Hypothesis

# The evolution of A-, F-, and V-type ATP synthases and ATPases: reversals in function and changes in the $H^+$/ATP coupling ratio

Richard L. Cross[a,*], Volker Müller[b]

[a] *Department of Biochemistry and Molecular Biology, SUNY Upstate Medical University, Syracuse, NY 13210, USA*
[b] *Institute of Microbiology, University of Frankfurt, 60439 Frankfurt, Germany*

Received 23 June 2004; revised 18 August 2004; accepted 30 August 2004

Available online 11 September 2004

Edited by Peter Brzezinski

**Abstract Members of the $F_oF_1$, $A_oA_1$ and $V_oV_1$ family of ATP synthases and ATPases have undergone at least two reversals in primary function. The first was from a progenitor proton-pumping ATPase to a proton-driven ATP synthase. The second involved transforming the synthase back into a proton-pumping ATPase. As proposed earlier [FEBS Lett. 259 (1990) 227], these reversals required changes in the $H^+$/ATP coupling ratio from an optimal value of about 2 for an ATPase function to about 4 for an ATP synthase function. The doubling of the ratio that occurred at the ATPase-to-Synthase transition was accomplished by duplicating the gene that encodes the nucleotide-binding catalytic subunits followed by loss of function in one of the genes. The halving of the ratio that occurred at the Synthase-to-ATPase transition was achieved by a duplication/fusion of the gene that encodes the proton-binding transporter subunits, followed by a loss of function in one half of the double-sized protein. These events allowed conservation of quaternary structure, while maintaining a sufficient driving force to sustain an adequate phosphorylation potential or electrochemical gradient. Here, we describe intermediate evolutionary steps and a fine-tuning of the $H^+$/ATP coupling ratio to optimize synthase function in response to different environments. In addition, we propose a third reversal of function, from an ATPase back to an ATP synthase. In contrast to the first two reversals which required a partial loss in function, the change in coupling ratio required for the third reversal is explained by a gain in function.**


*Keywords:* $A_oA_1$; $F_oF_1$; $V_oV_1$ ATP synthase; ATPase

## 1. Introduction

One of the best characterized members of the F-, A- and V-type family of ATP synthases and ATPases is the $F_OF_1$-ATP synthase from *Escherichia coli* (Fig. 1). The hydrophilic $F_1$ domain of this energy-transducing, membrane-embedded complex contains three catalytic β subunits that synthesize ATP from ADP and $P_i$ during oxidative phosphorylation. The hydrophobic $F_o$ domain conducts protons, or in some prokaryotes, sodium ions,[1] across the bilayer, down an electrochemical gradient. In coupling proton transport to ATP synthesis, the $F_o$ and $F_1$ domains function as a pair of rotary motors linked by a common central rotor ($\gamma\varepsilon c_{10\text{-}14}$) and a peripheral stator ($b_2\delta$). In the model shown in Fig. 1, rotation of the *c*-ring in $F_o$ allows protons to be carried by essential, *c*-subunit, mid-membrane carboxylates (*c*Asp61 in *E. coli*) between two partial subunit-*a* channels that lead to opposite sides of the membrane. As a result of the presence of the stator and the existence of a stable interaction between γε and the top of the *c*-subunit oligomer, rotation of the *c*-ring forces γ to rotate in the center of the $F_1$ domain. The rotation of γ indirectly drives net ATP synthesis by inducing cyclical conformational changes in the three alternating cooperative β subunits that result in the tight binding of substrate and release of product [1].

In 1990, Cross and Taiz [2] proposed a means by which this basic structure may have adapted, at critical points in its evolution, to reversals in primary function from a progenitor ATPase to an ATP synthase and then back to an ATPase. Two assumptions were made. The first was that in interconverting energy stored in the electrochemical gradient with that stored in ATP, the $H^+$/ATP coupling ratio, "*n*", should equal the ratio of proton-translocating carboxylates in the *c*-rings of $A_o$, $F_o$ and $V_o$ (i.e., with each 360° rotation of the ring, each carboxylate is expected to transport 1 $H^+$) to the number of catalytic subunits in $A_1$, $F_1$ and $V_1$ (i.e., with each 360° rotation of γ, each catalytic site is expected to produce or consume 1 ATP). The value of "*n*" determines the thermodynamic relationship between the two pools of stored energy according to the equation, $\Delta G(\mathrm{ATP}_{\mathrm{synth}}) = -n\Delta\mu_{\mathrm{H}+}$. The second assumption was that in order to function primarily as a synthase, a larger value for "*n*" (about 4) would be desirable as more protons consumed per ATP synthesized would assure maintenance of a high phosphorylation potential. In contrast, when functioning exclusively as an ATPase, a smaller "*n*" value (about 2) would be optimal as a larger electrochemical gradient would be maintained if less protons were pumped per ATP hydrolyzed.

