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Catalysis and energy coupling of H^+-ATPase (ATP synthase): molecular biological approaches

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Abstract

The molecular biological approach has provided important information for understanding the F_oF_1 H^+-ATPase. This article focuses on our recent results on the catalytic site in the β subunit, and the roles of α/β subunit interaction and amino/carboxyl terminal interaction of the γ subunit in energy coupling. Extensive mutagenesis of the β subunit revealed that βLys-155, βThr-156, βGlu-181 and βArg-182 are essential catalytic residues. βGlu-185 is not absolutely essential, but a carboxyl residue may be necessary at this position. A pseudo-revertant analysis positioned βGly-172, βSer-174, βGlu-192 and βVal-198 in the proximity of βGly-149. The finding of the roles of βGly-149, βLys-155, and βThr-156 emphasized the importance of the glycine-rich sequence (Gly-X-X-X-Gly-Lys-Thr/Ser, *E. coli* β residues between βGly-149 and βThr-156) conserved in many nucleotide binding proteins. The *A* subunits of vacuolar type ATPases may have a similar catalytic mechanism because they have conserved glycine-rich and Gly-Glu-Arg (corresponding to βGly-180–βArg-182) sequences. The results of these mutational studies are consistent with the labeling of βLys-155 and βLys-201 with AP3-PL, and of βGlu-192 with DCCD [15]. The DCCD-binding residue of a thermophilic *Bacillus* corresponds to βGlu-181, an essential catalytic residue discussed above. The defective coupling of the βSer-174 → Phe mutant was suppressed by the second mutation αArg-296 → Cys, indicating the importance of α/β interaction in energy coupling. The γ subunit, especially its amino/carboxyl interaction, seems to be essential for energy coupling between catalysis and transport judging from studies on γMet-23 → Lys or Arg mutation and second-site mutations which suppressed the γLys-23 mutation. Thus the conserved γMet-23 is not absolutely essential but is located in the important region for amino/carboxyl interaction for energy coupling.

Key words: ATPase, H^+-; ATPase, F_oF_1-; Energy coupling

1. Introduction

The F-type H^+-ATPase (F_oF_1) synthesizes ATP coupling with the transmembrane electrochemical proton gradient, and is found in membranes of mitochondria, chloroplasts, and bacteria. The *Escherichia coli* enzyme has been analyzed extensively at the level of amino acid residues taking advantage of easy gene manipulations in this organism, and the results have contributed significantly to understanding eukaryotic enzymes (for review, see Refs. [1–4]). Like organellar enzymes, the *E. coli* enzyme is composed of membrane extrinsic F_1 and intrinsic F_o sectors formed by different subunits with defined stoichiometries: F_1, $\alpha_3\beta_3\gamma\delta\epsilon$; F_o, ab_2c_{10}.

The catalytic site is located in the β subunit of the F_1 sector, and the proton pathway of F_o is formed from amino acid residues in the β and γ subunits. Purified F_1 can hydrolyse ATP following two kinetic modes. In unisite catalysis (ATP/F_1 ratio ≤ 1), ATP is hydrolyzed slowly, and the ratio of enzyme-bound ATP to enzyme bound ADP + P_i reaches approximate unity at equilibrium. In multisite (steady state) catalysis in the presence of excess ATP, the rates of release of ADP and P_i are 10^4–10^6 times higher as a result of catalytic cooperativity between multiple catalytic sites. The F_o sector becomes a passive proton pathway once F_1 is removed. Upon reconstitution of F_oF_1, ATP is synthe-

Abbreviations: DCCD, dicyclohexycarbodiimide, AP3-PL, adenosine triphosphopyridoxal; P_i, inorganic phosphate.

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sized with an electrochemical proton gradient as a driving force, and in a reverse direction, ATP hydrolysis is coupled with proton transport.

The molecular mechanism of ATP synthesis/hydrolysis and its coupling with proton translocation are still interesting, challenging questions. The mechanism may involve successive conformational changes of different domains in the β subunit followed by their transmission to other subunits. In this article we discuss our most recent results on the roles of amino acid residues in catalysis and those of the α and γ subunits in energy coupling.

