Journal of Bioenergetics and Biomembranes, Vol. 36, No. 1, February 2004 (© 2004)

An Exceptional Variability in the Motor of Archaeal A_1A_0 ATPases: From Multimeric to Monomeric Rotors Comprising 6–13 Ion Binding Sites

Volker Müller[1]

The motor domain of A_1A_0 ATPases is composed of only two subunits, the stator subunit I and the rotor subunit *c*. Recent studies on the molecular biology of the A_0 domains revealed the surprising finding that the gene encoding subunit *c* underwent several multiplication events leading to rotor subunits comprising 2, 3, or even 13 hairpin domains suggesting multimeric in different stoichiometry as well as monomeric rotors. The number of ion translocating groups per rotor ranges from 13 to 6. Furthermore, as deduced from the gene sequences H^+- as well as Na^+-driven rotors are found in archaea. Features previously thought to be distinctive for A_0, F_0, or V_0 are all found in A_0 suggesting that the differences encountered in the three classes of ATPases today emerged already very early in evolution. The extraordinary features and exceptional structural and functional variabilty in the rotor of A_1A_0 ATPases may have arisen as an adaptation to different cellular needs and the extreme physicochemical conditions in the early history of life.

KEY WORDS: Archaea; methanogens; A_1A_0 ATPase; rotor diversity; ion specificity.

INTRODUCTION

ATP synthases are present in every life form and are the most important enzymes for the energy metabolism of the cell (Boyer, 1997). They catalyze the formation of ATP at the expense of the transmembrane electrochemical ion gradient ($\Delta\mu_{ion}$) according to

$$ADP + P_i + n\ ions_{out} \rightarrow ATP + n\ ions_{in}$$

Bacteria, chloroplasts, and mitochondria employ the F_1F_0 ATPase, the best studied specimen of ATPases (Senior *et al.*, 1995). Most F_1F_0 ATPases use protons as coupling ions but the ones from the anaerobic bacteria *Propionigenium modestum* and *Acetobacterium woodii* use Na^+ instead (Dimroth, 1997; Müller *et al.*, 2001). The reaction catalyzed by F_1F_0 ATPases is reversible, and the direction of the reaction is controlled thermodynamically. At high $\Delta\mu_{ion}$, ATP synthesis driven by $\Delta\mu_{ion}$ is favored, but at low $\Delta\mu_{ion}$ ATP hydrolysis leading to the formation of $\Delta\mu_{ion}$ is favored. In contrast, the ATPase found in organelles of eukaryotes, the V_1V_0 ATPase, lost its ability to synthesize ATP, its function is to create steep ion gradients at the expense of ATP hydrolysis (Nelson, 1992). Archaea contain ATPases, the A_1A_0 ATPases, that are structurally similar to V_1V_0 ATPases but function as ATP synthases (Müller *et al.*, 1999).

The A_1A_0, F_1F_0, and V_1V_0 ATPases have the same overall architecture comprising a membrane-bound domain, $A_0/F_0/V_0$, that contains the motor and the ion channel, a central and a peripheral stalk, and an approximately spherical cytoplasmic domain, $A_1/F_1/V_1$, that contains the catalytic sites (Grüber *et al.*, 2001; Nishi and Forgac, 2002; Stock *et al.*, 2000) (Fig. 1). The ATPases arose from a common ancestor that underwent structural and functional changes leading to three distinct classes of enzymes present in three domains of life (Hilario and Gogarten, 1998; Müller *et al.*, 1999). Structurally, the V_1V_0 and A_1A_0 are more closely related to each other than to F_1F_0 (Müller *et al.*, 1999).

The ATPases do not only share the same overall architecture but also the same mechanism for coupling of

[1] Section Microbiology, Department Biology I, Ludwig-Maximilians-Universität München, Maria-Ward-Str. 1a, 80638 München, Germany; e-mail: v.mueller@lrz.uni-muenchen.de.

