F_oF_1-ATPase STATOR REGULATION STUDIED WITH A RESONANCE MODEL

Yao-Gen Shu and Zhong-Can Ou-Yang
Institute of Theoretical Physics, Chinese Academy of Sciences, Beijing 100190, China

Keywords: Molecular motor, F_oF_1-ATPase, Stator regulation, Binding change mechanism, Resonance model.

Abstract: The F_oF_1-ATPase activity was regulated through external links on the exposed stator. The regulation tendency of synthesis was the same with that of hydrolysis. A resonance model has been proposed to illustrate these regulation phenomena. The novel model not only has deepened our understanding of the "binding change mechanism", but also was very useful to develop the rotary motor into a biosensor.

1 INTRODUCTION

F_oF_1-ATPase is the ubiquitous rotary motor that uses the transmembrane electrochemical potential to synthesize ATP in bacteria, chloroplasts and mitochondria. The holoenzyme is a complex of two rotary motors, F_o and F_1, which are mechanically coupled by a common central stalk ("rotor"), c_n-ε-γ. The membrane embedded F_o unit converts the proton motive force(p.m.f) into mechanical rotation of the "rotor", thereby causing cyclic conformational change of $\alpha_3\beta_3$ crown ("stator") in F_1 and driving ATP synthesis. A striking characteristic of this motor is its reversibility. It may rotate in the reverse direction for ATP hydrolysis and utilize the excess energy to pump protons across the membrane. In F_1 motor, the eccentric rotation of γ subunit and the cyclic conformational change of $\alpha_3\beta_3$ crown, are mechanically coupled at near 100% efficiency(Boyer, 1997; Leslie and Walker, 2000; Weber and Senior, 2003; Kinosita et al., 2004; Junge, 2004; Wilkens, 2005; Nakamoto et al., 2008; Ballmoos et al., 2008; Yasuda et al., 1998; Shu et al., 2010).

In the proof-of-principle demonstrations, the holoenzyme activity was regulated by changing of the transmembrane gradient of proton(Pänke and Rumberg, 1996; Turina et al., 2003; Diez et al., 2004; Steigmiller et al., 2008). Moreover, the F_1 activity was studied in detail through an actin filament or a bead on the "rotor"(Yasuda et al., 1998; Itoh et al., 2004). On the other hand, several pioneering investigations(Boyer, 1993; Abrahams et al., 1994; Noji et al., 1997; Nishizaka et al., 2004) have revealed that the cyclic conformational change of $\alpha_3\beta_3$ crown "stator" is tightly coupled with the rotation of γ "rotor". A fascinating question arises: can we regulate the holoenzyme activity through a convenient method? Yue Group's recent experiments(Cheng et al., 2010) about virus detection have demonstrated that the holoenzyme activity can be regulated by the external links on the exposed α (or β) subunits. The external links not only inhibit the enzyme but also activate it, and the regulation tendency of synthesis is the same with that of hydrolysis. However, what is the implied mechanism?

It is impossible for the two movements to transfer energy at near 100% efficiency(Yasuda et al., 1998) unless they work in a resonance fashion through an elastic element. Actually, Boyer's "binding change mechanism"(Boyer, 1993), Walker's structure(Abrahams et al., 1994) and Oster's hinge bending model(Wang and Oster, 1998) have partly implied that F_1-ATPase works in a fashion of driven oscillation(Kargovsky et al., 2009; Wächter et al., 2011). The driving force is supplied by the eccentric rotation of γ subunit for synthase or by the binding of ATP for hydrolase, whereas the passive oscillation is the cyclic conformational change of $\alpha_3\beta_3$ crown, whose frequency is the driving frequency. If we regard α or β subunit as a harmonic oscillator, α and β subunits will vibrate at its natural frequency. Once the driving frequency matches the natural frequency, resonance occurs. At resonance frequency, the amplitude of steady state response is the biggest in proportion to the amount of driving force, which is the most convenient for binding of substrates and releasing of products. The driving force is essentially just pumping energy back into the system to compensate for the loss