2. Catalytic site of H^+-ATPase

2.1. Glycine-rich sequence and βLys-155 and βThr-156 residues

Our understanding of the catalytic site was initiated by the identification of a βAla-151 → Val mutant [5] of the β subunit and covalent affinity labeling of βLys-155 by an ATP analogue (AP3-PL, adenosine triphosphopyridoxal) [6]. The βVal-151 mutant F_1 showed low multisite catalysis and unisite catalysis with altered kinetics [5]. Binding of one mole of AP3-PL to the βLys-155 or βLys-201 residue resulted in loss of uni- and multi-site catalysis [6, 7]. The two residues βAla-151 and βLys-155 are in the glycine-rich sequence (Gly-Gly-Ala-Gly-Val-Gly-Lys-Thr, *E. coli* β subunit position 149–156) [8]. The consensus sequence (Gly-X-X-X-Gly-Lys-Thr/Ser) is conserved in many proteins capable of nucleotide binding. This sequence in crystalline *ras* protein [9] or adenylate kinase [10] forms a loop between an α helix and β sheet. We introduced mutations into positions 151 and 155, and analyzed the mutant enzymes in detail. The βAla-151 residue (non-conserved residue) was not essential because the βAla-151 → Pro mutant synthesized ATP as well as the wild type and had 2 fold higher membrane ATPase activty [11]. Consistent with the conservation, βLys-155 → Ala, Ser, or Thr, or βThr-155/βLys-156 mutants could not grow on succinate by oxidative phosphorylation and had very low membrane ATPase activities [12]. Purified βSer-155 and βAla-155 enzymes had very low unisite ($\leq$ 1.5% of the wild type) and multisite ($\leq$ 0.02% of the wild type) catalytic activities [12]. The $k+1$ (rate of ATP binding) values of the mutant enzymes for unisite catalysis were lower than that of the wild type: 10^2-fold lower for the βAla-155 and βSer-155 mutant enzymes. These results suggest that the βLys-155 is essential for catalysis.

An enzyme in which the entire glycine-rich sequence was replaced by that of the p21 *ras* protein (Gly-Ala-Gly-Gly-Val-Gly-Lys-Ser, residues 10–17) retained activity, indicating that βThr-156 can be replaced by a Ser residue [11]. This suggests that the β subunit sequence (between βGly-149 and βThr-156) forms a loop structure similar to that in the *ras* protein. Consistent with the results on the effect of introduction of the *ras* sequence into the β subunit, a βThr-156 → Ser mutant could grow on succinate by oxidative phosphorylation and had 1.5-fold higher membrane ATPase activity than the wild type [12]. However, βThr-156 → Cys, Ala, or Asp, or βAla-156/βThr-157 mutants had no membrane ATPase activity. Purified βAla-156 and βCys-156 mutants showed low unisite (less than 1.5% of the wild type) and multisite (less than 0.02% of wild type) catalysis and no detectable unisite $k+1$ values [12,13]. These results suggest that the βThr-156 residue is essential, although it can be replaced by Ser.

2.2. Domain near the glycine-rich sequence

The next obvious approach was to identify the amino acid residues interacting with the glycine-rich sequence. This could be done by isolating pseudo-revertants of a mutant of the glycine-rich sequence. We showed that the negative phenotype (no growth by oxidative phosphorylation) of the βSer-174 → Phe mutant was suppressed by a second-site mutation βGly-149 → Ser, Ala, or Cys in the glycine-rich sequence [13]. The membrane ATPase activity of the βPhe-174 single mutant was less than 10% of that of the wild type but increased to about 100% (βAla-149/βPhe-174) or 50% (βSer-149/βPhe-174, βCys-149/βPhe-174). However, the βGly-149 → Thr mutation did not suppress the βPhe-174 mutation. A single βGly-149 → Cys mutant could not grow by oxidative phosphorylation, indicating that the two defective mutations βCys-149 and βPhe-174 suppressed each other in the βCys-149/βPhe-174 mutant. The effect of βCys-149 mutation was suppressed by βGly-172 → Glu, βSer-174 → Phe, βGlu-192 → Val, or βVal-198 → Ala replacement [14]. These results suggest that βGly-149, βGly-172, βSer-174, βGlu-192 and βVal-198 are located close together in the catalytic site. Consistent with this possibility, F_1-ATPases with the double mutations βCys-149/βGlu-172, βCys-149/βPhe-174, βCys-149/βVal-192, and βCys-149/βAla-198 were less sensitive than the wild-type to DCCD (binding sites, βGlu-192) [15] and AP3-PL (binding site, βLys-155 and βLys-201) [6]. From these results we propose a model of the catalytic site near the ATP γ phosphate (Fig. 1) [14].