0145-479X/04/0200-0115/0 © 2004 Plenum Publishing Corporation

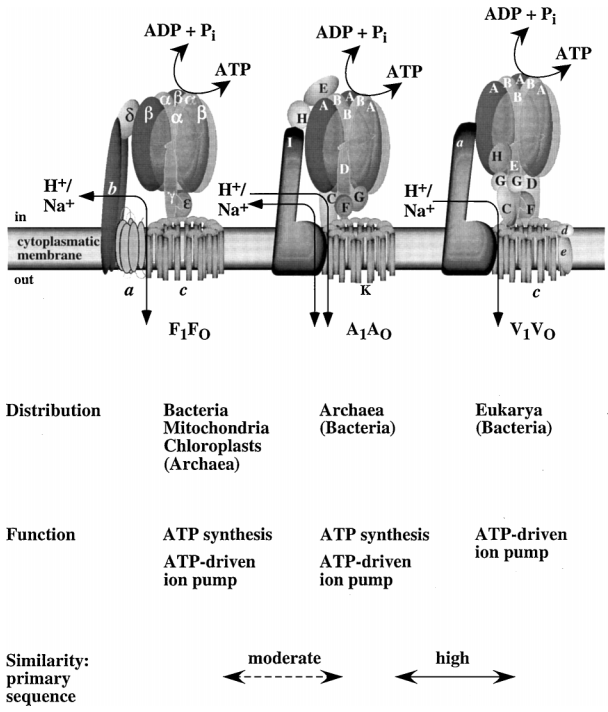

Fig. 1. *Structure and function of ATPases*. The subunit arrangement is given for the bacterial F_1F_0 ATPase (*left*), the A_1A_0 ATPase from *Methanosarcina mazei* (*middle*), and V_1V_0 ATPase from *Manduca sexta* (*right*). The subunit topology is based on molecular, biochemical, and structural data. Subunit K of A_1A_0 ATPases is synonymous with subunit *c*.

ion transport to ATP hydrolysis (Pedersen *et al.*, 2000). ATP hydrolysis drives rotation of subunits in the central stalk that concomittantly leads to rotation of a membrane-embedded motor, accompanied by the energetic uphill translocation of ions across the cytoplasmic membrane from the inside to the outside (Sambongi *et al.*, 1999; Yoshida *et al.*, 2001). Structural changes in the motor and variations of the number of ion translocating residues per motor unit affect the ion/ATP stoichiometry. This can change the physiological function of the enzyme dramatically as exemplified by the reversible, ATP synthesizing/hydrolyzing F_1F_0/A_1A_0 but unidirectional ATP hydrolyzing V_1V_0 ATPases (Schäfer *et al.*, 1999). Recent studies revealed an extraordinary variability of structure and function of motors of A_1A_0 ATPases and it is now evident that the class of A_1A_0 ATPases contains very unique but also motor components previously found only in either F_1F_0 or V_1V_0 ATPases. This review discusses the structure and function of archaeal ATPases in relation to F_1F_0 and V_1V_0 ATPases and will focuss on the extraordinary variability of the structure and function of the motor of the A_1A_0 ATPases. The heterogeneity of the motor of A_1A_0 ATPases sheds new light on the evolution of structure and function of ATPases and is discussed with respect to the evolution of life.

THE ATPases ARE ROTATORY ENZYMES

Biochemical as well as structural data suggested a rotary mechanism for the ATPases and this is now well supported by several lines of evidence for F_1F_0 and V_1V_0 ATPases (Abrahams *et al.*, 1994; Pänke *et al.*, 2000; Sambongi *et al.*, 1999; Stock *et al.*, 2000; Yoshida *et al.*, 2001). In F_1F_0 ATPases, hydrolysis of ATP drives rotation of the central stalk subunit γ in the cytoplasmic domain ($\alpha_3\beta_3$ hexagon). This requires a stator that is built by subunits *b* and δ. Subunit γ is connected to the membrane domain, and thus rotation of γ drives the motor embedded into the membrane.