Shu Y. and Ou-Yang Z..
FoF1-ATPase STATOR REGULATION STUDIED WITH A RESONANCE MODEL.
DOI: 10.5220/0003753401320137
In *Proceedings of the International Conference on Biomedical Electronics and Devices* (BIODEVICES-2012), pages 132-137
ISBN: 978-989-8425-91-1

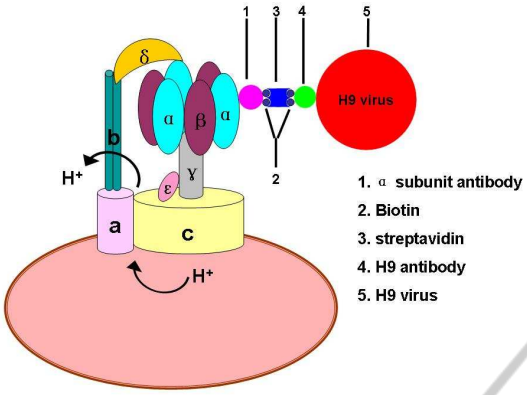

Figure 1: The schematic illustration of the activity regulation assay, showing the experimental geometry (not to scale). The other two same links are not painted. All the external links, α antibody, streptavidin, H9 antibody and H9 virus, are regarded as harmonic oscillators as shown in Fig.3.

of energy due to ATP synthesis or protons pumping as well as damping, which is the most efficient way for motor to work. The rotary speed of motor, which corresponds to the measurable holoenzyme synthesis activity, is equal to the resonance frequency of "stator".

2 EXPERIMENTAL RESULTS

A series of harmonic oscillators have been designed to link to the exposed α subunits. The schematic illustration of this activity regulation assay is shown in Fig.1. F_oF_1-ATPase is embedded in a chromatophore, in which the transmembrane p.m.f is stored. The first external harmonic oscillator is the α antibody, the second one is the streptavidin, the third one is H9 avian influenza virus (hereafter called H9 virus) antibody and the fourth one is the H9 virus.

The holoenzyme activity with different links has been measured as follows: Firstly, each α subunit was bound by an α antibody, the holoenzyme was inhibited, and the relative synthesis and hydrolysis activity decreased to 0.71 and 0.84 respectively. Secondly, the streptavidin was linked to α antibody through a small biotin, the enzyme was inhibited continuously, and the relative activities of synthesis and hydrolysis declined to 0.60 and 0.73 respectively. Thirdly, H9 antibody was linked to streptavidin through biotin, the holoenzyme was inhibited again, and the relative activities of synthesis and hydrolysis dropped to 0.51 and 0.61 respectively. However, when H9 virus bound to its antibody finally, the modified enzyme was activated, and its relative activities jumped to 0.90 and

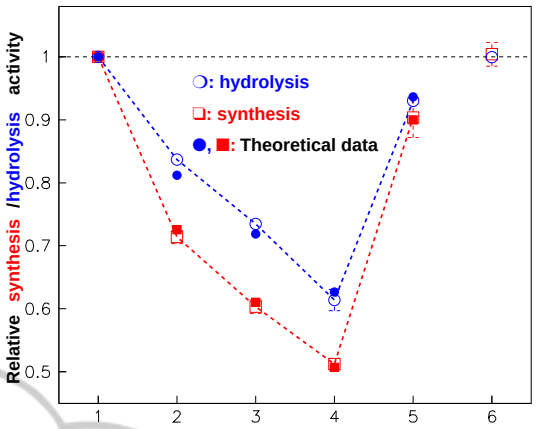

Figure 2: Different activities of enzyme with different links on α subunit shown in Fig.3. The statistical value (mean ± s.e.m.) of each system was computed by 50∼60 samples. The experiment has been repeated independently more than five times. The native F_oF_1-ATPase activity was taken as a control and others were expressed in proportion to the control. The solid dots are calculated by Eq.(4).

