Interactions among γR268, γQ269, and the β Subunit Catch Loop of Escherichia coli F1-ATPase Are Important for Catalytic Activity*

Matthew D. Greene and Wayne D. Frasch‡

From the Center for the Study of Early Events in Photosynthesis, School of Life Sciences, Arizona State University, Tempe, Arizona 85287-4501

Received for publication, September 8, 2003, and in revised form, October 1, 2003
Published, JBC Papers in Press, October 7, 2003, DOI 10.1074/jbc.M309948200

Removal of the ability to form a salt bridge or hydrogen bond between the β subunit catch loop (βY297-D305) and the γ subunit of Escherichia coli F1, F0-ATP synthase significantly altered the ability of the enzyme to hydrolyze ATP and the bacteria to grow via oxidative phosphorylation. Residues βT304, βD305, βD302, and γQ269 and γR268 were found to be very important for ATP hydrolysis catalyzed by soluble F1-ATPase, and the latter four residues were also very important for oxidative phosphorylation. The greatest effects on catalytic activity were observed by the substitution of side chains that contribute to the shortest and/or multiple H-bonds as well as the salt bridge. Residue βD305 would not tolerate substitution with Val or Ser and had extremely low activity as βD305E, suggesting that this residue is particularly important for synthesis and hydrolysis activity. These results provide evidence that tight winding of the γ subunit coiled-coil is important to the rate-limiting step in ATP hydrolysis and are consistent with an escape mechanism for ATP synthesis in which αβγ intersubunit interactions provide a means to make substrate binding a prerequisite of proton gradient-driven γ subunit rotation.

The F1F0-ATP synthase1 uses a non-equilibrium transmembrane proton gradient to catalyze the formation of ATP from ADP and inorganic phosphate. The enzyme consists of two protein complexes, the membrane embedded F0 complex, which couples proton translocation to the synthesis of ATP, and the membrane extrinsic F1 complex, which contains the catalytic sites. The F1 portion consists of five subunits that occur with a stoichiometric ratio of (αβγ)3F1. The α and β subunits are arranged alternately similar to the sections of an orange and contribute to the formation of the catalytic sites. The F1 portion can be isolated from F0 and function as an ATPase (1). The F1 functions as an ATP driven rotary motor (2), which moves through a complete 360° rotation in three discrete 120° steps. The binding of Mg2+-ATP to a catalytic site initiates a 90° rotation of the γ subunit to form an intermediate state. Following a 2-ms pause, a 30° rotation concurrent with product release completes the catalytic cycle (3).

Differences in the conformation of the γ subunit have been observed between ground state F1 structures (4, 5) and the (ADP-AlF4)2F1 structure by Menz et al. (6), a putative post-transition state structure that contains Mg2+-ADP and SO42− at the low affinity catalytic site and the transition state analog Mg2+-ADP-fluorooxaluminate bound at the other two catalytic sites. In this intermediate state structure, the position of the γ subunit used to attach the probe for rotation studies is rotated approximately 30° from its position in ground state structures.

As a consequence, the coiled-coil of the γ subunit is more tightly wound in the (ADP-AlF4)2F1 structure than in the ground state.

The major specific interaction between the helical coiled-coil of the γ subunit and the (αβγ)3F1 subcomplex occurs with γR268 and γQ269 in all of the F1 structures. These residues form a “catch” with a loop of the β subunit in the empty catalytic site conformation that encompasses residues 297–305 (Fig. 1A). In the ground state structure, this catch results from a salt bridge between γR268 and βD302 and from H-bonds among γQ269, βD302, and βT304 (Fig. 1B). In the (ADP-AlF4)2F1 structure, the catch loop of the catalytic site with bound Mg2+-ADP and SO42− also interacts with γR268 and γQ269. However, the interactions of these residues with the catch loop in (ADP-AlF4)2F1 differ from the ground state, such that γR268 and γQ269 are rotated approximately 15° as shown in Fig. 1C. Among other differences, γR268 forms a second salt bridge with βD305 of the catch loop in (ADP-AlF4)2F1.