The membrane-embedded motor is made of the stator and the rotor subunits and converts electrochemical energy into thrust by ion translocation from the medium into the cytoplasm of bacteria. The stator is built by subunits *a* (2 copies) and *b* (1 copy) in F_0 or by subunits *a* and I (presumably 1 copy each) in V_1V_0 and A_1A_0 ATPases, respectively (cf. Fig. 1). The rotor is a ring structure made by subunit *c* (also referred to as the proteolipid) that contains the ion binding site (Dimroth *et al.*, 1999; Fillingame *et al.*, 2000; Müller *et al.*, 1999; Wilkens and Forgac, 2001). The size of subunit *c* and thus the number of monomers and the number of ion translocating groups per F_0 rotor ranges from 10 to 14 in different organisms. For energetic considerations, the number of ion translocating groups per rotor is one of the most important properties of the enzyme. It determines the ion/ATP stoichiometry, and a change to this value can change the function of the enzyme dramatically from unidirectional ATP hydrolysis to bidirectional ATP hydrolysis/ATP synthesis. The proton is used by most ATPases as the coupling ion, and the H^+ binding residue is located in helix two of subunit *c*. This residue may be an aspartate or a glutamate and, therefore, this residue is often referred to as the "active carboxylate" (Deckers-Hebestreit and Altendorf, 1996).

How does the motor function, i.e., convert electrochemical energy into torque generation? A major advance of our understanding of rotor function comes from experiments done with the Na^+ F_1F_0 ATPase from *P. modestum* (Dimroth *et al.*, 1998, 2000; Kaim *et al.*, 1998) that are summarized in the following and illustrated in Fig. 2. The ring of *c* subunits is embedded into the cytoplasmic membrane. The ion binding sites are freely accessible from the cytoplasm but access from the periplasm is restricted and only possible by a not well-defined channel which leads to a negative charge on the rotor. In the close vicinity of

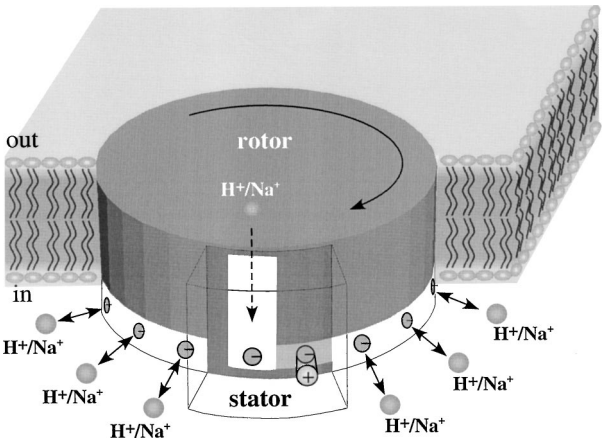

Fig. 2. *The motor of F_1F_0 ATPases.* The rotor is made from proteolipid oligomers, and the stator function is provided by subunit *a*. The ion binding sites on the rotor are indicated by the light gray dots. The positive charge on subunit *a* is the well-conserved arginine (Arg-210 in *E. coli*). For further explanations, see the text.

the negative charge of the rotor subunits is the highly conserved positive charge of the stator (Arg-210) of subunit *a*. This residue is close to the ion channel and connected to it by a hydrophilic sleeve. Arg-210 of subunit *a* and a negative charge of the rotor (the active carboxylate) form a salt bridge, thereby fixing the rotor to that position. To the left of the hydrophilic stretch is a hydrophobic zone that prevents rotation of the *c* ring with nonoccupied ion binding sites to the left. In the absence of a membrane potential, the rotor idles against the stator with no preference for direction of rotation; under these conditions, exchange of $^{22}Na^+_{in}/^{22}Na^+_{out}$ occurs. Upon generation of a membrane potential, the rotor is forced to rotate into one direction. The membrane potential, therefore, is an essential component for ATP synthesis, and cannot be substituted by a chemical ion gradient. The ion enters the channel at its periplasmic entrance and traps a negative charge of one *c* monomer. This allows the ion to enter the hydrophobic zone and prevents it from going backwards. Thereby, the *c* oligomer rotates to the left. The rotor will now donate the ion to the cytoplasm due to the lower chemical activity of the ion inside. Charge regeneration on the rotor also prevents the rotor from going backwards. In the reverse reaction, ATP hydrolysis drives ion extrusion by the same mechanism. This model is supported by several lines of experiments and discussed in detail by Dimroth *et al.* (2000). However, it should be mentioned in this connection that a second model is discussed which assumes two half channels giving access of the rotor to the cytoplasmic and periplasmic side, respectively (Junge *et al.*, 1997).