0.93 respectively. It is very amazing that the activity of the modified holoenzyme, in which a series of external proteins and virus are linking on "stator", can resume to a native level. These results are correspondingly indicated in Fig.2 No. 2, 3, 4 and 5. The activity of No. 5 is about two times that of No. 4, which means that the No. 4 complex is a sensitive biosensor of virus detecting.

To identify whether H9 virus affects F_oF_1-ATPase activity directly, we measured the activity of native buffer incubated with H9 virus. The result shown in Fig.2 No.6 has proved that the H9 virus doesn't have any direct effect on the holoenzyme activity no matter the enzyme functions as a synthase or hydrolase.

F_1 hydrolysis activity can be expectably regulated by varying the load on the "rotor"(Yasuda et al., 1998). With increasing of the length of filament attached on the "rotor", the damping force will increase, thereby inhibiting the F_1 hydrolysis activity, which has been approvingly interpreted by traditional models(Sun et al., 2004; Xing et al., 2005; Jonathan et al., 2006). Our experiment has revealed that the holoenzyme activity also can be regulated by the external links on the "stator". The regulation tendency of synthesis is the same with that of hydrolysis. However, it is very difficulty for us to understand this regulation mechanism using traditional model. Here, we introduce the resonance mechanism to statistically illustrate these phenomena.

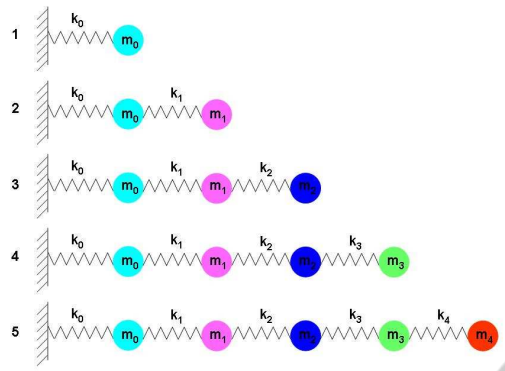

Figure 3: Resonance model. m_0, m_1, m_2, m_3 and m_4 represent the inertial mass(Dalton molecular weight) of α subunit, α antibody, streptavidin, H9 antibody and H9 virus respectively. k_i is the elastic coefficient of corresponded harmonic oscillator.

3 RESONANCE MODEL

The "stator" of F_1 motor is a crown with alternating α and β subunits. Each catalytic site located in β subunit is at different stage of the enzyme kinetic cycle: one binds ATP, another binds ADP, and the third keeps empty. All three sites coordinate for the next 120° step. The different stage of catalytic site is tightly coupled to the different conformation of β and α subunit(Nishizaka et al., 2004; Diez et al., 2004; Itoh et al., 2004). We define the cyclic conformational change of $\alpha_3\beta_3$ crown as an oscillation. The oscillations of three α subunits are identical with the exception of 120° phase. Hence, the activity regulation assay may be simply cartooned in Fig.3 with one α subunit. Each link, including α subunit, functions as a spring with its inertial mass, and is simply regarded as a harmonic oscillator. Five harmonic oscillators have constructed five systems. Each harmonic oscillator is a damped, driven harmonic oscillator. The dynamic equation of the *i*th system is

$$\mathbf{M}\ddot{\mathbf{X}} + \mathbf{K}\mathbf{X} + \Lambda\dot{\mathbf{X}} = \mathbf{F} + \Gamma \qquad (1)$$

where

$$\mathbf{M} = \mathbf{I}\begin{pmatrix} m_0 \\ \vdots \\ m_i \end{pmatrix}, \qquad \mathbf{X} = \begin{pmatrix} x_0 \\ \vdots \\ x_i \end{pmatrix},$$

