative phosphorylation is, for example, about 73% at the cytochrome bc_1 segment. The mechanical energy (force × displacement = 1 N × Å = 10^{-10} J = 100 pN·Å) required to synthesize ATP is expressed as pN·Å per mole, irrespective of the mechanism involved. Electrochemical energy is expressed in eV (1 eV = 1.6 × 10^{-19} J = 1.6 × 10^3 pN·Å; 1 ΔpH = 60 mV at room temperature).

The torque (T) to rotate the α-helical coiled-coil axis of γ in the $\alpha_3\beta_3$ oligomer during the release of ATP at the C site is estimated as shown below: From H^+-current measurement (Hirata *et al.*, 1986), the energy of one proton is 180 meV, and that of ATP is 550 mV per mole. Considering the reversibility of the reaction, the torque generated at c_{10} around γ is as high as 880 pN·Å × 3/2π = 420 pN·Å.

Is this torque comparable to that in a flagellar motor? If γ rotates in the water phase like a flagellar motor, T in the F_0F_1 is given by Eq. (1):

$$T = J(d\omega/dt) + b\eta\omega \qquad (1)$$

where $b\eta$ is the viscosity resistance coefficient and J is the moment of inertia. Assuming ω = 0 at t = 0, Eq. (2) is obtained from Eq. (1):

$$\omega = T/b\eta \, (1 - \exp[-b\eta t/J]) \qquad (2)$$

Thus the torque of the flagellar motor in the stationary state is $T = b\eta\omega$. A flagellar motor with three pitches of the flagella (J = 2 × 10^{-31} [kg·m²], and $b\eta$ = 2 × 10^{-21} [N·ms]) rotating at 100 Hz gives a torque T = 2 × 10^{-19} N·m = 200 pN·Å. In the flagellar motor, the diameter of S- and M-ring is 225 Å. Then the force at Mot A is of the order of only 2 pN. Similarly, in actin-myosin contraction (a linear motor), operating at highest efficiency, direct force measure-

ment indicated about 2 pN (load) per myosin head, and displacement of the actin filament of about 300 Å (Yanagida *et al.*, 1995). Thus, from both biomotors, a torque of 420 pN·Å of F_0F_1 is reasonable.

However, we cannot apply the analogy of biomotors to F_0F_1. In the case of F_0F_1, if the torque is used to drive against the viscosity, the energy is dissipated as heat, and hence ATP is not synthesized. Moreover, J is much smaller than that of a flagella. Thus, torque should be transferred directly from γ to the βDELSEED. In addition to the inertial term and viscosity term, there should be a conformational term proportional to the angular velocity ($F\omega$):

$$\frac{d\,(J\omega^2)}{dt} = T\omega - b\eta\omega^2 - F\omega \qquad (3)$$

But the torque T is concentrated at smaller contact points in F_0F_1 than in the myosin head (Rayment *et al.*, 1993) or flagellar motor (Berg, 1995). There are three points of contact between the γ axis and β: two in the C-terminal α-helix and one at γRGL (Fig. 2). Although C-terminal D273 of γ is muscle specific and may have a physiological function (Matsuda *et al.*, 1993), it is too slender to apply a torque of 420 pN·Å. The torsional rigidity is related to Young's modulus E in the form

$$\kappa = EJ/(1+\delta) \qquad (4)$$

where J is the moment of inertia, δ is the Poisson's ratio (about 0.5 in rod). J is given by $\pi r^4/4$, where r is the radius of the rod. A force of 168 pN (420/2.5) will cause a strong torsion to an α-helix (with a radius of 2.5 Å), which is about 114-fold stronger than the force generated at Mot A and the actin filament. For instance, the tension to break immunobiotin is only 85 ± 15 pN (Florin *et al.* 1994). In fact, the removal of 10 residues from the γC-terminal does not affect the activity (Futai *et al.*, 1995). Thus, the conserved basic sequence γ75RGLCG—K87—K90 interacting with the acidic βDELSEED sequence, 20 Å from the axis, is a possible major point of action in transmitting the torque that deforms C sites. γM23 may also contribute to the torque transfer to βDELSEED, because

its mutation into R or K decreased the H^+-transport without decreasing ATPase (Nakamoto et al., 1993). In addition to γ75RGLCG, the conserved γQ255 (γQ269 of EF1) may transfer some torque via βD316 and β318 (Fig. 2), because its mutation to L also decreased the H^+-transport without affecting ATPase (Futai et al., 1995).

