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The Chloroplast ATP Synthase Features the Characteristic Redox Regulation Machinery

Toru Hisabori,[1,2] Ei-Ichiro Sunamura,[1] Yusung Kim,[1] and Hiroki Konno[1,3]

Abstract

Significance: Regulation of the activity of the chloroplast ATP synthase is largely accomplished by the chloroplast thioredoxin system, the main redox regulation system in chloroplasts, which is directly coupled to the photosynthetic reaction. We review the current understanding of the redox regulation system of the chloroplast ATP synthase. ***Recent Advances:*** The thioredoxin-targeted portion of the ATP synthase consists of two cysteines located on the central axis subunit γ. The redox state of these two cysteines is under the influence of chloroplast thioredoxin, which directly controls rotation during catalysis by inducing a conformational change in this subunit. The molecular mechanism of redox regulation of the chloroplast ATP synthase has recently been determined. ***Critical Issues:*** Regulation of the activity of the chloroplast ATP synthase is critical in driving efficiency into the ATP synthesis reaction in chloroplasts. ***Future Directions:*** The molecular architecture of the chloroplast ATP synthase, which confers redox regulatory properties requires further investigation, in light of the molecular structure of the enzyme complex as well as the physiological significance of the regulation system. *Antioxid. Redox Signal.* 19, 1846–1854.

Introduction

THE F_oF_1-ATP SYNTHASE (F_oF_1) is ubiquitously found in energy transducing membranes such as chloroplast and cyanobacterial thylakoid membranes, mitochondrial inner membranes, and bacterial plasma membranes. This enzyme catalyzes the synthesis of ATP from ADP and inorganic phosphate using the electrochemical proton gradient formed across these membranes by photosynthetic or respiratory electron transfer reaction (7, 79). F_oF_1, a critical enzyme for energy conversion in the cell, consists of a membrane-peripheral component F_1, which in turn is formed of five different subunits with a stoichiometry of $\alpha_3\beta_3\gamma_1\delta_1\varepsilon_1$ (80), and a membrane-embedded part F_o, which consists of three different subunits with stoichiometry of $a_1b_2c_{10-15}$ (46, 51, 62). The soluble F_1 possesses ATP hydrolysis activity and is thus called F_1-ATPase. The minimum catalytic core of F_1 maintaining ATP hydrolysis activity is the $\alpha_3\beta_3\gamma$ complex (40), in which the F_1 catalytic sites are predominantly located on three β subunits. Based on the characterization of the catalytic cooperativity observed within these three catalytic sites, Boyer and his coworker proposed the rotational catalysis model (20), in which relative rotational movement of $\alpha_3\beta_3$ core against the γ subunit occurs during catalytic reaction, which was initially viewed with some skepticism. In 1994, however, determination of the crystal structure of the mitochondrial $\alpha_3\beta_3\gamma$ complex at 2.8 Å resolution (1) revealed an alternating hexagonal arrangement of three α and three β subunits around an α-helical domain containing the N- and C-terminal regions of the γ subunit, which occurs as a central axis in the complex. Since three β subunits in the structure showed different conformations due to different nucleotide binding situations at three catalytic sites, the reported structure strongly supported the idea of rotation of the γ subunit against the $\alpha_3\beta_3$ ring during catalysis. Since then, further experiments have allowed confirmation of the rotation of the γ subunit in the $\alpha_3\beta_3$ core (13, 55). Finally, direct visualization of the counter-clockwise continuous rotation of the γ subunit coupled with ATP hydrolysis reaction was observed under an optical microscope by attaching a fluorescent-labeled actin

[1]Chemical Resources Laboratory, Tokyo Institute of Technology, Yokohama, Japan.
[2]Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST), Tokyo, Japan.
[3]Imaging Research Division, Bio-AFM Frontier Research Center, Kanazawa University, Kanazawa, Japan.

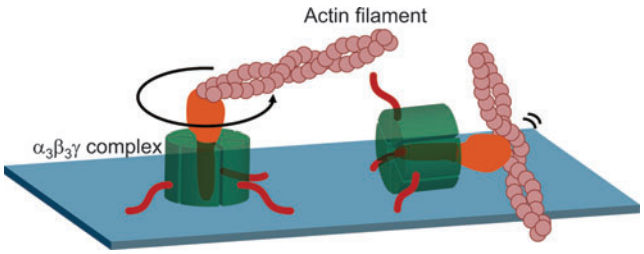

FIG. 1. Direct visualization of rotation of the γ subunit of F_1. A fluorescein-labeled actin filament was attached on the γ subunit of F_1 molecule by using biotin and streptavidin interaction. For this purpose, two cysteines were introduced into the γ subunit and biotinylated. Red lines are His-tags used to immobilize the protein on the glass surface. Counterclockwise rotation of the filament in the presence of MgATP was observed under the fluorescent microscope. F_1, membrane-peripheral part of ATP synthase.