STRUCTURE OF THE ROTOR OF F_1F_0 AND V_1V_0 ATPase

Subunit *c* in its simplest form has two transmembrane helices connected by a polar, cytoplasmic loop; this structure is often referred to as the hairpin or the 8-kDa proteolipid. The ion binding site is located in helix two (Fig. 3). Structural data show the *c* polypeptides of F_1F_0 ATPases arranged in a ring with a stoichiometry of 10, 11, and 14 in yeast (Stock *et al.*, 1999), *Ilyobacter tartaricus* (Stahlberg *et al.*, 2001; Vonck *et al.*, 2002), and chloroplasts (Seelert *et al.*, 2000), respectively (Fig. 3). Because biological function was not proven, it is not clear whether the different stoichiometries are the result of purification artifacts or indeed represent the in vivo structure. The structure of this ring is unknown but it is assumed that the subunit *c* monomers are arranged in a front-to-back type giving two concentric rings. It is still a matter of debate whether helix one or two makes the outer ring of the oligomer (Fillingame *et al.*, 2000; Groth and Walker, 1997; Jones *et al.*, 1998; Schnick *et al.*, 2000). However, since every subunit *c* contains one ion binding site, the rotor has 10, 11, and 14 ion binding sites in yeast, *I. tartaricus*, and chloroplasts, respectively, and the ion/ATP stoichiometry is 3.3, 3.6, and 4.6. Apparently, ion/ATP stoichiometries of 3.3–4.6 are sufficient for ATP synthesis and, of course, allow ion pumping coupled to ATP hydrolysis which is consistent with the finding that F_1F_0 ATPases are fully reversible.

The rotor of the V_0 domain is composed of one to three different proteolipids (Hirata *et al.*, 1997; Nishi and Forgac, 2002; Stevens and Forgac, 1997). However, the proteolipids of V_1V_0 ATPases have in common that they arose by duplication of an ancestral gene encoding one hairpin followed by fusion of the genes (Mandel *et al.*, 1988). Therefore, the proteolipid of V_1V_0 ATPases contains two hairpins and has a molecular mass of around 16 kDa. Most important, the ion binding residue was not conserved in hairpin one but only in hairpin two. It is generally believed that the motor of the V_1V_0 ATPases has six copies of the 16-kDa proteolipid, however, the rotor of the V_1V_0 ATPase of *Enterococcus hirae* was recently shown to contain seven monomers (Murata *et al.*, 2003). This gives six to seven ion-translocating groups per rotor in the V_1V_0 ATPase (Fig. 3) resulting in an H^+/ATP stoichiometry of only 2–2.3 which is too low to allow ATP synthesis at physiological electrochemical ion potentials. Therefore, V_1V_0 ATPases are incapable of synthesizing ATP. On the other hand, the reduction of the number of active carboxylates on the rotor makes the enzyme a better ion pump, a function required by the physiology of the eukaryotic cell. It should be noted that rotors with only half