To achieve a transition from its original role as a proton-pumping ATPase in an anaerobe (Table 1, stage 1) to an ATP

* Corresponding author. Fax: +1-315-464-8750.
*E-mail address:* crossr@upstate.edu (R.L. Cross).

*Abbreviations:* The sources of ATP synthases and ATPases are denoted as $A_oA_1$ for archaea; $F_oF_1$ for bacteria $MF_oF_1$ for mitochondria $CF_oF_1$ for chloroplasts; $V_oV_1$ for vacuolar membranes

[1] No species-specific distinctions are made between the transport of protons or sodium ions in the remaining text and the term "$H^+$/ATP coupling ratio" is applied to the transport of either ion.

0014-5793/$22.00 
doi:10.1016/j.febslet.2004.08.065

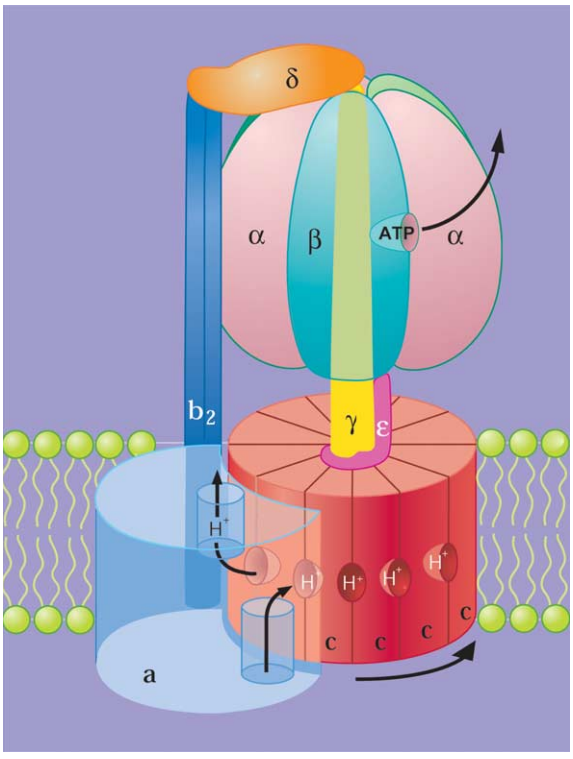


Fig. 1. A rotary binding-change model for the *E. coli* ATP synthase: In this model, the *a*-subunit contains two partial channels, each connected to a different side of the membrane. For a proton to traverse the membrane, it must move through one channel to the middle, bind to one of the *c* subunits and then be carried to the other partial channel by rotation of the *c*-ring. The *c* subunits are anchored to the γ-subunit (part of the rotor), whereas the *a*-subunit is anchored through $b_2\delta$ to the $\alpha_3\beta_3$ hexamer (part of the stator). Hence, rotation of the *c*-ring relative to the *a*-subunit in $F_o$ will drive the rotation of γ relative to the $\alpha_3\beta_3$ hexamer in $F_1$. Rotation of γ in turn drives conformational changes at the catalytic sites on the β subunits promoting the release of product and binding of substrates.

synthase in prokaryotes that had acquired electron transport chains or photosystems (Table 1, stage 2), it was proposed that the gene for the catalytic subunit (Fig. 1, β) underwent duplication with a loss of function in one of the two gene products (Fig. 1, α). This reduced the number of catalytic subunits from 6 in the progenitor ATPase to the 3 observed in extant synthases found in archaea and bacteria, as well as in mitochondria and chloroplasts. By halving the ATP synthesized during each revolution of the motor, the $H^+$/ATP ratio was doubled from 2 to a value of 4. It was further proposed that, at a later point on the evolutionary scale, some synthases reverted to ATPases. To meet the thermodynamic and kinetic requirements of its new role, the $H^+$/ATP ratio was halved (from 4 back to 2) by a duplication and fusion of the gene for the *c*-subunit with a loss of the functional carboxylate from one half of the double-sized protein. This functional transition appears to have occurred during the evolution of eukaryotic cells where an aerobic or photosynthetic symbiont assumed responsibility for synthesizing ATP, allowing the host archaeal ATP synthase to be transformed into an endomembrane vacuolar ATPase (Table 1, stage 3).