$$\mathbf{K} = \begin{pmatrix} k_0+k_1 & -k_1 & 0 & \dots & \dots \\ -k_1 & k_1+k_2 & -k_2 & 0 & \vdots \\ 0 & \ddots & \ddots & \ddots & 0 \\ \vdots & \dots & -k_{i-1} & k_{i-1}+k_i & -k_i \\ 0 & \dots & \dots & -k_i & k_i \end{pmatrix},$$

and

$$\Lambda = \mathbf{I}\begin{pmatrix} \lambda_0 \\ \vdots \\ \lambda_i \end{pmatrix}, \mathbf{F} = \begin{pmatrix} f_0(t) \\ \vdots \\ f_i(t) \end{pmatrix}, \Gamma = \begin{pmatrix} \Gamma_0(t) \\ \vdots \\ \Gamma_i(t) \end{pmatrix}.$$

I is an identity matrix. $\mathbf{M}\ddot{\mathbf{X}}$ is the inertial force and is usually ignored in linear motors motility study due to overdamping. $i = 0,1,2,3,4$ represents α subunit, α subunit antibody, streptavidin, H9 antibody and H9 virus respectively, the inertial mass, m_i, is in proportion to its Dalton molecular weight, that is, $m_i \propto$ 55, 170, 60, 170 and ∞ (KD) for i =0, 1, 2, 3, and 4 respectively, x_i is the displacement of the *i*th oscillator. $\mathbf{K}\mathbf{X}$ is the restoring force. $\Lambda\dot{\mathbf{X}}$ is the damping force. **F** is the driving force ($f_i(t) = 0$ for $i \geq 1$). Γ is the random force characterized by a Gaussian distribution with zero mean ($\langle\Gamma\rangle = 0$). The general solution of Eq.(1) is a sum of a transient solution that depends on initial conditions, and a steady state one that depends only on driving frequency, driving force, restoring force and damping force. Of course, we only discuss the latter.

F_oF_1 motor consists of two rotary motors acting in opposition: F_1 motor generates a mechanical torque using the hydrolysis energy of ATP, while F_o motor generates a rotary torque in the opposite direction using a transmembrane p.m.f. When the motor functions as a synthase, the driving force of α subunit, $f_{\gamma^+}(t)$, is generated by the eccentric rotation of γ subunit that is impelled by the transmembrane proton flux of chromatophore, whereas the incurred counterforce, $f_{\mathrm{Tr}}(t)$, comes of the releasing of ATP in β subunit, the net driving force then $f_0(t) = f_{\gamma^+}(t) - f_{\mathrm{Tr}}(t)$. When the motor functions as a hydrolase, the driving force of α subunit, $f_{\mathrm{Tb}}(t)$, is generated by the binding of ATP in β subunit, whereas the incurred counterforce, $f_{\gamma^-}(t)$, comes of the transmembrane protons pumping, the net driving force then $f_0(t) = f_{\mathrm{Tb}}(t) - f_{\gamma^-}(t)$. At steady state, F_oF_1-ATPase converts directly chemical energy at nearly 100% efficiency(Oster and Wang, 2000; Kinosita et al., 2000) between p.m.f and energy currency, ATP, which means that motor doesn't output any mechanical work and dissipates hardly heat in medium, hence, the net driving force $f_0(t) \approx 0$.

Resonance is the tendency of a system to oscillate with the maximum amplitude at a certain fre-