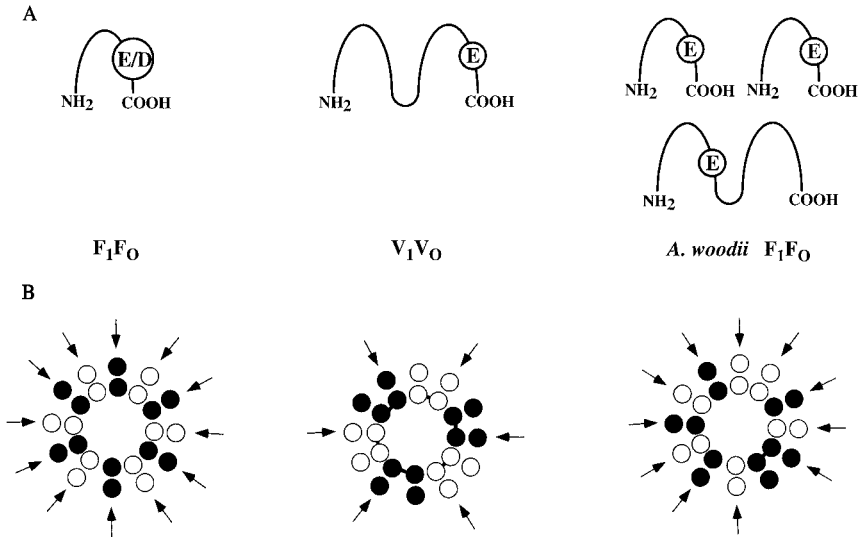

Fig. 3. *Diversity of proteolipids and rotors in F_1F_0 and V_1V_0 ATPases*. Panel A depicts the primary sequence (hairpin structure) of the proteolipids, the active carboxylate is indicated. Panel B depicts a top view on to the rotor. For the sake of simplicity, the F_1F_0 ATPase is shown to have 12 different monomers each with one hairpin arranged in a ring, the V_1V_0 ATPase is shown to have six different monomers each having two hairpins arranged in a ring, and the rotor of the F_1F_0 ATPase of *A. woodii* is a multimer containing 8- and 16-kDa proteolipids (the exact subunit stoichiometry is not known). The arrows denote the active carboxylates, i.e., the ion binding sites.

the number of ion translocating residues were considered for a long time as a unique feature of V_1V_0 ATPases but they are now also found in the Na^+ F_1F_0 ATPase from the bacterium *A. woodii* and, as we shall see later, also A_1A_0 ATPases.

The Na^+ F_1F_0 ATPase operon from *A. woodii* is the first known to contain a mixture of "bacterial" 8-kDa and "eukaryal" 16-kDa polypeptides, the latter with only one active carboxylate in two hairpin domains (Aufurth *et al.*, 2000; Müller *et al.*, 2001; Rahlfs *et al.*, 1999). However, the stoichiometry of the different polypeptides in the rotor has not yet been determined. A minimum of one subunit c_1 per rotor would give 11 ion binding sites per rotor, as in the case of *I. tartaricus*. The higher the number of subunit c_1 the lower will be the ion/ATP stoichiometry which will eventually lead to an enzyme incapable of ATP synthesis. This could be used as an alternative mechanism for the regulation of the ATPase.

CELLULAR FUNCTIONS OF THE A_1A_0 ATPases

Archaea represent the third domain in the universal tree of life beside the *Bacteria* and *Eukarya* (Woese *et al.*, 1990). To date, we can distinguish three physiological groups of archaea: the hyperthermophiles, the halophiles, and the methanogens. The physiological differences of these groups have to be considered while attempting to depict a universal model for the cellular function of archaeal ATPases. The methanogenic archaea (methanogens) are strictly chemiosmotic, which means that an ATP synthase is essential (Deppenmeier *et al.*, 1996; Müller *et al.*, 1993). The halophiles can perform respiration/photosynthesis but also fermentation; here we would expect an enzyme able to work in both directions, i.e., synthesis as well as hydrolysis (Bickel-Sandkötter *et al.*, 1996). Among the hyperthermophiles a number of strictly fermenting organisms are known, and, therefore, there is no need for the enzyme to synthesize ATP, hydrolysis would be sufficient to satisfy the cellular needs (Adams, 1994; Danson, 1993). However, recent data showed an additional ATP synthesis by ion gradient-driven phosphorylation in pyrococci (Sapra *et al.*, 2003). These considerations indicate that the rotor of A_1A_0 ATPases from different physiological groups differs considerably with respect to subunit composition and the number of ion translocating residues.