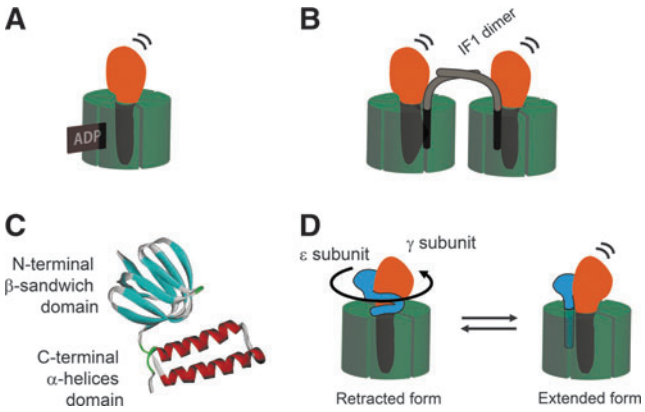

FIG. 2. Regulatory mechanism for ATP synthase. (A) Tightly bound ADP inhibits ATP hydrolysis activity. **(B)** IF_1 is the intrinsic ATPase inhibitor for MF_1. **(C)** The ε subunit is composed of two domains: the N-terminal β-sandwich domain and C-terminal α-helices domain. The latter domain is structurally variable. The NMR structure of the ε subunit from *Thermosynechococcus elongatus* BP-1 (PDB:2RQ6) is shown. For more detail, see Ref. (67). **(D)** The extended conformation of the ε subunit inhibits the ATPase. IF_1, intrinsic ATPase inhibitor protein; MF_1, F_1 part of mitochondrial ATP synthase.

filament to the γ subunit of the $\alpha_3\beta_3\gamma$ complex immobilized on a glass surface (48) (Fig. 1). Rotation analyses of this enzyme were then extensively studied, and the discrete 120° step rotation of γ per single molecule of ATP consumption, and 80° and 40° substeps within this 120° step, determined (78). In addition, further analyses revealed that ADP release occurs at the 240° position of γ rotated from the ATP binding position (2, 4). ATP synthase has since been acknowledged as a molecular motor enzyme in the cell.

Regulation of ATP Synthase Activity

Since ATP synthase can hydrolyze ATP when the electrochemical proton gradient across the membrane is not available for ATP synthesis, prevention of this wasteful reverse reaction seems to be critical for this enzyme complex, and multiple regulatory mechanisms exist for this purpose, irrespective of the origin of the enzyme. The most common regulatory mechanism for ATP synthase is known as ADP inhibition. Once MgADP has been produced by the hydrolysis reaction, it becomes tightly bound to the catalytic site, strongly preventing subsequent ATP hydrolysis, but not ATP synthesis (14, 45, 68) (Fig. 2A). To recover from this ADP inhibition, ATP binding to the noncatalytic sites on the α subunits is required (39). Some detergents, such as lauryldimethylamine oxide (15) and octylglucoside (50), and anions (11, 69), efficiently recover the enzyme from this inhibition. Based on the single-molecule analysis of the catalytic turnover of thermophilic F_1-ATPase using rotation assay, Hirono-Hara *et al.* clearly indicated that ADP inhibition can be observed as frequent long pauses at 80° within the 120° catalytic step during rotation (21).

ATP synthase is a multi-subunit complex and several accessory subunits participate in the regulatory function. Recently, dimerization of F_oF_1 molecule on the mitochondrial membranes has been reported to function as an inhibitory regulation mechanism. In the case of the mitochondrial F_oF_1, acidic pH-dependent dimerization of IF_1 (9) and its binding to the F_1-β subunit is known to achieve dimerization of F_oF_1 on the membrane, which inhibits ATP hydrolysis activity (17) (Fig. 2B). In contrast, the ε subunit works as an intrinsic inhibitor of ATP hydrolysis activity in bacterial and chloroplast ATP synthase. Based on the structural analysis of the ε subunit, the C-terminal helix-turn-helix domain undergoes a large conformational change from a retracted to an extended form (54, 77) (Fig. 2C, D). The conformational change of this ε subunit must be induced by a change in certain physiological conditions, such as ATP concentrations (28, 77) for bacterial ATP synthase, and membrane potential for chloroplast ATP synthase (24). The conformational change of the ε subunit in the C-terminal helices is linked to the inhibitory function of this subunit (27, 66). The ε subunit of the chloroplast ATP synthase is regarded as a stronger inhibitor for ATP hydrolysis activity than the bacterial one (53, 70). Single-molecule analysis of the function of the ε subunit of cyanobacterial F_1during rotation revealed that the ε subunit completely stops the rotation of the γ subunit of cyanobacterial F_1 (32), whereas the bacterial ε subunit decreases the average rotation speed in F_1-ATPase by way of increasing pause duration (59). In addition, based on rigorous rotation analysis on the difference between ADP inhibition and ε inhibition, we found that inhibition of the latter is mechanically more stable (31). Hence, inhibition of the ATP hydrolysis activity by the ε subunit is thought to be the regulatory mechanism, which also prevents futile ATP hydrolysis. These various shut-down mechanisms, which prevent ATP hydrolysis activity must work in a complementary fashion.