2.3. Roles of βGlu-181, βArg-182 and βGlu-185 residues in catalysis

As the catalytic residues seemed to be in βGly-172 - βVal-198 near the catalytic site, We next introduced a series of mutations between βGly-161 and βLys-201,

and found that βGlu-181 and βArg-182 are essential for enzyme catalysis [16]. None of the mutants at position 181 or 182 (βGlu-181 → Gln, Asp, Asn, Thr, Ser, Lys, or Ala; βArg-182 → Ala, Glu, Gln, or Lys) could grow by oxidative phosphorylation and they had essentially no membrane ATPase activity. Moreover, purified βGln-181, βAla-181, and βGln-182 F_1-ATPases showed very low multisite catalysis and slow rates ($\leq 1\%$ of the wild type) of unisite catalysis with greatly altered kinetics: e.g., their K_d $(k-1/k+1)$ values were two orders of magnitude higher than that of the wild-type.

The βGlu-185 → Ala or Gln mutant could not grow by oxidative phosphorylation and had no membrane ATPase, whereas the βGlu-185 → Asp mutant could grow and had 30% of the wild-type ATPase activity. Purified βGln-185 F_1-ATPase retained unisite catalysis with 1/3 of the wild-type rate and rate of ATP binding $(k+1)$, whereas βAsp-185 enzyme showed similar unisite catalysis to the wild type. These results suggest that βGlu-185 is not absolutely essential for catalysis but that a carboxyl moiety at this position may be essential.

3. Importance of α/β subunit interaction(s) for energy coupling

3.1. The βSer-174 → Phe mutant showed reduced coupling efficiency

As described above, we proposed a model of the catalytic site in which βGly-149, βGly-172, βSer-174, βGlu-192 and βVal-198 residues are located close together (Fig. 1) [14]. We introduced different residues (Gly, Ala, Thr, Leu, or Phe) at position 174, and found that the larger the side chain volume of the residue at this position, the lower the multisite activity became [17]. Thus mutation at this postion altered the conformation of catalytic residues, resulting in lower catalytic cooperativity. Of these mutants, only βPhe-174 was defective in growth by oxidative phosphorylation. Surprisingly, comparison of the βPhe-174 mutant with the βLeu-174 mutant showed that both had essentially the same membrane ATPase activity (about 10% of the wild-type activity). However, the βLeu-174 mutant could grow by oxidative phosphorylation, whereas the βPhe-174 could not. It is understandable that the βLeu-174 mutant could grow well because its membrane ATPase activity (coded by a multi-copy plasmid) was similar to that of the normal haploid strain carrying only the chromosomal operon for F_oF_1 [17]. Consistent with the lack of growth, membrane vesicles of the βPhe-174 mutant synthesized less ATP and formed a much lower ATP-driven proton gradient than those of the βLeu-174 mutant. These results indicated that the

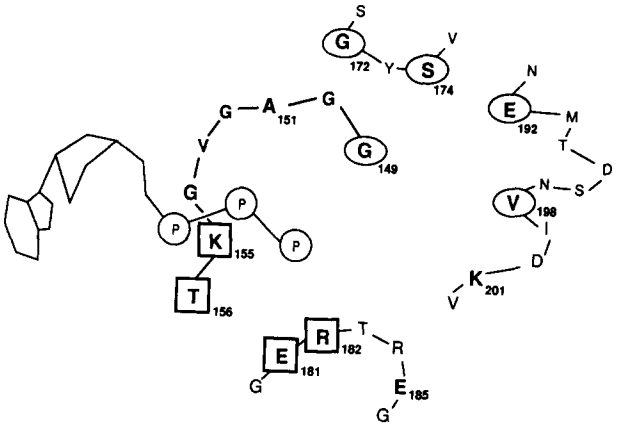

Fig. 1. Catalytic site of H^+-ATPase. A model of the catalytic site near the ATP γ phosphate is shown. The combined approaches of affinity labeling and analysis of mutants and their pseudo-revertants suggest amino acid residues in or near the catalytic site. Amino acid residues shown by thick letters are discussed in the text: residues in boxes are essential for catalysis. Residues in oval circles are suggested to be nearby from mutation/suppression studies. Modified from Iwamoto et al. [14].