STRUCTURE AND FUNCTION OF THE PARADIGM, THE A_1A_0 ATPase FROM METHANOGENS

The ATPases from methanogens are reversible enzymes and their physiological function is to synthesize

ATP. They are hitherto the best investigated specimen of this unique class of ATPases. The A_1A_0 ATPase has at least nine subunits (A_3:B_3:C:D:E:F:H:I:K_x), but the actual subunit stoichiometry and the cellular localization of most subunits is unknown (cf. Fig. 1) (Müller *et al.*, 1999; Müller and Grüber, 2003). The A_1 complex of archaeal ATPases possesses a pseudo-hexagonal arrangement of six peripheral globular masses, reflecting the major subunits A and B, as proposed from two dimensional images of the thermoacidophilic archaea *Sulfolobus acidocaldarius* and *Methanosarcina mazei* Gö1 (Lübben *et al.*, 1988; Wilms *et al.*, 1996). An A_1 subcomplex heterologously produced in *E. coli* made up of the five different subunits A, B, C, D, and F is asymmetric, with a headpiece that is approximately 94 Å long and 92 Å wide and a stalk with a length of approximately 84 and 60 Å in diameter (Grüber *et al.*, 2001; Lemker *et al.*, 2003). Subunits C and F are exposed in the complex, whereas subunit D is well protected from trypsin degradation (Grüber *et al.*, 2001). The shielding of subunit D from trypsin is an important finding since this subunit has been proposed as the structural and functional homolog of the γ subunit of F_1F_0 ATPases (Coskun *et al.*, 2002; Grüber *et al.*, 2001; Müller *et al.*, 1999).

The A_0 domain contains only two membrane-intrinsic subunits, I and K (cf. Fig. 1). The molecular mass of subunit I ranges from 72 to 76 kDa (Müller *et al.*, 1999); it is very similar to subunit *a* of V_1V_0 ATPases with a hydrophilic N-terminal and a hydrophobic C-terminal domain of apparent molecular masses of ~39 and ~33 kDa, respectively, in *M. mazei* Gö1. The hydrophilic domain is predicted to be highly α helical and assumed to be the functional homolog of the soluble domain of subunit *b* of F_1F_0 ATPases. The hydrophobic C terminus of subunit I is predicted to have seven transmembrane helices and is assumed to be functionally similar to subunit *a* of V_1V_0/F_1F_0 ATPases; however, similarity on amino acid sequence level is below 20%. The arginine essential for ion translocation (*a*Arg-735 in *S. cerevisiae* (Kawasaki-Nishi *et al.*, 2001)) is conserved in subunit I (equivalent to Arg-557 of *M. mazei* Gö1); this residue is most likely the essential positive charge on the stator in A_0. The second subunit of the A_0 domain is subunit K, which builds the rotor. Subunit K (synonymous with subunit *c*) or the proteolipid of A_1A_0 ATPases has an extraordinary variability that is discussed in detail in the following section.