Today, the basic tenets of this hypothesis remain viable. However, as discussed below, additional structural information is now available that provides further detail and suggests two intermediate steps, as well as the possibility of a third reversal of function.

## 2. Gene duplication events likely occurred well before the ATPase → Synthase and Synthase → ATPase transitions

Originally, it was thought that gene duplication with loss of function in one of the duplicated genes occurred at the time of each reversal of function. It now appears that the gene duplication events occurred much earlier, driven by other selective pressures.

Duplication of the gene for the catalytic subunit may have conveyed two advantages. First, it would aid in achieving expression of a sufficient number of catalytic subunits (six copies per progenitor ATPase, Table 1). Second, a doubling of the number of complementary surfaces between catalytic subunits would be expected to accelerate the rate of evolution of a stable hexamer. Stability is an important feature considering the mechanical stress exerted on the hexamer during rotation of its asymmetric core. This stabilizing process would have resulted

Table 1
Evolution of the F-, A-, and V-type family of ATPases and Synthases

| Stage | Function (examples) | Catalytic subunits | Functional *c*-subunit carboxylates | *a* subunits | $H^+$/ATP ratio (*n*) | Evidence |
|---|---|---|---|---|---|---|
| 1 | Progenitor anaerobic ATP-driven $H^+$-pump (probably no survivors) | 6 | 12 | 1 | 2 | α and β subunits arose from gene duplication. Thus, originally there were 6 catalytic subunits. |
| 2 | ATP Synthase ($A_oA_1$/$F_oF_1$/$MF_oF_1$/$CF_oF_1$) | 3 | 12[a] | 1 | 4 | Synthases that function in oxidative phosphorylation and photophosphorylation are well characterized. |
| 3 | ATP-driven $H^+$-Pump ($V_oV_1$/Some anaerobic $A_oA_1$ such as *Enterococcus hirae*) | 3 | 6[a] | 1 | 2 | Vacuolar ATPases have double-sized *c* subunits containing a single functional carboxylate as do some anaerobic $A_OA_1$. |
| 4 | ATP Synthase (*Pyrococci, C. fervidus*?) | 3 | 6 | 2 | 4 | Multiple stators have been observed for Cf. Synthases having double-sized *c*'s containing a single functional carboxylate might be expected to have a second *a*-subunit containing stator. |

[a] The *c*-subunit stoichiometries of 12 and 6 listed for stages 2 and 3 are average values for illustrative purposes. See Section 3 for further discussion.

in an alternating hexamer of the two catalytic-gene products. Thus primed, a subsequent ATPase → Synthase transition may have required only a single point mutation of a catalytically essential residue in one of the two genes. This would have resulted in a hexamer, where functional and non-functional subunits alternate as found in the $\alpha_3\beta_3$ complex of extant ATP synthases (Fig. 1). A possible candidate for the mutated essential amino-acid residue that rendered α subunits non-functional was suggested by the high-resolution structure of bovine $MF_1$ [3]. In the catalytic β subunits, E188 hydrogen bonds to a water molecule positioned for an in-line nucleophilic attack on the γ-phosphoryl of ATP. The equivalent α-subunit residue, Q208, cannot function as an acid–base catalyst.

The evidence for gene duplication preceding the second reversal-of-function transition (Synthase → ATPase) is even more compelling. Indeed, the *atp* operon of the hyperthermophilic sulfate-reducing archaeon, *Archaeoglobus fulgidus* contains two identical gene copies, each encoding a normal sized *c*-subunit with a proton-translocating carboxylate [4]. Furthermore, a double-sized *c*-subunit (*c*–*c*) retaining both proton-translocating carboxylates is seen in the ATP synthase from the archaeon, *Methanothermobacter thermoautotrophicus* [5]. Again, ease in achieving expression of a sufficient number of subunits would have been a selective advantage because only 6 of the double-sized *c*'s would be needed per *c*-ring instead of the original 12 copies. Also, the use of a double-sized fused protein as the building block might make it easier to avoid incorporating a non-optimal stoichiometry of subunits during assembly of the *c*-ring. A third advantage would be increased stability of the c-ring as half of the interfaces are now fused. A final advantage would be the potential for fine tuning the $H^+$/ATP coupling ratio in synthases as discussed below.