βPhe-174 mutant is defective in energy coupling between catalysis and proton transport, whereas in the βLeu-174 mutant this coupling is efficient.

3.2. Suppression of the βSer-174 → Phe mutant by an αArg-296 → Cys mutation

We found that the defect of energy coupling of the βPhe-174 mutant was suppressed by a second-site mutation in the α subunit [17]. Mutations were introduced randomly into the α subunit gene and ligated into the mutant plasmid (βSer-174 → Phe). Oxidative phosphorylation positive strains carried αArg-296 → Cys and βSer-174 → Phe mutations. The αCys-296/βPhe-174 mutant grew well in liquid medium by oxidative phosphorylation giving essentially the same growth yield as βLeu-174. Consistent with these results, membrane vesicles of the αCys-296/βPhe-174 mutant formed a much higher electrochemical gradient than those of the βPhe-174 mutant. These results indicate that the defective energy coupling resulting from the βPhe-174 mutation was suppressed by the αCys-296 mutation. Thus α/β subunit interaction is essential for energy coupling. The region around αArg-296 is highly conserved in the α subunit of various organisms, and many random mutants have been mapped in this region (αPro-281 → Leu, αAla-285 → Val, αGlu-299 → Lys, αArg-303 → Cys, αAla-303 → Val) [18–20]. Furthermore, this region is not conserved in the β subunit and has been proposed to be a non-catalytic nucleotide binding domain of the α subunit [21]. Thus the interaction between the catalytic domain including βSer-174 and the proposed nucleotide domain of the α subunit may be required for the energy coupling.

4. Role of the γ subunit in regulation of F_oF_1

4.1. Three unique regions of the γ subunits

On aligning the known γ subunit sequences to obtain maximal homology, we found three unique regions (Fig. 2): (*A*) a stretch of about thirty amino acids in the chloroplast γ subunit of spinach [22], *Arabidopsis thaliana* [23] and other plants, but not in those of mitochondria or bacteria [24]; (*B*) the amino terminal region between γIle-19 and γVal-26, which is conserved in all γ subunits of various origins; (*C*) a carboxyl terminal region betwen γGlu-238 and γAla-285, which is highly conserved. The 22 out of conserved 28 residues are in the region *B* and *C*.

Region *A* of the chloroplast enyme has two cysteine residues γCys-199 and γCys-205 (spinach enzyme) forming a disulfide bond in the dark [25]. Upon illumination of the chloroplasts, the disulfide bond becomes accessible to the thioredoxin/feredoxin system and the reduction induces activation of the enzyme. This reduction/oxidation is the regulatory mechanism of the chloroplast enzyme. An obvious question is whether the γ subunits of mitochondrial or bacterial enzymes have a similar regulatory role(s), although they lack region *A*. Our early studies indicated that deletion of the sequence between γLys-21 and γAla-27 (region *A*) caused defective assembly of the F_1 sector in membranes [26]. This finding supports the results of assembly studies showing that the γ subunit is required to obtain the minimal assembly ($\alpha_3\beta_3\gamma$) having ATPase activity [27].

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   240        250        260        270        280    286
   *    ** **  *   *       *     *  ***  ** *  *    **
···EQA ARMVAMKAAT DNGGSLIKEL QLVYNKARQA SITQELTEIV SGAAAV
        ▼                            ▼▼   ▼▼     ▼▼▼
        C                            RV   TS     GTA
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Fig. 3. γ Subunit second-site mutations that suppress γMet-23 → Lys. The carboxyl-terminal sequence of the *E. coli* γ subunit is shown. Amino acid replacements that suppressed the γMet-23 → Lys mutation are indicated by arrowheads. Positions with identical residues in all the γ subunits so far sequenced are indicated by asterisks. Modified from Nakamoto et al. [24].