Chloroplast ATP Synthase Is a Thiol Enzyme, Which Is Subject To Redox Regulation by Thioredoxin

Chloroplast ATP synthase (CF_oCF_1) is classed as a thiol enzyme; a disulfide bond located on the γ subunit is reduced by reducing equivalents supplied by thioredoxin (Trx), which is, in turn, reduced by the photosynthetic electron transfer reaction (44) (Fig. 3). Trx was first identified in *Escherichia coli* as a ribonucleotide reductase cofactor and supplier of reducing equivalents (37). The significance of Trx in chloroplasts as a mediator of reducing equivalents was first characterized by the Buchanan's group (8, 73), leading to the establishment of

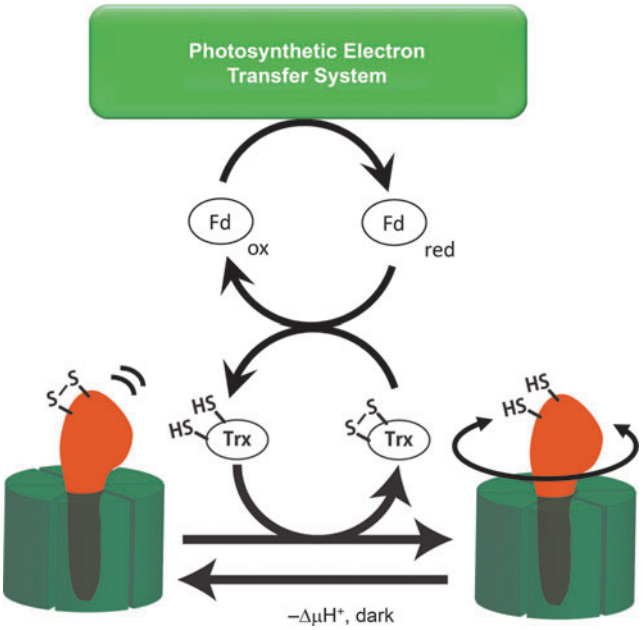

FIG. 3. Trx-dependent activation of CF_1-ATPase. The reduced Trx reduces CF_1- γ subunit. Under suboptimal conditions for ATP synthesis, the γ subunit is immediately oxidized to prevent ATP hydrolysis activity. CF_1, F_1 part of CF_oCF_1; Trx, thioredoxin.

the idea of thiol modulation of chloroplast enzymes (57, 73). In the case of the catalytic moiety of the chloroplast ATP synthase, CF_1, activation of the ATP hydrolysis activity of the isolated enzyme was first demonstrated by chemical reduction (41). McKinney *et al.* then reported that the reduced form Trx can activate the isolated CF_1 ATPase (42). Hereafter, critical cysteines (Cys199 and Cys205) for this thiol modulation were determined as part of four cysteines on the γ subunit of spinach CF_1 (43, 47). Interestingly, the region containing the two regulatory cysteines, the so-called inserted sequence, is comprised of a total of about 40 amino acid residues, and is only present in the γ subunit of chloroplast ATP synthases obtained from higher plants and green algae (23). In contrast, the γ subunit of cyanobacteria includes the insertion sequence consisting of 30 amino acids, although the region lacks 9 amino acids, including two regulatory cysteines (Fig. 4) (71).