ANALOG-TO-DIGITAL CONVERSION VIA ELASTICITY AROUND βDELSEED

Torque is an analog quantity, while moles ATP synthesized is a digital quantity. Thus, F_0F_1 is an analog-to-digital converter, not a rigid gear. According to the scheme of Vik and Antonio (1994), there is a wheel-shaped oligomer (c_{10}) composed of 10 c subunits in F_0, yet $\alpha_3\beta_3$ is three-fold symmetric. If the rotor composed of (γ-c_{10}) rotates relative to $\alpha_3\beta_3$ during ATP synthesis, one H^+ can be translocated per $\pi/5$ rotation, while ATP is released per $2\pi/3$ rotation. It is obvious that DELSEED of β_{ATP} is hit by γ different rotational angles during the cycle of 30 rotations. Loose coupling in F_0F_1 proposed by Oosawa and Hayashi (1986) may overcome this analog-digital difficulty by some black box mechanism. This inconsistent stoichiometry must involve an elastic energy around βDELSEED to store transiently the analog quantity (ω) until the strain reaches a threshold (corresponding to about $3H^+$ translocation) to release an ATP molecule (digital quantity). Since the definition of conformational energy is vague, the idea of elastic energy of a reversibly deformed flexible domain is used here (Fig. 1, right). In fact, the direct measurement of the elasticity of single actin filament by nanomanupulation revealed that 50% of the compliance is due to extensibility (elasticity) of the thin filament (Kojima et al., 1994). In sarcomere, there is a highly elastic titin (Maruyama, 1994).

The rotation of c_{10} (wheel of 20 α-helixes, the radius may be 15–25 Å) is driven by the H^+-current supplied from the a subunit of F_0. Two cD61s of EF_0 (cE56 of TF_0) simultaneously interact with aE219 and aR210 of EF_0. Although aE219 is not conserved in MF_1 (H), the flow of H^+ from aR210 to cD61 will rotate c_{10}. Reconstitution of an active F_0 from the a, b, and c subunits was prevented by addition of as little as 0.05–0.2 of a cD61N or cD61G mutant of the c subunit of EF_0 per wild type c subunit (Dmitriev et al., 1995). This is very different from results in a reconstitution experiment on a flagellar motor, showing stepwise increase of torque on expressing the MotB

gene in E. coli lacking MotB (Berg, 1995). Cross-linking of ϵE31C and cQ42C of EF_1 via an S–S bond leads to inhibition of ATPase coupled H^+ transport (Zhang and Fillingame, 1995). Thus, protonation/deprotonation of the conserved cD61 causes conformational change of the polar loop of the same subunit, and the change is transferred to this subunit via direct interaction of EF_1 (Zhang and Fillingame, 1995).

ANGULAR VELOCITY AND THERMAL FLUCTUATION

The velocity of rotation of γ is 120 Hz, estimated from the V_{max} of ATPase activity. The rotation of flagellar motors is proportional to the $\Delta\mu H^+$ applied (DC motor). Likewise, the voltage applied to F_0F_1 proportionally increases the V_{max} (Muneyuki et al., 1989), and hence the angular velocity is the rate-limiting step. If F_1 is removed from F_0F_1, the load to the torque generated in F_0-γ portion is lost. F_0 alone shows 1 pS = 2×10^5 H^+ per sec (Groth and Junge, 1995) per ten c subunits in F_0. Thus, the value is 2×10^4 Hz, which is extremely high, compared with 100 Hz for F_0F_1. A rotation of 10^3 Hz is possible in a flagellar motor (Muramoto et al., 1995). There are two important differences between the flagellar motor and F_0F_1: (1) F_0F_1 allows no proton current even without the load (ADP + Mg + Pi) (Muneyuki et al., 1989). (2) The proton current is reversed in F_0F_1, while a "Brownian rachet" mechanism blocks a reverse rotation with the reverse proton flux (Berg, 1995).