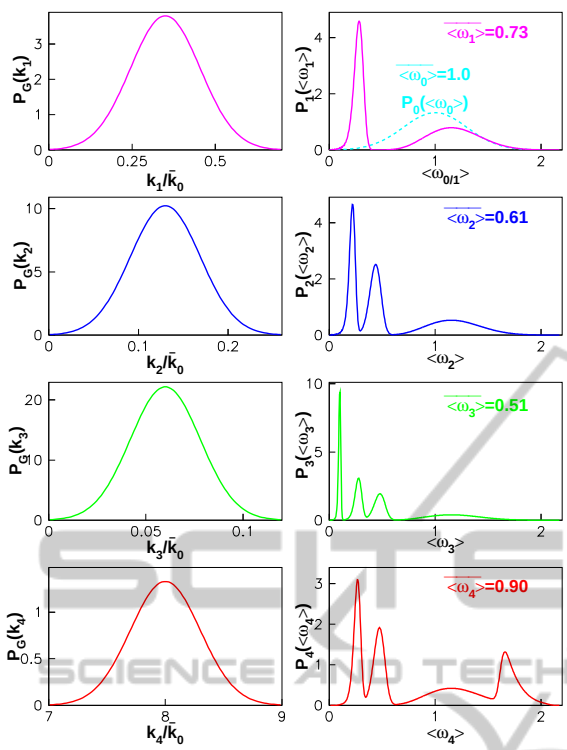

Figure 4: Right: Distribution of time average resonance frequency. The ensemble statistic average value is calculated by Eq.(3).Left: Distribution of elastic coefficient. All of them are assumed Gaussian distribution. The fitted average values k_i/k_0= 0.35, 0.13, 0.06 and 8 for $i = 1,2,3,4$ respectively. The standard deviation, σ_i, is not sensitive to the average value. Here, σ_i/k_0=0.1, 0.04, 0.02 and 0.3 for $i = 1,2,3,4$ respectively, and $\sigma_0/\overline{\langle\omega_0\rangle}$=0.3 for $P_0(\langle\omega_0\rangle)$. $\bar{k}_0 \equiv m_0\overline{\langle\omega_0\rangle}^2$.

quency. At this resonance frequency, even small periodic driving force can produce large amplitude vibration because the system stores vibrational energy. When damping is small, the resonance frequency is approximately equal to the natural frequency of the system(Ogata, 2004). So, if we only consider the time average resonance frequency of the ith system at steady state for small damping, Eq.(1) may be simplified into

$$\mathbf{M}\langle\ddot{\mathbf{X}}\rangle = -\mathbf{K}\langle\mathbf{X}\rangle \qquad (2)$$

where $\langle\cdot\rangle$ means time average value. Eq.(2) can be used to determine the distribution of the time average resonance frequency of ith system, $P_i(\langle\omega_i\rangle)$, if that of native holoenzyme, $P_0(\langle\omega_0\rangle)$, has been given. Considering the diversity of motors and external links, we assume $\langle\omega_0\rangle$ and all k_i ($i \geq 1$) are Gaussian distribution as inserted in right top and shown in the left row of Fig.4. The right row of Fig.4 is the corresponding $P_i(\langle\omega_i\rangle)$. The system that comprises n harmonic oscillators in series possesses n natural frequencies, that is, motor has n frequencies to match for resonance. The frequency spectrum indicated in Fig.4 can be directly tested by a mass of single molecule experiments. The ensemble average resonance frequency of $\alpha_3\beta_3$ crown is equal to the average holoenzyme activity. For simplest, we consider that motor equiprobably matches every resonance frequency, the ensemble statistic time average resonance frequency of ith system can be calculated statistically by

$$\overline{\langle\omega_i\rangle} = \int\dots\int\int P_i(\langle\omega_i\rangle)\langle\omega_i\rangle \mathrm{d}\langle\omega_i\rangle \mathrm{d}k_1\dots\mathrm{d}k_i \qquad (3)$$