The region of the γ subunit that interacts with the β subunit catch loop was one of three γ subunit locations where second site mutations suppressed the deleterious effects of ATP synthase activity caused by the F1 γM23K mutant (7). Based on these observations, Al-Shawi et al. (7) concluded that γM23K decreases the coupling efficiency of F1, F0 because of an increase in the energy of interaction between β and γ subunits. Catch loop residue βY297 is approximately 5.5 Å from the terminal phosphate in the conformation of the catalytic site that binds Mg2+-AMPPNP (4).

Site-directed mutants of this residue in Chlamydomonas chloroplast F1 decreased Mg2+-ATPase activity and changed the electron paramagnetic resonance spectrum of vanadyl bound as VO2+-ATP to the low affinity catalytic site (8). These changes indicated that this functional surrogate for the Mg2+ cofactor used βY297 as a metal ligand during the initial binding of metal-nucleotide to the empty catalytic site. Based on these observations, it was proposed that intersubunit H-bonds between the γ subunit and the (αβγ)3 ring prevent rotation driven by the proton gradient until the empty catalytic site binds substrate. Deformation of the catch loop-γ subunit...
resulted in the strain AN87. Construction of pXL1 was performed by insertion of a six-histidine residue tag immediately after the start codon of unc A, which encodes the F1 α subunit, using the Stratagene Chameleon double-stranded site-directed mutagenesis kit. The mutagenic primer was 5'-TAAGGGGACTTGAGCACCTACATCACCATCAGGCGGTGACGCTCCCGCGGAA-3' and its complement 5'-CTCTAGATCTGGGCTAGCCGTTACG-3'. The underlined base insertions add a His6 tag, whereas the boldface base change introduces a PvuI site (CAA to CAG). The selection primer utilized was 5'-CTCTAGATCTGGGCTAGCCGTTACG-3' and its complement 5'-CTCTAGATCTGGGCTAGCCGTTACG-3'. The desired mutations were first identified by screening with PvuI and then confirmed by DNA sequencing using ABI prism automatic sequencing as were the sequences of all of the other mutations. Strain AN87 was transformed via insertion of pXL1 by electroporation. A further mutation, ψ193C, was made to facilitate attachment of rotation probes for future studies. The mutagenic primer utilized was 5'-CTTGCGGTTCACGGCATCGGTATGATGATCGT-3' and its complement 5'-CATCACGGGAAAAGCTCTTCCGGGGCGG-3'. The resulting cell line that contains the His6 tag, ψ193C mutation, and XmnI deletion is referred to as XL10.

The ψR268L, ψQ269L, β3D02V, β3D04A, β3D02T, β3D05V, and β3D05E mutants were prepared in plasmid pXL1 using the Stratagene QuickChange XL site-directed mutagenesis kit with the oligonucleotide primers as follows: 5’-GGATATACAAACAAGTCCTACGCGCC-3’ and its complement 5’-CTCTAGATCTGGGCTAGCCGTTACG-3’ and its complement 5’-CTCTAGATCTGGGCTAGCCGTTACG-3’ for the creation of ψR268L; 5’-GTATAAGGGGACTTGAGCACCTACATCAGGCGGTGACGCTCCCGCGGAA-3’ and its complement 5’-CTCTAGATCTGGGCTAGCCGTTACG-3’ for the creation of ψQ269L; 5’-GTATAAGGGGACTTGAGCACCTACATCAGGCGGTGACGCTCCCGCGGAA-3’ and its complement 5’-CTCTAGATCTGGGCTAGCCGTTACG-3’ for the creation of ψQ269L; 5’-GGATATACAAACAAGTCCTACGCGCC-3’ and its complement 5’-CTCTAGATCTGGGCTAGCCGTTACG-3’ for the creation of ψQ269L; 5’-GGATATACAAACAAGTCCTACGCGCC-3’ and its complement 5’-CTCTAGATCTGGGCTAGCCGTTACG-3’ for the creation of ψQ269L; 5’-GGATATACAAACAAGTCCTACGCGCC-3’ and its complement 5’-CTCTAGATCTGGGCTAGCCGTTACG-3’ for the creation of ψQ269L; 5’-GGATATACAAACAAGTCCTACGCGCC-3’ and its complement 5’-CTCTAGATCTGGGCTAGCCGTTACG-3’ for the creation of ψQ269L; 5’-GGATATACAAACAAGTCCTACGCGCC-3’ and its complement 5’-CTCTAGATCTGGGCTAGCCGTTACG-3’. The resulting plasmid was used to transform the AN87 strain.