Of course, the kinetic rotational energy of F_1 ($= J\omega^2$) is too small to synthesize ATP ($= 7$ kT), and the thermal fluctuation is of the order of kT. Therefore we must calculate the rotational relaxation time (ϕ) of the protein. TF_1 in water phase showed $\phi = \eta V/kT = 40$–150 nsec, where V is the volume of TF_1 (Kinoshita et al., 1982). Thus, the resistance of F_1 against viscosity of water ($\eta = 10^{-3}$ Pa·sec) is very small. The value of ϕ is much larger in F_0F_1 in a liposome: $\phi = 4\pi a^2 h\eta/kT = 100$–180 μsec, where a is the radius of F_0, h is the thickness of the membrane (45 Å), and η is the viscosity of the membrane lipid ($\eta = 0.2$ Pa·sec) (Musier-Forsyth and Hammes, 1990). This rapid rotation (ϕ) of γ specifically labeled with erythrosin isothiocyanate in active F_0F_1 in liposome was unaffected by ATP or ADP + Pi, and ΔpH (Musier-Forsyth and Hammes, 1990). This kind of thermal fluctuation is too rapid to explain the ATP-driven rotation, and does not exclude the possibility

that slower rotations of γ than measured by them are important for the function. On the other hand, a slow rate of ATP-dependent association of the $\alpha_3\beta_3$ oligomer has been established by synchrotron radiation (Harada *et al.*, 1991; Kagawa *et al.*, 1992; Sato *et al.*, 1995). In the absence of load, γ in the isolated F_1 rotates freely, unless γ is cross-linked to DELSEED, and dissipate energy during ATPase reaction. F_0F_1 is a reversible motor, yet in the absence of a load, why does F_0F_1 not allow proton translocation? In TF_0F_1, addition of the $\gamma\delta\varepsilon$ complex to F_0 can block the loadless proton flux (Kagawa, 1978). Moreover, in order to prevent futile ATP hydrolysis by F_0F_1, ATPase inhibitor, a DELSEED homolog (Staut *et al.*, 1993; Ichikawa *et al.*, 1996), blocks the rotation. Even in the absence of the inhibitor, ADP converts F_1 into an inhibited state, and the proton current through F_0F_1 is inhibited (Muneyuki *et al.*, 1989).

CANDIDATES FOR A STATOR: F_0-b δ, OSCP, AND F_6

Since the viscosities of water and lipid are very low, the torque applied to the $\alpha_3\beta_3$ complex from γ-c_{10} (the rotor; see Vik and Antonio, 1994) will rotate the $\alpha_3\beta_3$-γ-c_{10} complex together but will not effectively cause the conformational change needed to release ATP. The rotor of a motor must be attached to the membrane somewhere, or else the torque that it can generate cannot be applied. Thus, a stator of the motor to block futile rotation to dissipate energy by the viscosity resistance is needed. Minor subunits which are peripheral proteins not cross-linked to the rotor, and which connect the $\alpha_3\beta_3$ complex to the a subunit of F_0, are candidates for component of the stator. In F_1, part of the stator might be δ of TF_1 (OSCP of MF_1), because it can form a $\alpha_3\beta_3\delta$ complex (Kagawa, 1984) and bind to F_0 in the absence of γ. Both OSCP and F_6 can be removed by trypsin (Racker's T-particles) and are essential for [32P]-ATP synthesis in reconstituted MF_0F_1-liposomes (Kagawa and Racker, 1971). They were shown to be the only components that form a stable binary component, and the quaternary stoichiometric complexes F_1-OSCP-b'-F_6 and F_1-OSCP-bI-F_6 (Collinson *et al.*, 1994a). Chemical cross-linking revealed that the minor subunits that are bound to α/β but not to γ are F_6, F_0-b, F_0-d and OSCP (Belogrudov *et al.*, 1995). Since F_0-d and F_6 are not found in TF_1 or EF_0, the universal components of the stator may be F_0-b and OSCP. F_0-b, to which these