The regulatory role of the carboxyl terminal region *C* was first suggested by nonsense mutants isolated randomly. The two mutants γAla-283 → end and γThr-277 → end had 63 and 14% of the wild-type membrane ATPase activity, respectively [28]. These mutants grew at reduced rates by oxidative phosphorylation, indicating that the ten residues are dispensable for catalysis. However, the γGln-269 → end mutant could not grow by oxidative phosphorylation, although the mutant F_1 was assembled in membranes [29]. On the other hand, the γGln-261 → end mutant did not show normal assembly of the enzyme. We introduced mutations between γGln-269 and γLeu-278 [28]. Mutants with γGln-269 → Glu or Leu, γThr-273 → Val or Gly, and γGlu-275 → Lys or Glu showed assembly of the enzyme but decreased membrane ATPase activity and growth by oxidative phosphorylation. These results suggest that the carboxyl terminal residues are essential for normal activity. Since the γ subunit does not have catalytic residues (or at least does not bind ATP), these mutational studies suggest that the γ subunit is important for regulation of ATPase activity.

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                           **   *  *                                            *
Ec      AGAKEIRSKI ASVQNTQKIT KAMEMVAASK MRKSQDRMAA SRPYAETMRK VIGHLA-HGN L-EYKHPYLE DRDVKRVGYL
mit     ATLKDITRRL KSIKNIQKIT KSMKMVAAAK YARAERELKP ARVYGVGSLA LYEKAD---- ---IKTP--E DK--KK--HL
chl     ANLRELRDRI GSVKNTQKIT EAMKLVAAAK VRRAQEAVVN GRPFSETLVE VLYNMNEQLQ TEDVDVP-LT KIRTVKKVAL
                           ________
                               B

                **  *                                *
Ec      VV--STDRGL CGGLNINLFK KLLAEMKTWT DKGVQCDLAM IGSKGVSFFN SVGGNVVAQV TGMGDN--PS LSELIGPVKV
mit     IIGVSSDRGL CGAIHSSVAK QMKSEAANLA AAGKEVKIIG VGDKIRSILH RTHSDQFLVT FKEVGRRPPT FGDASVIALE
chl     MVV-TGDRGL CGGFNNMLLK KAESRIAELK KLGVDYTIIS IGKKGNTYFI RRPEIPVDRY FD-GTNL-PT AKEAQAIADD

Ec      MLQAYDEGRL DKLYIVSNKF INTMSQVPTI SQLLPLPASD ---------- ---------- ---------- ----DDDLKH
mit     LLNSGYE--F DEGSIIFNRF RSVISYKTEE KPIFSLDTI- ---------- ---------- ---------- ---SSAESMS
chl     VFSLFVSEEV DKVEMLYTKF VSLVKSDPVI HTLLPLSPKG EICDINGKCV DAAEDELFRL TTKEGKLTVE RDMIKTETPA
                                                ____________________________________
                                                                  A

                       *                        *    ** **  *   *       *     *  ***  ** *  *    **
Ec      KSWDYLYEPD PKALLDTLLR RYVESQVYQG VVENLASEQA ARMVAMKAAT DNGGSLIKEL QLVYNKARQA SITQELTEIV SGAAAV  286
mit     -----IYDDI DADVLRNYQE YSLANIIYYS LKESTTSEQS ARMTAMDNAS KNASEMIDKL TLTFNRTRQA VITKELIEII SGAAAL  272
chl     FSPILEFEQD PAQILDALLP LYLNSQILRA LQESLASELA ARMTAMSNAT DNANELKKTL SINYNRARQA KITGEILEIV AGANACV 323
                                              _____________________________________________
                                                                     C
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Fig. 2. Alignment of γ subunit sequences from *E. coli*, mitochondria and chloroplast. Amino acid sequences of the γ subunits from *E. coli* (Ec), bovine mitochondria (mit) and spinach chloroplasts (chl) are shown. Positions with identical amino acid residues in all the γ subunits so far sequenced are indicated by asterisks. Three unique regions (A, B and C) discussed in the text are indicated. Modified from Nakamoto et al. (24).

4.2. Loss of energy coupling by the γ subunit mutations

A possible role(s) of the γ subunit in coupling was suggested by studies of mutants mapped in the carboxyl terminal region. γGln-269 → Leu, γGlu-275 → Lys, γThr-277 → end mutants and a frame shift mutant had similar ATPase activity (about 15% of that of the wild type), but showed different degrees of ATP-dependent proton gradients [28]. Control experiments indicated that the differences in the proton gradients were not due to increased nonspecific proton leakage of the membranes, and supported the idea that the mutations resulted in various degrees of defective coupling between ATP hydrolysis and proton translocation. These results prompted us to introduce mutations in other regions especially the amino terminus, another conserved region between position 19 and 33 (Fig. 2).