The F1-ATPase was purified from E. coli using a modified form of the procedure described by Wise (10). Cells were grown on LB agar plates enriched with 10 mM MgSO4 and 20 mM glucose containing 50 μg/ml ampicillin and 34 μg/ml chloramphenicol. Single colonies were picked and transferred to 250-ml flasks containing LB medium with the same supplements and grown overnight at 37 °C and shaken at 250 rpm. Overnight cultures were then transferred to 2-liter baffled flasks containing 1 liter of minimal medium (60 mM K2HPO4, 40 mM NaH2PO4, 15 mM (NH4)2SO4) containing 50 μg/ml ampicillin and 34 μg/ml chloramphenicol, and glucose to a final concentration of 30 mM. Cells were grown until late log phase at 37 °C and shaken at 250 rpm. The cultures were then harvested by centrifugation at 6000 × g. 

Prior to dissociation of F1, from F1 membranes, membranes were washed once with 50 mM TESE (pH 8.0), 40 mM e-aminoacaproic acid, 5% (v/v) glycerol, and 4.8 mM para-aminobenzamidine. Removal of F1, was accomplished with washing of membranes with 30 mM MES (pH 6.0), 20 mM e-aminoacaproic acid, 5% (v/v) glycerol, and 1.0 mM EDTA. Dithiothreitol was exchanged from all of the buffers to avoid reducing the nickel column material. Membranes were then centrifuged at 100,000 × g, and the supernatant containing F1, was concentrated down to ~5 ml via pressure dialysis using an Amicon YM100 membrane. Nickel affinity chromatography was utilized to purify the His6-tagged F1 under native conditions as described in the Qiagen nickel-nitrirotioc acid Superflow® protocol manual. Proteins removed from membranes in earlier steps were exchanged into nickel column binding Buffer (25 mM Tris-HCl (pH 8.0), 150 mM NaCl, 10% glycerol, 1% imidazole, 1 mM ATP). Buffer exchange was accomplished by concentrating the crude F1, extract to <5% of the original volume by pressure dialysis using an Amicon YM100 membrane followed by dilution in Binding Buffer. Diluted F1, was stirred in the presence of nickel-nitrirotioc acid column material for 1 h and packed into a column. Unwanted proteins were removed by flushing the column in Wash Buffer (25 mM Tris-HCl (pH 8.0), 150 mM NaCl, 10% glycerol, 10% imidazole, 1 mM ATP). The purified His6-tagged F1, was then removed with Elution Buffer (25 mM
Tris-HCl (pH 8.0), 150 mM NaCl, 10% glycerol, 100 mM imidazole, 1 mM ATP). Succinate-dependent growth measurements were determined by growing single colonies picked from LB agar plates overnight at 37 °C and shaken at 250 rpm in 50-ml cultures of minimal medium described above with 50 μg/ml ampicillin, 34 μg/ml chloramphenicol, 30 mM succinate, and 600 mg/liter casein hydrolysate. Overnight cultures were then inoculated into 1-liter cultures of the same medium, grown at 37 °C, and shaken at 250 rpm. Absorbance at 600 nm was used as a measure of cell density, and the doubling time of each culture was determined in the log phase of growth.