minor subunits are cross-linked (Belogrudov *et al.*, 1995), has a long hydrophilic α-helix (Dunn, 1992) extending from F_0-A6L and binds to α/β. In EF_0, F_0-b is a dimer that also connects F_0-a and F_1 (Dunn, 1992). Since δ of MF_1 is equivalent to ε of TF_1 and not cross-linked to these minor subunits, it may be a part of the rotor. The rotation of F_0-b in F_0F_1 was proposed by Cox *et al.* (1984), but the evidence is still not solid. If the stator fixes the $\alpha_3\beta_3$ complex to F_0-a, both the first term (inertial of rotor = γ-c_{10}) and second term (water viscosity resistance of only the rotor) of Eq. (3) will become very small. Thus, the energy loss is minimized during the rotational energy transmission to the elastic deformation of the DELSEED domain.

CONCLUSIONS

There is no *a priori* reason to believe the occurrence of intramolecular rotation of F_0F_1, because there are many pumps like Ca-ATPase that have no rotating structure. The rotational energy ($= J\omega^2$) of F_1 is too small to synthesize ATP, and the viscosity resistance of the $\alpha_3\beta_3$ oligomer should dissipate energy. But recent X-ray crystallographic analyses of MF_1 and TF_1, the chemical cross-linking of βDELSEED to γ, and results on the kinetics of F_0F_1 suggest a rotation mechanism (Fig. 1). In contrast to flagellar motors, the rotation of γ does not rotate the $\alpha_3\beta_3$ oligomer, but transmits the torque to release ATP. The tight binding of ATP is favorable for the synthesis without external energy at the P-loop of β_{ATP}. The rapid ATP synthesis using catalytic cooperativity of F_1 is also important in oxidative phosphorylation. These two events may require an efficient ATP release cycle via rotation in F_0F_1.

1. *ATP to Conformation Energy.* Detailed X-ray analysis of crystals of $\alpha_3\beta_3$ and F_1 revealed intersubunit interaction and conformation change. The very tight binding of Δ, β, γ-ATP-Mg to P-loop-βE188 was estimated by kinetic and ESR studies. The $\alpha\beta$ interface was shown to be 9.6 Å by use of 2,8-diN$_3$-ATP. A conformational change was induced by the addition of nucleotides to these complexes. The cooperativity of the $\alpha_3\beta_3$ oligomer in F_0F_1 was established.

2. *Proton to Conformation Energy.* Although no X-ray crystallographic data on F_0 are available, a mutagenesis study revealed that 10 c subunits/F_0 are required for proton translocation. The electronics of reversible energy transduction between ATP and $\Delta\mu H^+$ (and the H^+ current) in F_0F_1 incorporated into a lipid

bilayer revealed $3H^+/ATP$ stoichiometry. Since the V_{max}, but not the $K_{m(ATP)}$, is affected by the external voltage applied, the rate-limiting step may be the rotational velocity (ω). The torque of γ-c_{10} rotation to induce an elastic deformation in a domain of F_1 will be 420 pN·Å.