The most interesting mutants were γMet-23 → Lys and γMet-23 → Arg [30]. They could grow only slowly by oxidative phosphorylation and could be clearly identified as strains unable to grow on succinate solid medium. However, the membranes from γLys-23 and γArg-23 mutants had 65% and 100% of the wild-type membrane ATPase activity, and formed low ATP-dependent proton gradients of 16 and 40%, respectively, of that of wild-type membranes. Control experiments indicated that the mutant membranes had the same levels of respiratory proton gradients and that the F_o sector functioned normally when combined with wild-type F_1. These results suggest that the γMet-23 → Lys or Arg mutant enzymes are defective in energy coupling between catalysis and proton translocation. In contrast with these two mutants, other mutants including γMet-23 → Asp, Glu, or Leu mutants showed wild-type level proton gradients dependent on ATP, membrane ATPase activities and growths by oxidative phosphorylation.

4.3. Mutations in the carboxyl terminal region suppressing the effect of γLys-23 mutation

The defective couplings of the γLys-23 and γArg-23 mutants suggest that the γ subunit is important for energy coupling. If the properties of the γLys-23 mutation are due to a defect in the intrinsic properties of the γ subunit, it could be suppressed by a second-site mutation(s) in the same subunit. The neighboring amino acid residues in the higher-ordered structure could be identified from such suppressor mutations. We screened second site mutations in the γ subunit which conferred growth by oxidative phosphorylation to the γ Lys-23 mutant [31]. Eight such mutations were identified: γArg-242 → Cys, γGln-269 → Arg, γAla-270 → Val, γIle-272 → Thr, γThr-273 → Ser, γGlu-278 → Gly, γIle-279 → Thr, and γVal-280 → Ala in combination with γMet-23 → Lys. These mutants were able to grow on succinate by oxidative phosphorylation. Efficient ATP-dependent proton transport was restored in membranes from these double mutants.

The single mutations γGln-269 → Arg and γThr-273 → Ser caused slow growth by oxidative phosphorylation. However, growth was substantially recovered, when one of these mutations was combined with the γLys-23 mutation. Furthermore, strains carrying γLys-23, γArg-269, or γSer-273 as a single mutation were temperature sensitive, showing slower growth by oxidative phosphorylation than that of the wild-type at 37°C but increased growth at 25°C. Consistent with this temperature sensitive growth, membranes of these single mutants showed lower ATP-dependent proton transport at 37°C compared with that at 25°C. On the other hand, the double mutants γLys-23/γArg-269 and γLys-23/γSer-273 showed higher growth yields at 37°C and 25°C (about 80% of the wild type). The membranes of the double mutants showed higher ATP-dependent proton transport. These results suggest that γMet 23, γArg-242 and the region γGln-269 - γVal-280 are close to each other and interact to mediate coupling between ATP synthesis/hydrolysis and proton translocation. It is noteworthy that the amino acid changes that suppressed the γLys-23 mutation do not fall into a pattern such as from large to small or non-polar to polar residues. Thus a γLys-23 mutation destabilized the subtle interaction between the amino and carboxyl terminal regions, and a second-site mutation or low temperature restored the efficient energy coupling. Other possibilities have been discussed previously [24].

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References

[1] Futai, M., Noumi, T. and Maeda, M. (1989) Annu. Rev. Biochem. 58, 111–136.
[2] Fillingame, R.H. (1990) in The Bacteria (Krulwich, T.A., ed.), pp. 345–391, Academic Press, New York.
[3] Futai, M., Iwamoto, A., Omote, H., Orita, Y., Shin, K., Nakamoto, R.K. and Maeda, M. (1992) J. Exp. Biol. 172, 443–449.
[4] Senior, A.E. (1990) Annu. Rev. Biophys. Chem. 19, 7–41.
[5] Hsu, S.Y., Noumi, T., Takeyama, M., Maeda, M., Ishibashi, S. and Futai, M. (1987) FEBS Lett. 218, 222–226.
[6] Ida, K., Noumi, T., Maeda, M., Fukui, T. and Futai, M. (1991) J. Biol. Chem. 266, 5424–5429.