Systematic kinetics study of F_oF_1-ATPase(Shu and Lai, 2008) has derived the formula of saturated rotary speed. The rates of ATP synthesis and hydrolysis have been given by

$$v_i^{\mathrm{s}} = 3k_{i,\mathrm{T}}^-, \qquad v_i^{\mathrm{h}} = \frac{3k_{i,\mathrm{D}}^- k_{i,\mathrm{P}}^-}{k_{i,\mathrm{D}}^- + k_{i,\mathrm{P}}^-}$$

respectively, where $k_{i,}^-$ is the releasing rate of corresponding product, and T, D and P represent ATP, ADP and phosphate respectively. Because phosphate is too small to be blocked in the catalytic cleft, we consider that the releasing rate of phosphate, $k_{i,\mathrm{P}}^-$, doesn't rely on the conformation of β subunit and is constant, k_{P}^-. The releasing of ATP(ADP) is tightly coupled to the conformational change of β subunit because the size of ATP(ADP) can be compared with the size of catalytic cleft, that is, $k_{i,\mathrm{T}}^-(k_{i,\mathrm{D}}^-) = \overline{\langle\omega_i\rangle}/2\pi$. The relative synthesis and hydrolysis activities are defined as

$$\rho_i^{\mathrm{s}} \equiv \frac{v_i^{\mathrm{s}}}{v_0^{\mathrm{s}}} = \frac{\overline{\langle\omega_i\rangle}}{\overline{\langle\omega_0\rangle}}, \qquad \rho_i^{\mathrm{h}} \equiv \frac{v_i^{\mathrm{h}}}{v_0^{\mathrm{h}}} = \rho_i^{\mathrm{s}}\frac{1+b}{\rho_i^{\mathrm{s}}+b} \qquad (4)$$

respectively, where $b \equiv k_{\mathrm{P}}^-/(\overline{\langle\omega_0\rangle}/2\pi)$. Eq.(4) predicts that $\rho_i^{\mathrm{h}} > \rho_i^{\mathrm{s}}$ if $\rho_i^{\mathrm{s}} < 1$ as indicated in Fig.2, otherwise, $\rho_i^{\mathrm{h}} < \rho_i^{\mathrm{s}}$ which has also been demonstrated by our recent experiment with links on β subunit. The fitted $b = 1.58$, a reasonable value.

4 DISCUSSIONS

Different from previous activity regulation assays(Yasuda et al., 1998; Pänke and Rumberg, 1996; Turina et al., 2003; Diez et al., 2004; Steigmiller et al., 2008; Itoh et al., 2004), our regulation based on the stator links. The external links not only inhibit the enzyme but also resume it, and the regulation tendency of synthesis is the same with that of hydrolysis. It is very difficulty for power stroke model(Wang and Oster, 1998) to interpret this regulation mechanism. The

resonance mechanism, however, has satisfactorily illustrated these phenomena. Each external link serves as a harmonic oscillator. The more oscillators are linked in series, the more resonance frequencies may be selected. If the system does in a resonance fashion, the motor works in a most efficient way. Here, we consider that motor will equiprobably match every resonance frequency. The average resonance frequency is statistically equal to the ensemble experimental activity. Actually, the lowest resonance frequency will be closer and closer to zero with adding of oscillator or the increasing of inertial mass. However, it is impossible for F_1 motor to stop in virtue of the driving of F_o or binding of ATP. This lowest frequency that is close to zero has to be cancelled in statistics and the average resonance frequency then will jump. This is one of reasons that the value of No.5 is higher than that of No.4 in Fig.2. The critical lowest resonance frequency is waiting to be determined by further experiments. Our novel resonance model together with systematic kinetics study(Shu and Lai, 2008) has predicted that the relative activity of hydrolysis is higher(lower) than that of synthesis if the latter is less(higher) than 1, which has been experimentally demonstrated.

The frequency spectrum indicated in Fig.4 is waiting to be tested by a mass of single molecule experiments. If there is enough single molecule experimental data, we can firstly confirm whether the distribution of activity of single motor is the same with that of our model. Secondly, we can affirm whether the motor equiprobably matches every resonance frequency or not. Finally, we can estimate the standard deviation, σ_i, for each Gaussian distribution. Here, for simplest, we consider that motor equiprobably matches every resonance frequency. The fitted elastic coefficient can be used to estimate the elasticity of linking protein. It seems reasonable that k_4 is much higher than others in view of the stiff capsid of H9 virus. The fitted rate of P_i releasing is also logical because it is higher than $\overline{\langle\omega_0\rangle}/2\pi$.