When organisms with a *c*-*c* fusion returned to anaerobic fermentation as their primary ATP source, as in *Enterococcus hirae* [6], or became hosts for ATP-producing symbionts, as in eukaryotes [7], all that was needed to lower their $H^+$/ATP coupling ratio from 4 to 2 was a single point mutation resulting in loss of the functional carboxylate from one half of the *c*-*c* molecule.

## 3. Fine tuning the $H^+$/ATP coupling ratio in ATP synthases

In addition to halving or doubling the $H^+$/ATP coupling ratio at times of change in primary function, smaller changes in the ratio appear to have occurred as synthases optimized performance in response to the thermodynamics dictated by their unique environments. One way to accomplish this would be to alter the number of subunits in the *c*-ring [8]. Since the size of the ring appears to be determined solely by the intrinsic properties of the *c*-subunit [9], this would have involved mutations in *c*-subunit interfaces that altered the number of units incorporated during assembly. Yeast $MF_o$ has been reported to contain 10 *c*'s per ring [10], as is likely the case for *E. coli* $F_o$ [11]. In contrast, *Ilyobacter tartaricus* [12] and *Propionigenium modestum* [13] $F_o$'s have 11 *c*'s per ring, and chloroplast $CF_o$ has 14 [[14], but see [15]]. The stoichiometry of the *c*-ring in the synthase from the archaeon *Methanopyrus kandleri* was recently, and surprisingly, revealed by the gene sequence. This organism's supersized *c*-gene shows 13 fused *c* subunits with each unit retaining the functional carboxylate [16,17].

The *c*-ring stoichiometries discussed above predict $H^+$/ATP coupling ratios of 10/3, 11/3, 13/3, and 14/3. The different coupling ratios derived for mitochondria (10/3) and chloroplasts (14/3) may reflect differences in the organelles' topography. $MF_1$ is attached to the inner surface of the inner mitochondrial membrane facing the matrix space. During one complete turnover of the synthase, membrane transporters use 3 $H^+$ to move 3 ADP/$P_i$ into, and 3 ATP out of, the matrix against concentration gradients. This results in a higher phosphorylation potential in the cytosol than in the matrix space. Hence, the effective coupling ratio in yeast, with respect to cytosolic ATP, would be (10 + 3)/3. In contrast, thylakoid membranes have the opposite orientation, placing $CF_1$ towards the cytosol and eliminating the need for active transport of substrates and product. Because of this, an additional 3 *c*'s would be required in $CF_o$ to maintain the same cytosolic phosphorylation potential [18]. Thus, when comparing $H^+$/ATP coupling ratios with respect to cytosolic ATP, the yeast (13/3) and plant cell (14/3) values are very similar. The higher coupling ratio for *M. kandleri* (13/3) compared to *E. coli* (10/3) may be necessitated by the environment in which this extreme thermophile lives. At high temperatures, the magnitude of the electrochemical gradient will be reduced due to increased leakage rates [19]. With less energy available per proton transported, the $H^+$/ATP coupling ratio would need to be higher to maintain the same phosphorylation potential.

A second means of optimizing the $H^+$/ATP ratio in synthases would be through duplication of the *c*-subunit gene with partial loss of function, although, in this fine-tuning process, less of a loss would occur than that which accompanied the Synthase → ATPase transition. Multiple gene duplication events have occurred in the anaerobic bacterium *Acetobacterium woodii* to give two 8-kDa *c*'s of identical amino acid sequence, as well as one 16-kDa fused dimer [20]. The double-sized subunit, *c*–*c*x, has a single functional carboxylate in the N-terminal half ("x" indicates the absence of a functional carboxylate in the second *c* unit). Although the stoichiometry of *c*-subunit gene products in the assembled *c*-ring is not known [21], an $H^+$/ATP ratio of 10/3 or 8/3 would be achieved if they assemble as five or four repeats of *c*/*c*–*c*x, respectively. Similarly, a triple-*c* fusion with loss of the N-terminal carboxylate, as found in synthases from the methanogenic archaea *Methanococcus maripaludis* (GenBank Accession member NC_005791) and *M. jannaschii* [22], would give a coupling ratio of 10/3 or 8/3 depending on whether five or four of the *c*x–*c*–*c*'s assemble to form a ring.