[7] Noumi, T., Tagaya, M., Miki-Takeda, K., Maeda, M., Fukui, T. and Futai, M. (1987) J. Biol. Chem. 262, 7686–7692.
[8] Walker, J.E., Saraste, M., Runswick, M.J. and Gay, N.J. (1982) EMBO J. 1, 945–951.
[9] Pai, E.F., Kabsch, W., Krengel, U., Holmes, K.C., John, J., and Wittinghofer, A. (1989) Nature 341, 209–214.
[10] Dreusicke, D., Karplus, P.A. and Schulz, G.A. (1988) J. Mol. Biol. 199, 359–371.
[11] Takeyama, M., Ihara, K., Moriyama, Y., Noumi, T., Ida, K., Tomioka, N., Itai, A., Maeda, M. and Futai, M. (1990) J. Biol. Chem. 265, 21279–21284.
[12] Omote, H., Maeda, M. and Futai, M. (1992) J. Biol. Chem. 267, 20571–20576.
[13] Iwamoto, A., Omote, H., Hanada, H., Tomioka, N., Itai, A., Maeda, M. and Futai, M. (1991) J. Biol. Chem. 266, 16350–16355.
[14] Iwamoto, A., Park, M.Y., Maeda, M. and Futai, M. (1993) J. Biol. Chem. 268, 3156–3160.
[15] Yoshida, M., Allison, W.S., Esch, F.S. and Futai, M. (1982) J. Biol. Chem. 257, 10033–10037.
[16] Park, M.Y., Omote, H., Maeda, M. and Futai, M. (1994) in press.
[17] Omote, H., Park, M.Y., Maeda, M. and Futai, M. (1994) J. Biol. Chem. 269, 10265–10269.
[18] Soga, S., Noumi, T., Takeyama, M., Maeda, M. and Futai, M. (1989) Arch. Biochem. Biophys. 268, 643–648.
[19] Maggio, M.B., Pagan, J., Parsonage, D., Hatch, L. and Senior, A.E. (1987) J. Biol. Chem. 262, 8981–8984.
[20] Pagan, J. and Senior, A.E. (1990) Arch. Biochem. Biophys. 277, 283–289.
[21] Maggio, M.B., Parsonage, D. and Senior, A.E. (1988) J. Biol. Chem. 263, 4619–4623.
[22] Miki, J., Maeda, M., Mukohata, Y. and Futai, M. (1988) FEBS Lett. 232, 221–226.
[23] Inohara, N., Iwamoto, A., Moriyama, Y., Shimomura, S., Maeda, M. and Futai, M. (1991) J. Biol. Chem. 266, 7333–7338.
[24] Nakamoto, R.K., Shin, K., Iwamoto, A., Omote, H., Maeda, M. and Futai, M. (1992) Ann. NY Acad. Sci. 671, 335–344.
[25] McCarty, R.E. (1982) Ann. NY Acad. Sci. 402, 84–90.
[26] Kanazawa, H., Hama, H., Rosen, B.P. and Futai, M. (1985) Arch. Biochem. Biophys. 241, 364–370.
[27] Futai, M. (1977) Biochem. Biophys. Res. Commun. 79, 1231–1237.
[28] Iwamoto, A., Miki, J., Maeda, M. and Futai, M. (1990) J. Biol. Chem. 265, 5043–5048.
[29] Miki, J., Takeyama, M., Noumi, T., Kanazawa, H., Maeda, M. and Futai, M. (1986) Arch. Biochem. Biophys. 251, 458–464.
[30] Shin, K., Nakamoto, R.K., Maeda, M. and Futai, M. (1992) J. Biol. Chem. 267, 20835–20839.
[31] Nakamoto, R.K., Maeda, M. and Futai, M. (1993) J. Biol. Chem. 268, 867–872.
[32] Inatomi, K., Eya, S., Maeda, M. and Futai, M. (1989) J. Biol. Chem. 264, 10954–10959.
[33] Yoshida, M., Poser, J.W., Allison, W.S. and Esch, F.S. (1981) J. Biol. Chem. 256, 148–153.