3. *Coupling Between Torque and ATP Release.* Rotation and ATPase of $\alpha_3\beta_3$ is blocked by cross-linking of the βDELSEED to the γ axis. Considering the torsion of the α-helix of the C-terminal of γ, the βDELSEED sequence is the major site for energy transmission. It interacts with γRGL—K—K during the rotation. This converts the torque into a conformational change that opens the P-loop-βE188 to release bound ATP (Figs. 1 and 2). The structures of both DELSE(D)ED and the P-loop are also conserved in V-ATPase. The inconsistent stoichiometry (10 H^+ per rotation of c_{10}-γ in $\alpha_3\beta_3$ produce ATP per $3H^+$) must involve an elastic energy around βDELSEED to store the torque (analog quantity until the deformation reaches a threshold to release an ATP molecule (digital quantity).

4. *Stator Problem.* Considering the very low viscosity and small inertia of the rotor, there should be a stator in the motor. The subunits fixing $\alpha_3\beta_3$ to F_0-a during rotation of the rotor (γ-c_{10}) may be a complex of F_0-b, OSCP (δ of TF_1) and F_6 which have already been shown to be essential for ATP synthesis. The torque is applied to the fixed $\alpha_3\beta_3$, in which the energy is transiently stored as an elastic energy until ATP is released.

ACKNOWLEDGMENTS

NOTE ADDED IN PROOF

After this article was submitted, "real time (100ms) of the intersubunit rotation in active F_1-ATPase" has been measured by photobleaching of eosin-labled γ by D. Sabbert, S. Engelbrecht, and W. Junge (*Nature* **381**:623, 1996). Their result supports our conclusions. There are two reservations: i.e. direction of the rotation, and the partial dissociation of the $\alpha_3\beta_3$ induced by the ATPase reaction (Harada *et al.*, 1991).

REFERENCES

Abrahams, J. P., Leslie, A. G. W., Lutter, R, and Walker, J. E. (1994). *Nature* **370**, 621–628.

Aggeler, R., Haughton, M. A., and Capaldi, R. A. (1995). *J. Biol. Chem.* **270**, 9185–9191.

Akiyama, S., Matsuda, C., and Kagawa, Y. (1995). *Biochemistry of Cell Membranes* (Papa, S., and Taber, J. M., eds.), Birkhauser, Verlag, Basel, pp. 167–178.

Aloise, P. Kagawa, Y., and Coleman, P. S. (1991). *J. Biol. Chem.* **266**, 10368–10376.

Amman, R., Ludwig, W., Laubinger, W., Dimroth, P., and Schleifer, K. H. (1988). *FEMS Microbiol. Lett.* **56**, 253–260.

Belogrudov, G. I., Tomich, J. M., and Hateft, Y. (1995). *J. Biol. Chem.* **270**, 2053–2060.

Berg, H. C. (1995). *Biophys. J.* **68**,163s–167s.

Bianchet, M., Ysern, X., Hullihen, J., Pedersen, P. L., and Amzel, L. M. (1991). *J. Biol. Chem.* **266**, 21197–21201

Boyer P. D. (1993). *Biochim. Biophys. Acta* **1140**, 215–250.

Burgard, S., Nett, J. H., Sauer, H. E., Kagawa, Y., Schäfer, H-J., Wise, J. G., Vogel, P. D., and Trommer. W. E. (1994). *J. Biol. Chem.* **269**, 17815–17819.

Burgard, S., Kagawa, Y., Wise, J. G., Trommer, W. E., and Vogel, P. D. (1995). *Biophys. J.* **68**, A320.

Capaldi, R. A. (1994). *Trends Biochem. Sci.* **19**, 284–289.

Collinson, I. R. van Raaij, M. J., Runswick, M. J., Fearnley, I. M., Skehel, J. M., Orriss, G. L., Miroux, B., and Walker, J. E. (1994a). *J. Mol. Biol.* **242**, 408–421.

Collinson, I. R. Runswick, M. J., Buchanan, S. K., Fearnley, I. M., Skehel, J. M., van Raaij, M. J., Griffiths, D. A., and Walker, J. E. (1994b). *Biochemistry* **33**, 7971–7978.