It is clear that the mechanical movement of F_1 comprises the eccentric rotation of γ subunit and the conformational change of the $\alpha_3\beta_3$ crown, and they are tightly coupled with each other. However, previous theoretical study only focus on the rotation of γ subunit. In fact, the conformational change of the $\alpha_3\beta_3$ crown is very important for the entire motor, and plays an active role in hydrolysis. On the other hand, the role that α subunit plays in the conformational change of the $\alpha_3\beta_3$ crown has not been studied although the structure of α subunit resembles that of β subunit with the exception of catalytic site. This work is try to investigate what α subunit functions in the rotary motor and has expanded the "binding change mechanism".

In contrast with Langevin equation that ignores the inertial force due to overdamping, our resonance model does neglect the damping force because the entire motor doesn't output any mechanical work and dissipates hardly heat in medium. Additionally, the scale of conformational change of the $\alpha_3\beta_3$ crown is very small, thereby the average velocity of oscillation is so low that $\lambda_i\langle\dot{x}_i\rangle \ll m_i\langle\ddot{x}_i\rangle$, which is possible although the values have not been estimated. It should be noted that the elastic coefficient is independent of the additional links. Although the elastic coefficient can in fact depend on the additional links due to electrostatic interaction, in the simplest version of the model, we assume that all elastic coefficients are links-independent.

ACKNOWLEDGEMENTS

This work is partially supported by the NSFC grant No.91027045 and No.11075015, and the National Basic Research Program of China (973 Program) under the grant No. 2007CB935903.

REFERENCES

Abrahams, J. P., Leslie, A. G. W., Lutter, R., and Walker, J. E. (1994). Structure at 2.8å resolution of F_1-ATPase from bovine heart mitochondria. *Nature*, 370:621–628.

Ballmoos, C. V., Cook, G. M., and Dimroth, P. (2008). Unique rotary ATP synthase and its biological diversity. *Annu. Rev. Biophys.*, 37:43–64.

Boyer, P. D. (1993). The binding change mechanism for ATP synthase-some probabilities and possibilities. *Biochim. Biophys. Acta.*, 1140:215–250.

Boyer, P. D. (1997). The ATP synthase-a splendid molecular machine. *Annu. Rev. Biochem.*, 66:717–749.

Cheng, J., Zhang, X. A., Shu, Y. G., and Yue, J. C. (2010). F_oF_1-ATPase activity regulated by external links on β subunits. *Biochem. Biophys. Res. Commun.*, 391:182–186.

Diez, M., Zimmermann, B., Börsch, M., König, M., Schweinberger, E., Steigmiller, S., Reuter, R., Felekyan, S., Kudryavtsev, V., Seidel, C. A. M., and Gräber, P. (2004). Proton-powered subunit rotation in single membrane-bound F_oF_1-ATP synthase. *Nat. Struct. Mol. Biol.*, 11:135–141.

Itoh, H., Takahashi, A., Adachi, K., Noji, H., Yasuda, R., Yoshida, M., and Jr, K. K. (2004). Mechanically driven ATP synthesis by F_1-ATPase. *Nature*, 427:465–468.

Jonathan, L. E., Chakraborty, A. K., and Oster, G. (2006). Simple models for extracting mechanical work from the ATP hydrolysis cycle. *Biophys. J.*, 90:4281–4294.

Junge, W. (2004). Protons, proteins and ATP. *Photosynthesis Res.*, 80:197–221.

Kargovsky, A. V., Romanovsky, Y. M., and Tikhonov, A. N. (2009). F_1-ATPase as an auto-oscillatory system. *Biophysics*, 54:1–7.

Kinosita, J. K., Yasuda, R., Noji, H., and Adachi, K. (2000). A rotary molecular motor that can work at near 100% efficiency. *Phil. Trans. R. Soc. Lond. B*, 355:473–489.