In higher plant chloroplasts, four Trx isoforms, Trx-*f*, Trx-*m*, Trx-*x*, and Trx-*y*, have been identified to date [see reviews such as (38, 57)]. Among these, Trx-*f* and Trx-*m* were found before the genome project of *Arabidopsis thaliana* (3), and have been well studied (61, 74). For activation/reduction of CF_1, Trx-*f* rather than Trx-*m* is regarded to act as the physiological reductant (58), based on kinetic analysis of thiol modulation of CF_oCF_1 in the thylakoid membranes. The efficiency and rate of the dithiol–disulfide exchange reaction within the reduced form Trx and an oxidized form target protein must be defined by the protein–protein interaction between Trx isoforms and target proteins (18). Several critical amino acid residues have been identified on the Trx molecule, which are important for interaction with the target proteins. However, no crystal structures of cocrystals of chloroplast Trx and target protein have been obtained to date; thus, the critical interacting amino acid residues remain to be identified. Interestingly, the mutant

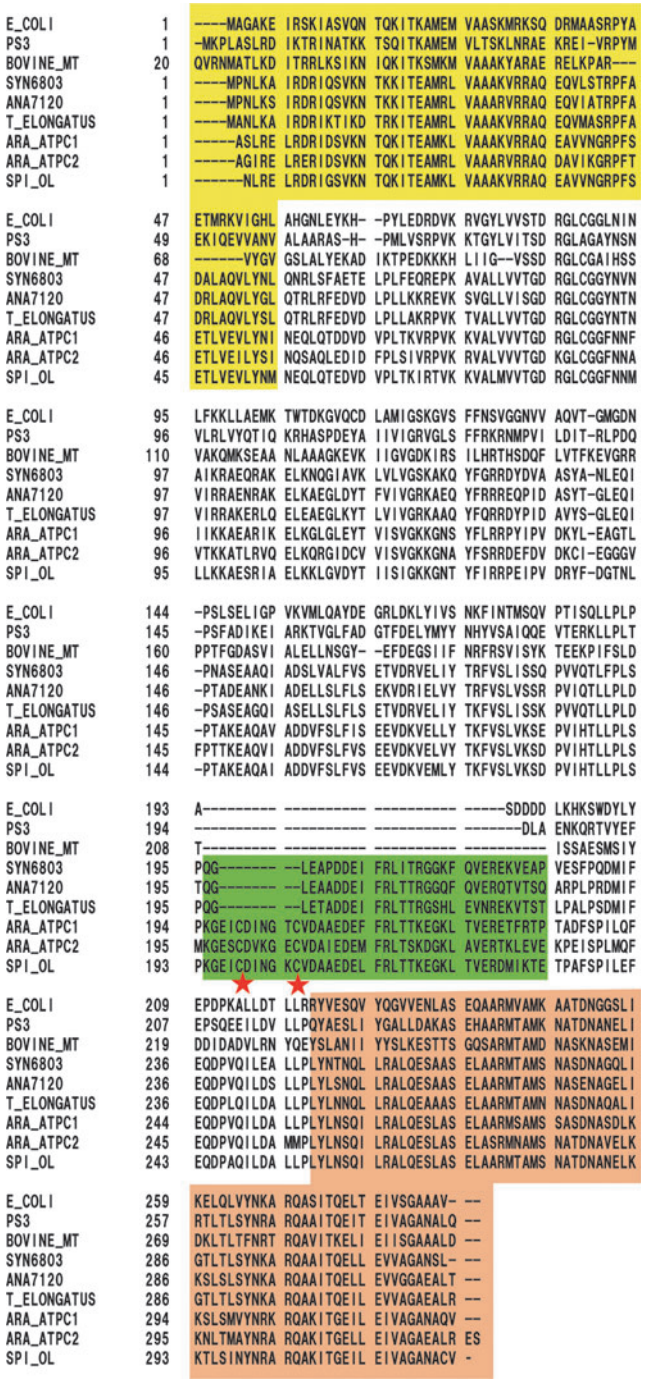

FIG. 4. Alignment of the γ subunit of ATP synthase from various organisms. The N-terminal α-helix is shaded in yellow, and the C-terminal α-helix in orange. The insertion region observed only in photosynthetic organisms is shaded in green. The colorless region is mainly the globular domain. Two cysteines for redox regulation are labeled with red stars. E_COLI, *Eschericia coli*; PS3, thermophilic *Bacillus* PS3; BOVINE_MT, mitochondria from *Bos taurus*; SYN6803, cyanobacteria *Synechocystis* sp. PCC6803; ANA7120, cyanobacteria *Anabaena* sp. PCC7120; T_ELONGATUS, cyanobacteria *T. elongatus*; ARA_ATPC1, *Arabidopsis thaliana* chloroplast type 1; ARA_ATPC2, *A. thaliana* chloroplast type 2; SPI_OL, chloroplast form *Spinacia oleracea*.

Trx, which lacks two critical cysteines can assist the reduction of the disulfide bond on the γ subunit in the presence of high concentrations of DTT. We therefore concluded that Trx displays an affinity to this region and induces the conformational change of a certain region on the γ subunit, which may assist the reduction of the disulfide bond, although the interaction between Trx and the regulatory region of the γ subunit has not been fully elucidated (63).