Cox, G. B., Jans, D. A., Fimmel, A. L., Gibson, F., and Hatch, L. (1984). *Biochim. Biophys. Acta* **768**, 201–208.

Dmitriev, O. Y., Altendorf, K., and Fillingame, R. H. (1995). *Eur. J. Biochem.* **233**, 478–483.

Duncan, T. M., Bulygin, V. V., Zhou, Y., Hutcheon, M. L., and Cross, R. L. (1995). *Proc. Natl. Acad. Sci. USA* **82**, 10964–10968.

Dunn, S. D. (1992). *J. Biol. Chem.* **267**, 7630–7636.

Florin, E-L., Moy, V. T., and Gaub. H. E. (1994). *Science* **264**, 415–417.

Futai, M., Omote, H., and Maeda, M. (1995). *Biochem. Soc. Trans.* **23**, 785–789.

Groth, G., and Junge, W. (1995). *Biochemistry* **34**, 8589–8596.

Harada, M., Ito Y., Sato, M., Aono, O., Ohta, S., and Kagawa, Y. (1991). *J. Biol. Chem.* **266**, 11455–11460.

Hayashi, S., and Oosawa, F. (1984). *J. Phys. Soc. Jpn.* **53**, 1575–1579.

Hirata, H., Ohno, K., Sone, N., Kagawa, Y., and Hamamoto, T. (1986). *J. Biol. Chem.* **261**, 8939–8943.

Ichikawa, N., Yoshida, Y., Hashimoto, T. and Tagawa, K. (1996). *J. Biochem.* **119**, 193–199.

Jault, J-M., Matsui, T., Jault, F. M., Kalbara, C., Muneyuki, E., Yoshida, M., Kagawa, Y., and Alison, W. S. (1995). *Biochemistry* **34**, 16412–16418.

Jeanteur-De Beukelaer, C., Omote, H., Iwamoto-Kihara, A., Maeda, M., and Futai, M. (1995). *J. Biol. Chem.* **270**, 22850–22854.

Kagawa, Y. (1972). *Biochim. Biophys. Acta* **265**, 297–338.

Kagawa, Y. (1978). *Biochim. Biophys. Acta* **505**, 45–93.

Kagawa, Y. (1984). Protonmotive ATP synthesis. In (Ernster, L. ed.) *Bioenergetics, New Comprehensive Biochemistry,* Elsevier, Amsterdam, pp. 149–186.

Kagawa, Y., and Racker, E. (1966). *J. Biol. Chem.* **241**, 2475–2482.

Kagawa, Y., and Racker, E. (1971). *J. Biol. Chem.* **246**, 5477–5487.

Kagawa, Y., Ishizuka, M., Saishu, T., and Nakao, S. (1986). *J. Biochem.* **100**, 923–934.

Kagawa, Y., Ohta, S., and Otawara-Hamamoto, Y. (1989). *FEBS Lett.* **249**, 67–69.

Kagawa, Y., Ohta, S., Harada, M., Sato, M., and Ito, Y. (1992). *Ann. N. Y. Acad. Sci.* **671**, 366–376.

Kaibara, C., Matsui, T., Hisabori, T. and Yoshida, M. (1996). *J. Biol. Chem* **271**, 2433–2438.

Kandpal, R. P., and Boyer, P. D. (1987). *Biochim. Biophys. Acta* **890**, 97–105.

Kayalar, C., Rosing, J., and Boyer, P. D. (1977). *J. Biol. Chem* **252**, 2486–2491.

Kinoshita, K., Jr., Ikegami, A., Yoshida, M., and Kagawa, Y. (1982). *J. Biochem.* **92**, 2043–2046.

Kojima, H., Ishijima, A. and Yanagida, T. (1994). *Proc. Natl. Acad. Sci. USA* **91**, 12962–12966.

Maruyama, K. (1994). *Biophys. Chem.* **50**, 73–85.