The rate of ATP hydrolysis was measured with an ATP-regenerating coupled assay that consisted of 50 mM Tris-HCl (pH 8.0), 10 mM KCl, 2.5 mM phosphoenolpyruvate, 0.15 mM-0.3 mM NADH, 50 μg/ml pyruvate kinase, 50 μg/ml lactate dehydrogenase, and 3 mM F1 with 2 mM Mg2+-ATP. The rate was determined as the change in absorbance at 340 nm using a Varian Cary 50 Bio UV-visible spectrophotometer equipped with a stirred cell Peltier temperature control. The reaction was initiated by the addition of F1 to the assay mixture. Reaction rates were calculated from data collected 8–10 min after initiation of the reaction to allow for dissociation of the ε subunit and to minimize inhibition by entrapped Mg2+-ADP (11). Arrhenius analyses of data to determine the entropic and enthalpic components of the free energy of activation were performed using standard Equations 1–3 (12),

\[ E_A = \Delta H^0 + RT \]  
\[ \Delta G^0 = \Delta H^0 - T\Delta S^0 \]  
\[ \Delta G^0 = -RT\ln(Nh/RTk_{cat}) \]

where \( k_{cat} \) is the turnover number, \( E_A \) is the Arrhenius activation energy, \( N \) is Avogadro’s number, and \( \Delta H^0 \) and \( \Delta S^0 \) are the enthalpic and entropic components of the changes in Gibbs free energy of activation (\( \Delta G^0 \)). All of the Mg2+-ATPase assays were accomplished within 5 days of the date at which the F1-ATPase preparation was completed. After this period, the preparations were found to lose activity as the result of an increase in the entropy of activation (data not shown).

RESULTS

With the exception of βD305V and βD305S, the yield of F1 purified from the site-directed mutants to remove γ subunit-catch loop interactions was approximately the same as that isolated from the XL10 strain. Intact F1-ATPase that contained the βD305V or the βD305S mutants was never successfully isolated, and these mutations were presumed to interfere with assembly of the F1F0-ATP synthase. The F1Fo-ATPase isolated from E. coli with the other mutations (γR268L, γQ269L, βD302T, βD302V, βT304A, or βD305E) contained all five subunits as determined by SDS-polyacrylamide gel electrophoresis (data not shown). These results suggest that these latter mutations did not significantly affect the synthesis and assembly of the enzyme. The relative ability of the mutant and wild type strains to grow via oxidative phosphorylation on minimal medium in the presence of succinate was assessed by determining growth curves. The inability of βD305V and βD305S mutants to assemble intact F1-ATPase coupled with their inability to grow on minimal medium with succinate was utilized as a negative control in which the growth rate was dependent on the rate of ATP synthesis catalyzed by the F1F0-ATP synthase. The doubling times calculated from the growth curves are summarized in Table I.

The F1F0 demonstrated very little tolerance for the substitution of βD305. In addition to the fact that substitution of this carboxyl for a hydroxyl prevented assembly of F1, the most conservative mutation, βD305E, completely impaired the succinate-dependent growth rate. The strain that contained the γQ269L mutation was also unable to grow on succinate, indicating that these residues are very important for ATP synthase activity. The ability of the strains that contained γR268L, βD302V, or βD302T mutants to grow on succinate was also significantly decreased. The doubling time of the strain containing γR268L was nearly twice that of XL10, whereas that of the strains that contained mutants of βD302 increased by approximately 1.5-fold. However, the rate of growth of the βT304A mutant on succinate was not affected. None of the mutations made to either the β or γ subunits affected the ability of the bacteria to grow on minimal medium in the presence of glucose. These results suggest that the poor growth on minimal medium with succinate was the result of impairment of the ability of the F1F0 complex to synthesize ATP.