Molecular Switch on the γ Subunit Governed by Trx

The reduction of the disulfide bond located on the γ subunit has been a subject of extensive biochemical studies predominantly with the isolated CF_1 (10, 60), leading to the elucidation of the link between the enzyme activity and the redox state of the regulatory cysteines. Following the rotation experiments reported by Noji *et al.* (48), we proceeded to visualize the rotation of the γ subunit in the authentic CF_1 complex and successfully observed the unidirectional counterclockwise rotation (22). The redox regulation of rotation of the γ subunit was then studied using the chimeric thermophilic bacterial $\alpha_3\beta_3\gamma$ complex designated as $\alpha_3\beta_3\gamma_{TCT}$, the γ subunit being constructed by fusion of the N- and C-terminal regions of γ from thermophilic bacteria, separated by a central region composed of the regulatory region of the spinach γ (5) (Fig. 5A). By using this $\alpha_3\beta_3\gamma_{TCT}$ complex, the relation between the

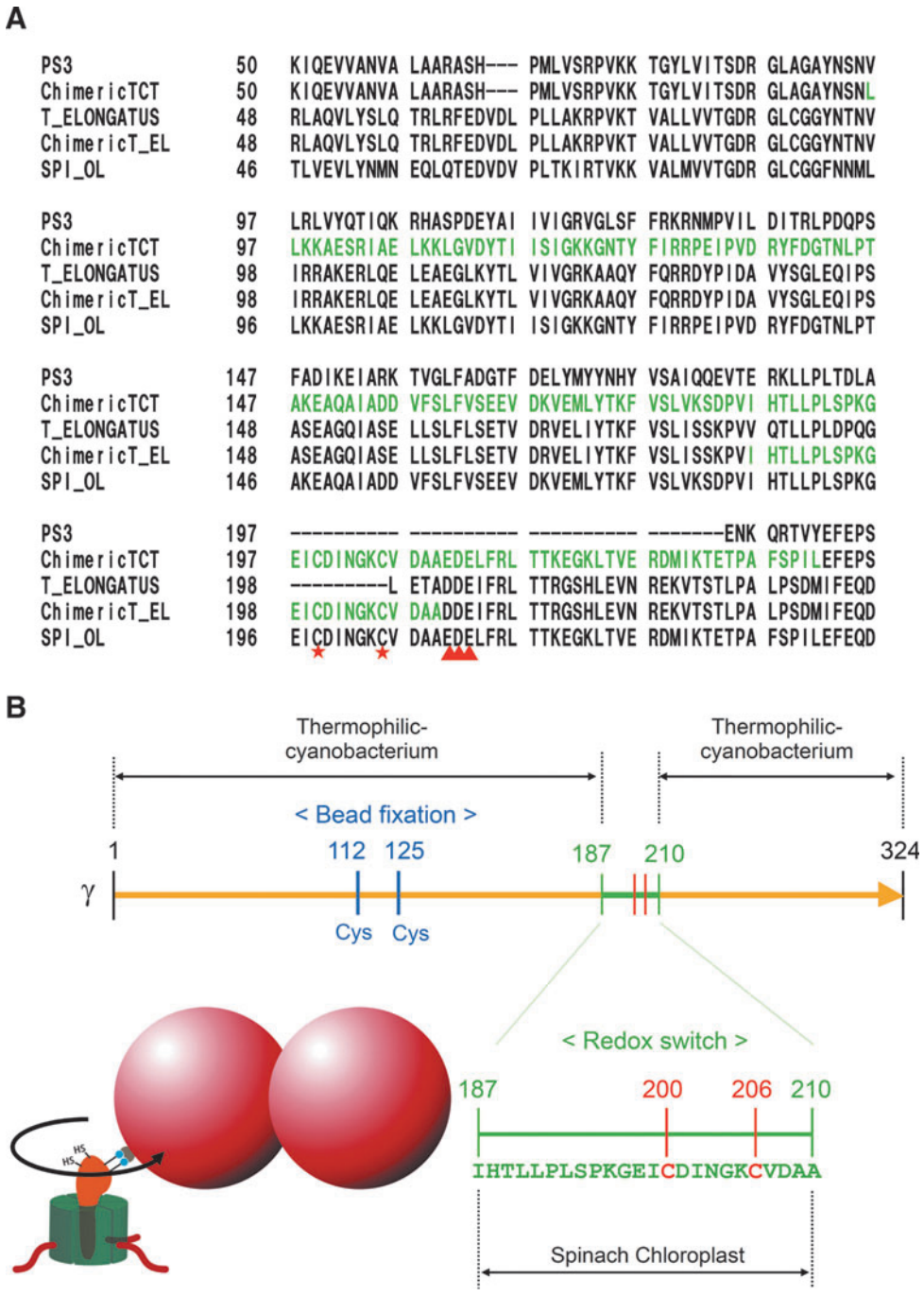

FIG. 5. Analysis of the redox regulation of rotation using the cyanobacterial $\alpha_3\beta_3\gamma$ complex containing the regulatory region of the γ subunit of spinach CF_1. **(A)** Sequence alignment of the chimeric γ subunit. The chimeric construct part alone is shown. Chimeric TCT is the γ subunit constructed with thermophilic *Bacillus* PS3 and spinach γ subunit (5). Chimeric T_EL is the γ subunit constructed with *T. elongatus* and spinach γ subunits (29). In both sequences, those from spinach γ subunit are shown in green letters. Other labels are same as those shown in Fig. 4. Two cysteines for redox regulation are labeled with red star and EDE sequence with red triangle. **(B)** Experimental setup for analysis of the redox regulation of rotation. Four cysteines were introduced on the γ subunit; two cysteines at positions of 112 and 125 were for the fixation of the probe beads and those at 200 and 206 (originally 199 and 205 on the spinach γ) were for redox regulation. Amino acid numbering is based on the sequence of *T. elongatus* BP-1. Due to the limit of the restriction sites on DNA, 24 amino acids were introduced from spinach γ. Rotation of the γ subunit was observed using the attached polystyrene beads on the γ subunit. For more detail, see Ref. (29).

suppressed enzyme activity under oxidizing conditions and the suppressed rotation movement was characterized, although further experiments were difficult due to the poor stability of this chimeric complex (6). More recently, the regulatory region of the spinach γ subunit was introduced into the $\alpha_3\beta_3\gamma$ complex of thermophilic cyanobacterial F_1, and the redox regulation of rotation was thoroughly investigated (29) (Fig. 5A, B). Thanks to the optimal properties of this stable redox switch harboring chimeric complex, the pause position during rotation under oxidizing conditions could be assigned to 80° within the 120° catalytic step, which is the same as the position of ADP inhibition. In addition, the lower activity and rotation frequency of the oxidized form of the enzyme was attributed to a longer pause duration and a relatively shorter rotation duration [see Fig. 6 of Ref. (29)]. Thus, suppression of ATP hydrolysis activity under oxidizing conditions, which will occur in the dark in the natural enzyme, must be accomplished using the ADP inhibition properties.