Although an average value of 12 was used for the *c*-subunit copy number in illustrating how the $H^+$/ATP coupling ratio may have been halved or doubled at times of reversal of function (Table 1, stage 2), there are, in fact, no confirmed examples of a dodecameric *c*-ring. Taking into account the fact that ATP synthases function as rotary motors, it has been argued that "mismatched symmetry" between the hexameric rings in $F_1$ and $A_1$ and the *c*-rings in $F_o$ and $A_o$ may help avoid rate-limiting energy minima that could conceivably occur with a symmetrical 6:12 ring-component ratio [10]. In the case of vacuolar ATPases (Table 1, state 3), it had long been thought that the *c*-ring consisted of 6 double-sized *c*'s [23]. However, recently the possibility of 7*cx*-*c*'s per $V_o$ has been considered [24] and a stoichiometry of 7*c*x–*c*'s per ring has been demonstrated for the $A_oA_1$ ATPase from the anaerobic bacterium, *Enterococcus hirae* [25]. This is equivalent to the 14 regular-

sized *c*'s found in $CF_o$ [14]. Although too few *c*-rings have been characterized to drawn any final conclusions, the fact that rings of known structure containing 10, 11, 13, and 14 *c*-subunit units skirt the value of 12, suggests that kinetics, as well as thermodynamics, may be an important determinant of ring size.

## 4. A third reversal-of-function transition (ATPase → Synthase) may have occurred

In V- and A-type complexes, the gene for the *a*-subunit is fused with a hydrophilic peptide that appears to function as part of the peripheral stator [26,27]. Having part of the stator covalently tethered to a large hydrophobic subunit, would increase its stability and eliminate the need for the hydrophobic transmembrane helix in the *b*-like subunit [28]. Electron microscopy images showing two stators in $A_oA_1$ from the bacterium *C. fervidus* [29] allow for the possible presence of two *a* subunits. This is supported by an estimate of subunit stoichiometry which gave a *cx*-*c* to *a* ratio of about 5 to 2 and led the authors to propose a model for $A_o$ that includes two *a* subunits [30]. A second *a*-subunit would provide a second proton conduction pathway, thus doubling the $H^+$/ATP ratio. This might allow greater efficiency in pumping protons against the smaller electrochemical gradients that can be maintained at very high temperatures [19]. Alternatively, a second *a*-subunit would allow *C. fervidus* to make ATP using a $\Delta\mu_{H+}$ established by electrogenic end-product efflux or antiport mechanisms as observed in lactic acid bacteria [31]. The same may be true for *Pyrococcus furiosus* which has a *cx*–*c* type proteolipid but is apparently able to synthesize ATP. The acquisition of a proton-translocating hydrogenase by this organism may have led to a reversal of function from an ATPase → Synthase [32]. Considering the size of the *c*-ring and that of the *a*-subunit (Fig. 1), there should be an ample room to accommodate a second *a*-subunit. It is of interest that there is a precedent for multiple *a*-like subunits (MotA) in the bacterial flagellar motor [33].

*Acknowledgements:* This work was supported by Research Grant GM23152 from the National Institutes of Health, USPHS to R.L.C. and the Deutsche Forschungsgemeinschaft (MU 801/10) to V.M.

From these considerations, it appears that this versatile complex has proven remarkably adept over the last few billion years in responding to changes in environment and function, thereby enabling it to maintain a continuous and significant role in cellular bioenergetics.