Matsuda, C., Endo, H., Ohta, S., and Kagawa, Y. (1993). *J. Biol. Chem.* **268**, 24950–24958.

Mitchell, P. (1979). *Science* **206**, 1148–1159.

Muneyuki, E., Kagawa, Y., and Hirata, H. (1989). *J. Biol. Chem.* **264**, 6092–6096.

Muramoto, K., Kawagishi, I., Kudo, S., Magariyama, Y., Imae, Y., and Homma, M. (1995). *J. Mol. Biol.* **251**, 50–58.

Musier-Forsyth, K. M., and Hammes, G. G. (1990). *Biochemistry* **29**, 3236–3241.

Nakamoto, R. K., Maeda, M., and Futai, M. (1993). *J. Biol. Chem.* **268**, 867–872.

Ohta, S., Yohda, M., Ishizuka, M., Hirata, H., Hamamoto, T., Otawara-Hamamoto, Y., Matsuda, K., and Kagawa, Y. (1988). *Biochim. Biophys. Acta* **933**, 141–155.

Oosawa, F., and Hayashi, S. (1986). *Adv. Biophys.* **22**, 151–183.

Paik, S. R., Yokoyama, K., Yoshida, M., Ohta, T., Kagawa, Y., and Allison, W. S. (1993). *J. Bionerg. Biomemb.* **25**, 679–684.

Penefsky, H. S. (1985). *Proc. Natl. Acad. Sci. USA* **82**, 1589–1593.

Rayment, I., Rypniewskei, W. R., Schmidt-Bäse, Smith, R., Tomchick, D. R., Benning, M. M., Winkelmann, D. A., Wesenberg, G., and Holden, H. M. (1993). *Science* **261**, 50–58.

Saika, K., and Yoshida, M. (1995). *FEBS Lett.* **368**, 207–210.

Sato, M., Ito, Y., Harada, M., Kihara, H., Tsuruta, H., Ohta, S., and Kagawa, Y. (1995). *J. Biochem.* **117**, 113–119.

Schäfer, H-J., Rathgeber, G., and Kagawa, Y. (1995). *FEBS Lett.* **377**, 408–412.

Senior, A. E. (1990). *Annu. Rev. Biophys. Chem.* **19**, 7–41.

Senter, P., Eckstein, F., and Kagawa, Y. (1983). *Biochemistry* **22**, 5514–5518.

Shirakihara, Y., Leslie, A., Abrahams, J. P., Walker, J., Ueda, T., Sekimoto, Y., Kambara, M., Saiga, K., Odaka, M., Yoshida, M., and Kagawa, Y. (1995). *Proc. Jpn. Bionerg.* **21**, 30–31.

Skulachev, V. P. (1988). *Membrane Bioenergetics,* Springer-Verlag, Berlin–Heidelberg, pp. 19–25.

Staut, J. S., Partridge, B. E., Dibbern, D. A., and Schuster, S. M. (1993). *Biochemistry* **32**, 7496–7502.

Steinert, K., Kroth-Panicic, P. G., and Bickel-Sandkötter, S. (1995). *Biochim. Biophys. Acta* **1249**, 137–144.

Van Walraven, H. S., Strotmann, H., Schwarz, O., and Rumberg, B. (1996). *FEBS Lett.* **379**, 309–313.

Vik, S. B., and Antonio, B. J. (1994). *J. Biol. Chem.* **269**, 30364–30369.

Yanagida, T., and Ishijima, A. (1995). *Biophys. J.* **69**, 312s–315s.

Yohda, M., Kagawa, Y., and Yoshida, M. (1986). *Biochim. Biophys. Acta* **850**, 429–435.

Yohda, M., Ohta, S., Hisabori, T., and Kagawa, Y. (1988). *Biochim. Biophys. Acta* **833**, 156–164.

Zhang, Y., and Fillingame, R. H. (1995). *J. Biol. Chem.* **270**, 24609–24614.