Although, as described above, the majority of the molecular mechanism of redox regulation of rotation of this motor enzyme has already been characterized, there remain a number of interesting features relating to this regulatory system. For example, deletion of three negatively charged residues on the insertion region (^{210}Glu-^{211}Asp-^{212}Glu) (Fig. 5A, red triangles) from the whole γ subunit sequence of spinach CF_1 results in an apparent reversal of redox regulation of the enzyme activity; apparently, inactivated by reduction and activated by oxidation (34, 67). Correlation between the redox state of the redox switch and the inhibitory effect by the ε subunit has also been studied (12, 60). In the case of the isolated CF_1, reduction of the γ subunit enhances the dissociation of the ε subunit from γ. Although structural aspects of the regulatory region of the γ subunit are extrapolated based on the calculation of the molecular model of the globular domain of the γ subunit (56), there remains limited information pertaining to the conformational change of the γ subunit by reduction and oxidation of two regulatory cysteines. Therefore, it is still difficult to explain the change in affinity of the ε subunit to the γ subunit in a redox-dependent manner. Hence, further structural insights into this motor enzyme are required to fully understand the molecular machinery that underpins regulation.

Molecular Evolution of the Thiol Switch of ATP Synthase; The Cyanobacterum Is the Ancestor of the Chloroplast

As described above, the γ subunit of cyanobacterial F_oF_1 also bears the inserted sequence found in spinach CF_1-γ, although the sequence lacks 9 amino acids including two regulatory Cys residues involved in redox regulation (72) (Fig. 4). This insertion region is not present in the bacterial and mitochondrial F_1 (MF_1)-γ counterparts. Phylogenetic tree analysis also shows that the γ subunits of photosynthetic organisms have a shared ancestor (23), even though the cyanobacterial γ subunit lacks part of the regulatory region (72). The γ subunits of proteobacterial F_1 are phylogenetically close to photosynthetic organisms, and the γ subunits of the mitochondrial ATPase form the original subfamily (23). This independence is in good agreement with the observed difference in the ε subunit as well. In the MF_1, the counterpart of the ε subunit of the bacterial and chloroplast F_1 is the δ subunit, and MF_1 possesses the additional unique ε subunit, which binds to the γ subunit just on the upper region of the δ subunit binding domain (19). The ε subunit of MF_1bound to the γ subunit is thought to disturb the conformational change of the δ subunit in the F_1-ATPase complex. The apparent difference in the mitochondrial γ subunit on the phylogenetic tree from those of bacteria and chloroplast γ subunit [see Fig. 3 of Ref. (23)] might be reflected in this characteristic structural difference.