## References

[1] Boyer, P.D. (1997) Annu. Rev. Biochem. 66, 717–749.
[2] Cross, R.L. and Taiz, L. (1990) FEBS Lett. 259, 227–229.
[3] Abrahams, J.P., Leslie, A.G., Lutter, R. and Walker, J.E. (1994) Nature 370, 621–628.
[4] Klenk, H.P. et al. (1997) Nature 390, 364–370.
[5] Ruppert, C., Schmid, R., Hedderich, R. and Müller, V. (2001) FEMS Microbiol. Lett. 195, 47–51.
[6] Takase, K., Kakinuma, S., Yamato, I., Konishi, K., Igarashi, K. and Kakinuma, Y. (1994) J. Biol. Chem. 269, 11037–11044.
[7] Gogarten, J.P. et al. (1989) Proc. Natl. Acad. Sci. USA 86, 6661–6665.
[8] Schemidt, R.A., Qu, J., Williams, J.R. and Brusilow, W.S. (1998) J. Bacteriol. 180, 3205–3208.
[9] Müller, D.J. et al. (2001) FEBS Lett. 504, 219–222.
[10] Stock, D., Leslie, A.G. and Walker, J.E. (1999) Science 286, 1700–1705.
[11] Jiang, W., Hermolin, J. and Fillingame, R.H. (2001) Proc. Natl. Acad. Sci. USA 98, 4966–4971.
[12] Stahlberg, H., Müller, D.J., Suda, K., Fotiadis, D., Engel, A., Meier, T., Matthey, U. and Dimroth, P. (2001) EMBO Rep. 2, 229–233.
[13] Meier, T., Matthey, U., von Ballmoos, C., Vonck, J., Krug von Nidda, T., Kühlbrandt, W. and Dimroth, P. (2003) J. Mol. Biol. 325, 389–397.
[14] Seelert, H., Poetsch, A., Dencher, N.A., Engel, A., Stahlberg, H. and Müller, D.J. (2000) Nature 405, 418–419.
[15] Turina, P., Samoray, D. and Graber, P. (2003) EMBO J. 22, 418–426.
[16] Slesarev, A.I. et al. (2002) Proc. Natl. Acad. Sci. USA 99, 4644–4649.
[17] Lolkema, J.S. and Boekema, E.J. (2003) FEBS Lett. 543, 47–50.
[18] Ferguson, S.J. (2000) Curr. Biol. 10, R804–R808.
[19] Speelmans, G., Poolman, B., Abee, T. and Konings, W.N. (1994) J. Bacteriol. 176, 5160–5162.
[20] Rahlfs, S., Aufurth, S. and Müller, V. (1999) J. Biol. Chem. 274, 33999–34004.
[21] Aufurth, S., Schagger, H. and Müller, V. (2000) J. Biol. Chem. 275, 33297–33301.
[22] Ruppert, C. et al. (1999) J. Biol. Chem. 274, 25281–25284.
[23] Arai, H., Terres, G., Pink, S. and Forgac, M. (1988) J. Biol. Chem. 263, 8796–8802.
[24] Arata, Y., Nishi, T., Kawasaki-Nishi, S., Shao, E., Wilkens, S. and Forgac, M. (2002) Biochim. Biophys. Acta 1555, 71–74.
[25] Murata, T., Arechaga, I., Fearnley, I.M., Kakinuma, Y., Yamato, I. and Walker, J.E. (2003) J. Biol. Chem. 278, 21162–21167.
[26] Kawasaki-Nishi, S., Nishi, T. and Forgac, M. (2001) Proc. Natl. Acad. Sci. USA 98, 12397–12402.
[27] Müller, V. and Grüber, G. (2003) Cell. Mol. Life Sci. 60, 474–494.
[28] Hunt, I.E. and Bowman, B.J. (1997) J. Bioenerg. Biomembr. 29, 533–540.
[29] Boekema, E.J., van Breemen, J.F., Brisson, A., Ubbink-Kok, T., Konings, W.N. and Lolkema, J.S. (1999) Nature 401, 37–38.
[30] Ubbink-Kok, T., Boekema, E.J., van Breemen, J.F., Brisson, A., Konings, W.N. and Lolkema, J.S. (2000) J. Mol. Biol. 296, 311–321.
[31] Konings, W.N., Lolkema, J.S. and Poolman, B. (1995) Arch. Microbiol. 164, 235–242.
[32] Sapra, R., Bagramyan, K. and Adams, M.W. (2003) Proc. Natl. Acad. Sci. USA 100, 7545–7550.
[33] Berg, H.C. (1995) Biophys. J. 68, 163S–166S.