The mutations that interfered with γ subunit-catch loop interactions also affected the ATPase activity of isolated F1, significantly. Fig. 2 shows an Arrhenius plot the Mg2+-ATPase activity. With the exception of the γQ269L mutant, the mutations decreased the temperature stability of the enzyme. Although XL10-F1 was stable to 50 °C, βT304A-F1 and βD305E-F1 were stable only to 32.5 °C, whereas βD302T-F1 and γR268L–F1 exceeded their stability limits at 40 and 27.5 °C, respectively. Consequently, a direct comparison of the effects of the mutations on \( k_{cat} \) was made at 25 °C where all of the enzymes were stable as shown in Table I.

The F1-ATPase that contained the βD302V mutation had no detectable ATP hydrolysis activity over the temperature range of 5–50 °C despite the fact that this mutant retained partial ability to grow on minimal medium with succinate (Table I). The more conservative βD302T mutation retained approximately 3% of the XL10-F1 activity. The effect of this mutation on ATPase activity was substantially greater than its effect on succinate-dependent growth. The \( k_{cat} \) values of βD305E-F1 and γQ269L-F1 were only 4 and 1% XL10-F1, respectively, which were comparable to their effects on the succinate-dependent growth rate. It is noteworthy that the temperature stability of the γQ269L-F1 mutant was comparable to XL10-F1, despite the fact that both ATP hydrolysis and the growth rate on succinate were significantly lower than XL10-F1. The difference in ATPase activity and succinate-dependent growth of the βT304A and γR268L mutations were 2.5–3-fold with \( k_{cat} \), values for ATP hydrolysis that were 45 and 16% XL10-F1, respectively.

When the data are plotted as in Fig. 2, the activation energy (\( E_A \)) is determined directly from the slope. The values for enthalpy of activation, \( \Delta H^0 \), of ATP hydrolysis are directly proportional to \( E_A \) as per Equation 1 and were calculated from the data indicated by the solid lines in the Arrhenius plot as summarized at 25 °C in Fig. 3. For every mutant that retained Mg2+-ATPase activity, \( \Delta H^0 \) was significantly lower than ob-
Catch Loop Mutations of E. coli F_{1}-ATPase

Fig. 2. Arrhenius analysis of Mg-ATPase activity catalyzed by soluble XL10-F_{1}\(\bullet\), βD302T-F_{1}\(\bigcirc\), βT304A-F_{1}\(\bigcirc\), γR268L-F_{1}\(\bigcirc\), βD305E-F_{1}\(\bigcirc\), and γQ269L-F_{1}\(\bigcirc\). The concentration of F_{1} used was 3 nm for XL10-F_{1}, βD305E-F_{1}, and βT304-F_{1}, mutants, 9 nm for γR268L-F_{1}, 40 nm for γQ270L-F_{1}, and 25 nm for βD302T-F_{1}. ATPase activities were assayed at 2 mM Mg^{2+}-ATP every 2.5 °C from 5–50 °C as described under “Experimental Procedures.” The lines plotted were generated by linear least squares regression of the data.

DISCUSSION

The results presented here imply that hydrogen bonds and salt bridges between the β subunit catch loop and the γ subunit are very important to the catalytic function of the enzyme. Residues βT304, βD305, βD302, γQ269, and γR268 were found to be very important for ATP hydrolysis catalyzed by soluble F_{1}-ATPase, and the latter four residues were also very important for oxidative phosphorylation. At a resolution of 2.4 Å, typical of the available F_{1} crystal structures, it is difficult to determine whether a hydrogen bond is present or assesses its relative strength based on structural information alone. It is remarkable that single mutations to remove the possibility to make a single hydrogen bond or salt bridge outside the catalytic site can have such large effects on catalytic activity.

Several of the residues in the catch loop make a more important contribution to ATP hydrolysis than to ATP synthesis. The greatest differential effects were observed with mutants to βD302, although the γR268 and βT304 mutants also affect hydrolysis to a greater extent than synthesis. Even though mutations to these latter residues caused 2.5–3-fold differences in the decrease of hydrolysis and synthesis, the results indicate that γR268 was much more important to synthesis than βT304. These differential effects probably result from the fact that ATP hydrolysis was measured with isolated F_{1}, whereas the competency of ATP synthesis was assessed by the growth rate of the E. coli strains on succinate. In the latter case, the fully assembled F_{1},F_{0}-ATP synthase may have a different rate-limiting step than isolated F_{1}.