To understand the structural evolution of the γ subunit and the evolution of the regulatory mechanism, we have focused on the insertion region of the cyanobacterial $F_1\gamma$ subunit, and have prepared a mutant complex with the subunit γ lacking this insertion (32, 64). We have then studied the special characteristics of the mutant complex at both the single-molecule and whole-cell levels. Deletion of this insertion region from the γ subunit results in acceleration of the ATP hydrolysis activity (32). By detailed analysis of rotation behavior of the mutant complex, we concluded that this region confers the ability to frequently shift into an ADP-inhibition state; this is a highly conserved regulatory mechanism, which prevents ATP synthase from carrying out the reverse reaction (64). We then prepared the mutant strain of *Synechocystis* sp. PCC6803, whose γ subunit of ATP synthase lacks the insertion region. The intracellular ATP levels in cyanobacterial cells under light and dark conditions were then measured to determine the physiological significance of ADP inhibition in preventing the intrinsic ATP hydrolysis activity of CF_1. The intracellular ATP level decreased sharply in both the wild-type and the mutant cells in darkness. Amounts of ATP in the wild-type cells decreased to 50% of the original level measured in the light when transferred into dark, whereas those in the insertion deletion mutant cells decreased to 20% or less. When the cells were subjected to light again, the ATP levels of both cells recovered to the original levels immediately [see Fig. 6 of Ref. (64)]. We therefore concluded that the physiological significance of this insertion must be in conferring the special capability to shift into the ADP inhibition state, which allows intracellular ATP levels to be maintained due to the shut-down of ATP hydrolysis activity, a property that must be especially critical for photosynthetic organisms.

Why can the insertion region located on the bottom of the γ subunit affect the ADP inhibition, which occurs at the catalytic site located on the β subunit? To examine the possible signal transfer pathway from the γ subunit to the catalytic sites, we recently introduced multiple Cys residues into the applicable positions on two central α-helices of the γ subunit, which enable us to restrict the relative movement of the α-helices and conformational change of γ (65). Consequently, we found that the conformational changes of both the central α -helices and the lower globular domain of the γ subunit are important for regulation of the activity.

Physiological Significance of Redox Regulation of ATP Synthase

The relation between ATP synthesis activity in chloroplasts and the redox state of the chloroplast ATP synthase was established first in class II chloroplasts, that is, the chloroplasts lacking outer membranes (26). In the study, four forms of the enzyme were suggested, namely, reduced active, reduced inactive, oxidized active, and oxidized inactive. By illumination in the presence of reductants, the oxidized inactive form enzyme, whose activity is supposed to be the basal level after

the preparation of the chloroplasts, turns into the reduced active form enzyme. This form shows significant activities both in ATP hydrolysis and ATP synthesis. In contrast, the inactive species show neither ATP hydrolysis nor synthesis activities. Although the activation process was clearly described in their study, the physiological impact of the redox regulation of ATP synthase was not. Further progress in the study of regulation of chloroplast ATP synthase is well summarized in Ref. (49) especially in light of the electrochemical potential, the oxidation state of the γ subunit, and the bound nucleotide(s) at the catalytic sites.

In 1994, Ort and coworkers carried out a random mutagenesis study of *A. thaliana* in which mutant plants were selected that grew poorly under low irradiance, but performed satisfactorily at high irradiance (16). From this study, they found that the *cfq* mutant, impaired in effective reduction of the γ subunit by Trx, showed a lower efficiency of ATP formation (75, 76). The *cfq* mutant showed a phenotype in which the γ subunit was poorly reduced. Proper redox regulation must therefore be important for ATP synthesis as well, although there are several reports that ATP synthase can become active and support high rates of ATP synthesis even in the oxidized state (26, 52). Similar to the *cfq* mutant, a Trx-insensitive γ subunit isoform, ATPC2, in *A. thaliana* was recently identified (30) (see Fig. 4). The mutant plant expressing only ATPC2 showed high ATP synthesis activity in the light and dark although the change in the internal ATP level was not measured *in vivo*. The physiological significance of this γ subunit isoform should be further investigated as the authors showed that expression of ATPC2 occurs in root and that it is involved in root development.