Kinosita, K. J., Adachi, K., and Itoh, H. (2004). Rotation of F_1-ATPase: How an ATP-driven molecular machine may work. *Annu. Rev. Biophys. Biomol. Struct.*, 33:245–268.

Leslie, A. G. W. and Walker, J. E. (2000). Structural model of F_1-ATPase and the implications for rotary catalysis. *Phil. Trans. R. Soc. Lond. B*, 355:465–472.

Nakamoto, R. K., Scanlon, J. A. B., and Al-Shawi, M. K. (2008). The rotary mechanism of the ATP synthase. *Arch. Biochem. Biophys.*, 476:43–50.

Nishizaka, T., Oiwa, K., Noji, H., Kimura, S., Muneyuki, E., Yoshida, M., and Jr, K. K. (2004). Chemomechanical coupling in F_1-ATPase revealed by simultaneous observation of nucleotide kinetics and rotation. *Nat. Struct. Mol. Biol.*, 11:142–148.

Noji, H., Yasuda, R., Yoshida, M., and Kinosita, K. (1997). Direct observation of the rotation of F_1-ATPase. *Nature*, 386:299–302.

Ogata, K. (2004). *System Dynamics*. University of Minnesota, Minnesota, 4th edition.

Oster, G. and Wang, H. (2000). Why is the efficiency of the F_1 ATPase so high? *J. Bioenerg. Biomembr.*, 332:459–469.

Pänke, O. and Rumberg, B. (1996). Kinetic modelling of the proton translocating CF_oCF_1-ATP synthase from spinach. *FEBS Lett.*, 383:196–200.

Shu, Y. G. and Lai, P. Y. (2008). Systematic kinetics study of F_oF_1-ATPase. *J. Phys. Chem. B*, 112:13453–13459.

Shu, Y. G., Yue, J. C., and Ou-Yang, Z. C. (2010). F_oF_1-ATPase, rotary motor and biosensor. *Nanoscale*, 2:1284–1293.

Steigmiller, S., Turina, P., and Gräber, P. (2008). The thermodynamic H^+/ATP ratios of the H^+-ATPsynthases from chloroplasts and *Escherichia coli*. *Proc. Natl. Acad. Sci. USA*, 105:3745–3750.

Sun, S. X., Wang, H., and Oster, G. (2004). Asymmetry in the F_1-ATPas and its implications for the rotational cycle. *Biophys. J.*, 86:1373–1384.

Turina, P., Samoray, D., and Gräber, P. (2003). H^+/ATP ratio of proton transport-coupled ATP synthesis and hydrolysis catalysed by CF_oCF_1-liposomes. *EMBO J.*, 22:418–426.

Wächter, A., Bi, Y. M., Dunn, S. D., Cain, B. D., Sielaff, H., Wintermann, F., Engelbrecht, S., and Junge, W. (2011). Two rotary motors in F-ATP synthase are elastically coupled by a flexible rotor and a stiff stator stalk. *Proc. Natl. Acad. Sci. USA*, 108:3924–3929.

Wang, H. and Oster, G. (1998). Energy transduction in the F_1 motor of ATP synthase. *Nature*, 396:279–282.

Weber, J. and Senior, A. E. (2003). ATP synthesis driven by proton transport in F_1F_0-ATP synthase. *FEBS Lett.*, 545:61–70.

Wilkens, S. (2005). Rotary molecular motors. *Adv. Protein Chem.*, 71:345–382.

Xing, J., Liao, J. C., and Oster, G. (2005). Making ATP. *Proc. Natl. Acad. Sci. USA*, 102:16539–16546.

Yasuda, Y., Noji, H., Kinosita, K., and Yoshida, M. (1998). F_1-ATPase is a highly efficient molecular motor that rotates with discrete 120° steps. *Cell*, 93:1117–1124.