Relationship between Catch Loop-γ Subunit Interactions and ATP Hydrolysis—The rate-limiting step of the ATPase reaction of isolated F_{1} occurs after a 90° rotation of the γ subunit induced by Mg^{2+}-ATP binding (3). The catalytic cycle is completed by a 30° rotation of the γ subunit concurrent with product release. In the (ADP-AlF_{4})_{2}F_{1} crystal structure, the portion of the γ subunit used to attach the rotation probe is rotated approximately 30° from the ground state structure (6). This crystal structure contains Mg^{2+}-ADP and SO_{4}^{2-} at the low affinity catalytic site and the transition state analog Mg^{2+}-ADP-fluoroorolamine at the other two catalytic sites.

Although the foot region of the γ subunit, the point of attachment for the rotation probe, was found to be rotated about 30° in (ADP-AlF_{4})_{2}F_{1}, the C terminus of this subunit is in nearly the same position as the other F_{1} structures. Consequently, the helical coiled-coil of the γ subunit is wound more tightly in (ADP-AlF_{4})_{2}F_{1} than the ground state structure. This implies that some of the torque on the γ subunit generated upon substrate binding may be used to wind the coiled-coil more tightly by inducing a 120° rotation of the C terminus while the foot of the γ subunit rotates 90°. Relaxation of the coiled-coil during the final 30° rotation could contribute to the energy needed to complete the rate-limiting step of the reaction.

The results presented here are consistent with a role for the intermolecular interactions of the catch loop serving to promote the formation of the tightly wound form of the coiled-coil in a...
manner that provides energy for the final 30° rotation during the rate-limiting step. Because product release is rate-limiting to F_{1}-ATPase activity (3), the thermodynamic parameters measured here provide information concerning this step. Despite the fact that all of the mutants decreased $\Delta H^\circ$, the changes in entropy of activation more than compensated for the more favorable values of $\Delta H^\circ$, thereby significantly lowering $k_{cat}$. These changes in $T_\Delta S$ can be explained if the elimination of the salt bridges and/or H-bonds between the $\beta$ subunit-catch loop and $\gamma$ subunit increased the number of allowable conformations of the F_{1} subunits during the rate-limiting step. Although some of these additional conformations dramatically lower the activation energy barrier, and thus $\Delta H^\circ$, from that of XL10-F_{1}, many more of them are nonproductive. The additional time needed for the enzyme to adopt a productive conformation in the absence of the salt bridge or H-bond leads to the decrease in $k_{cat}$. It is noteworthy that $\beta D305E$ residues tolerated mutations least, forms a salt bridge to the $\gamma$ subunit in (ADP-AlF_{4})_{2}F_{1}, which may represent the rate-limiting conformation, but not in the ground state structures.

**Relationship between Catch Loop-$\gamma$ Subunit Interactions and ATP Synthesis**—The results presented here also indicate that catch loop-$\gamma$ subunit interactions are important to ATP synthesis. The succinate-dependent growth rate of the $\gamma Q269L$ and $\beta D305E$ strains was 1% of wild-type, suggesting that these residues were essential for ATP synthase activity. The $\gamma R268L$ and both $\beta D302$ mutants also decrease the growth rate by 4- and 2-fold, respectively. These results are consistent with the hypothesis that the residues at the catch loop serve as an escapement mechanism during ATP synthesis (12). In this mechanism, the trans-membrane proton gradient provides constant torque to the $\gamma$ subunit (via the c-subunit ring). The interactions between the $\gamma$ and $\alpha\beta$ subunit rings prevent this rotation until the empty catalytic site binds substrate. When the H-bonds and salt bridges at the catch loop are broken as the result of substrate binding, the torque on the $\gamma$ subunit is greater than the energy in the remaining H-bonds, such that rotation of the $\gamma$ subunit induces the conformational changes in the catalytic sites necessary for ATP synthesis. The results presented here suggest that residues $\gamma Q269$, $\gamma R268$, $\beta D305$, and $\beta D302$ contribute to the restraint of the rotation of the $\gamma$ subunit during ATP synthesis prior to the binding of substrate.