Irrespective of the significance of the redox regulation switch for CF_oCF_1, there had been no *in vivo* reports pertaining to the reduction of the disulfide bond on the γ subunit of the CF_oCF_1 complex by reducing equivalents generated *in vivo*. For example, photosynthetic activation of CF_oCF_1 was indirectly measured using the electrochromic shift of carotenoids in chloroplasts (35). By using this technique, activation of the sunflower CF_oCF_1 in the light, which was assessed from the measurement of electrochromic shifts in leaf thylakoids, was reported. The authors then concluded that the catalytic activation of CF_oCF_1 is not rate limiting for the induction of carbon assimilation under field conditions (36). Although the actual redox states of CF_1 could not be determined in their measurements, this study is certainly the first effort to determine the redox state of CF_oCF_1 *in vivo*. In contrast, the relevance of the electrochemical proton gradient in chloroplasts to activation of ATP synthase was studied using a combined method of spectrophotometric and fluorescence measurements (25). In this study, the authors suggested that the Calvin-Benson cycle is predominantly controlled by the ATP concentration in chloroplasts, and thiol enzymes in chloroplasts are in the reduced active state even in the dark-adapted leaves, based on their kinetic analysis.

We then directly assessed the reduction level of the γ subunit in the leaf and in intact chloroplasts using the thiol modifier 4-acetoamido-4′-maleimidylstilbene-2,2′-disulfonate and a specific antibody against the γ subunit (33). In this study, we clearly showed that the γ subunits in the dark-adapted leaves and in the intact chloroplasts exist in an oxidized state. In addition, we reported that the reduction of the γ subunit in chloroplasts is not a prerequisite for efficient ATP synthesis in the light, as previously suggested (26). Furthermore, we found that the disulfide bond formation on the γ subunit occurs spontaneously in chloroplasts, irrespective of the light/dark conditions and the reduced form Trx conclusively forces to shift the redox state of the γ subunit to the reduced form. The observed tendency of the equilibrium shift to the oxidation state of CF_1 in chloroplasts must provide an advantage in conferring the ability to shut down prejudicial ATP hydrolysis activity, thus avoiding futile ATP hydrolysis under inappropriate conditions for photophosphorylation (Fig. 6).

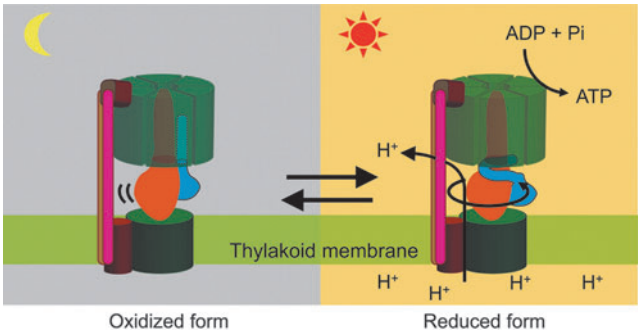

FIG. 6. Oxidized enzyme in the dark is the inactive enzyme for ATP hydrolysis. In the light, CF_oCF_1 synthesize ATP using $\Delta\mu H^+$ across thylakoid membranes. In contrast, inactivation of the enzyme in the dark is critical to maintain ATP levels in the cell. CF_oCF_1, chloroplast ATP synthase.

Conclusions

The chloroplast ATP synthase is a unique molecular motor possessing a redox switch to control its motion. The role of this switch is important in carrying out efficient ATP synthesis and avoiding futile ATP hydrolysis under the transient light/dark conditions in nature. However, the physiological significance of this switch is not understood very well due to the unclear phenotype of the redox switch mutants analyzed to date. In contrast, recent research progress in the control of the molecular motion of F_1 motor by this redox switch has been remarkable using mainly the F_1 subcomplex $\alpha_3\beta_3\gamma$. Various properties of cyanobacterial ATPase come down to those of the chloroplast enzyme except the redox regulation system and by mere introduction of the redox switch into the γ subunit, the cyanobacterial enzyme is able to adopt major characteristics of the chloroplast ATPase. Hence, we successfully analyzed the significance of the redox regulation system *in vitro*. For a complete understanding of this regulatory system, insights into the conformational change of the redox region as well as the structural information of the whole regulated enzyme complex are required.

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Address correspondence to:
Dr. Toru Hisabori
Chemical Resources Laboratory
Tokyo Institute of Technology
Nagatsuta 4259-R1-8
Midori-Ku
Yokohama 226-8503
Japan

E-mail: thisabor@res.titech.ac.jp

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Abbreviations Used

CF_1 = F_1 part of CF_oCF_1
CF_oCF_1 = chloroplast ATP synthase
F_1 = membrane-peripheral part of ATP synthase
F_o = membrane-embedded part of ATP synthase
IF_1 = intrinsic ATPase inhibitor protein
MF_1 = F_1 part of mitochondrial ATP synthase
Trx = thioredoxin