Zhou et al. (13) demonstrated that substrate binding was a prerequisite of proton gradient-driven rotation in E. coli F,F_{o} by direct observation of rotation via the FLAG epitope. Mutations to residues in the catch loop have been found in naturally occurring second site revertants of the inhibitory $\gamma M23K$ mutant (7). The $\gamma M23K$ mutation was postulated to form an additional H-bond to the DELSEED region of the $\beta$ subunit. We note that in every case the reported revertants cause the loss or weakening of a salt bridge or an H-bond either in the catch loop or near the $\gamma$ subunit C terminus. Second site mutations to restore ATP synthase activity to $\gamma Q269E$ or $\gamma T273V$ had similar effects and identified a third important location in the $\gamma$ subunit N terminus (14). We note that several intersubunit H-bonds are present at the N terminus of the $\gamma$ subunit. These results suggest that the sum of the energy in the intersubunit H-bonds and salt bridges, regardless of their location, must not be above or below a certain value for the enzyme to function.

If the steps in ATP synthesis are the reverse of those during hydrolysis, the first synthesis step involves a 30° rotation of the $\gamma$ subunit that forms the tightly wound coiled-coil. The available data suggest that the intersubunit H-bonds and salt bridges appear to be stronger in this conformation. Consequently, the proton-motive force may be capable of driving the 30° rotation, even though substrate has not bound to the empty catalytic site. With the mutants described here, the energy of the proton gradient may be sufficient to induce rotations greater than 30° that induce conformations of the catalytic sites not conducive to product formation.

**Acknowledgments**—We thank Jennifer Sniegowski and David S. Lowry for assistance with construction of mutants, Kathryn Boltz for development of methods, and Laura Rodriguez for excellent technical support.

**REFERENCES**

1. Frasch, W. D. (2000) *Biochim. Biophys. Acta* **1458**, 310–325
2. Noji, H., Yasuda, R., Yoshida, M., and Kinosita, K., Jr. (1997) *Nature* **386**, 299–302
3. Yasuda, R., Noji, H., Yoshida, M., Kinosita, K., Jr., and Itoh, H. (2001) *Nature* **410**, 898–904
4. Abrahams, J. P., Leslie, A. G., Lutter, R., and Walker, J. E. (1994) *Nature* **370**, 621–628
5. Braig, K., Menz, R. I., Montgomery, M. G., Leslie, A. G., and Walker, J. E. (2000) *Structure Fold Des.* **8**, 567–573
6. Menz, R. I., Walker, J. E., and Leslie, A. G. (2001) *Cell* **106**, 331–341
7. Al-Shawi, M. K., Ketchum, C. J., and Nakamoto, R. K. (1997) *J. Biol. Chem.* **272**, 2300–2306
8. Chen, W., and Frasch, W. D. (2001) *Biochemistry* **40**, 7729–7735
9. Sambrook, J., Frasch, E. F., and Mannatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
10. Wise, J. G. (1990) *J. Biol. Chem.* **265**, 10403–10409
11. Kato, Y., Sasayama, T., Muneyuki, E., and Yoshida, M. (1995) *Biochim. Biophys. Acta* **1231**, 275–281
12. Al-Shawi, M. K., and Senior, A. E. (1988) *J. Biol. Chem.* **263**, 19640–19648
13. Zhou, Y., Duncan, T. M., and Cross, R. L. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 10583–10587
14. Nakamoto, R. K., al-Shawi, M. K., and Futai, M. (1995) *J. Biol. Chem.* **270**, 14042–14046