STRUCTURE OF THE PROTEOLIPIDS FROM ARCHAEA

The proteolipids from methanoarchaea are very similar to each other (≈50%), to those from other archaea (30–52% identity) and to proteolipids of V_1V_0 ATPases from bacteria or eukarya, the degree of identities range from 26.7 to 33%. A leader sequence is present in *Halobacterium salinarium* (Ihara *et al.*, 1997) and *S. acidocaldarius* (Lübben and Schäfer, 1989). Proteolipids have been purified and characterized from some archaea and in almost every case they were shown to be of $M_r \approx 8000$ with two transmembrane helices (Ihara *et al.*, 1997; Inatomi *et al.*, 1989; Steinert *et al.*, 1997; Wilms *et al.*, 1996). Furthermore, with the exceptions mentioned below genome sequences predict 8-kDa proteolipids in archaea. This size corresponds to the size of the proteolipid from F_1F_0 ATPases and was hitherto assumed to be the reason for the F_1F_0-like properties of the A_1A_0 ATPases, i.e., their function as ATP synthases. However, *Methanothermobacter thermautotrophicus* and *Methanocaldococcus jannaschii* (formerly *Methanococcus jannaschii*) have duplicated and triplicated proteolipids with two and three hairpin domains, respectively. Apparently, the proteolipids from *M. thermautotrophicus* and *M. jannaschii* arose by gene duplication and triplication, respectively, with subsequent fusion of the genes (Ruppert *et al.*, 1998, 1999). In case of *M. thermautotrophicus*, the ion binding site is conserved in helix two and four, but in *M. jannaschii* it is only conserved in helix four and six, in helix two it is substituted by a glutamine residue. The genome sequence of *Methanopyrus kandleri* revealed another extraordinary feature: the A_1A_0 ATPase genes are located in one cluster, but the gene encoding the proteolipid is 13 times the size of the gene encoding an 8-kDa proteolipid. The sequence predicts a proteolipid of 97.5 kDa comprising 13 covalently linked hairpin domains (Slesarev *et al.*, 2002). These domains have a highly conserved sequence (55.9–86.3%), and the ion binding site is conserved in helix two of every hairpin domain. However, posttranscriptional and posttranslational modifications cannot be excluded and, therefore, the extraordinary size of the proteolipid has to be verified by other means.

The pyrococci *Pyrococcus furiosus*, *Pyrococcus horikoshii*, and *Pyrococcus abyssi* are anaerobic archaea that have a fermentative metabolism. Interestingly, their proteolipid genes arose by duplication and subsequent fusion of a precursor gene coding for one hairpin (Kawarabayasi *et al.*, 1998; Robb *et al.*, 2001). The duplicated proteolipid with two covalently linked hairpins has an ion binding site in hairpin two but not in one. Therefore, the proteolipid of the A_1A_0 ATPases from pyrococci is identical to the 16-kDa proteolipid of eukaryal V_1V_0 ATPases. This finding gives further evidence that 16-kDa proteolipids with only one ion binding site in two hairpins are not an exclusive feature of eukarya.

A SODIUM ION BINDING MOTIF IN A_1A_0 ATPases

The F_1F_0 ATPases of the bacteria *P. modestum* and *A. woodii* and the A_1A_0/V_1V_0 ATPases of *E. hirae* and *Calaromator fervidus* use Na^+ as coupling ion (Kakinuma and Igarashi, 1995; Laubinger and Dimroth, 1988; Reidlinger and Müller, 1994; Speelmans *et al.*, 1994), and an Na^+ binding site has been defined in the proteolipid (Kaim and Dimroth, 1995; Rahlfs and Müller, 1997; Zhang and Fillingame, 1995). Three residues (Gln in helix one, Glu and Ser or Thr in helix two) were shown experimentally to be involved in Na^+ binding, and a fourth residue (Pro in helix one) is suggested by sequence comparisons (Fig. 4(A)). In the proteolipid of the Na^+ V_1V_0 ATPase from *E. hirae*, the Gln, Glu, and Ser residues of the motif are conserved, but Pro is substituted by a Ser (Kakinuma *et al.*, 1993). However, the free electron pair of the hydroxyl group of serine could also serve for complexation of Na^+. Therefore, the Na^+ binding motif of the proteolipid from F_1F_0 ATPases and V_1V_0 ATPases is suggested to contain four residues in the order: P/SXXXQ (motif I, helix one)—ET/S (motif II, helix two).

So far, the ion specificity of A_1A_0 ATPases is not settled, and this question was only addressed in methanogenic archaea. The strictly anaerobic methanogenic archaea are unique among organisms because they couple the pathway of energy conservation with the primary extrusion of both Na^+ and H^+ (Deppenmeier *et al.*, 1996; Schäfer *et al.*, 1999). Claims have been made for H^+- as well as Na^+-driven ATP synthesis in *M. mazei* Gö1 and *M. thermautotrophicus* and inhibitor studies suggested the presence of H^+ coupled A_1A_0 and Na^+ coupled F_1F_0 ATPases (Becher and Müller, 1994; Smigan *et al.*, 1992, 1994, 1995). In contrast, genome studies clearly excluded the presence of F_1F_0 ATPase genes in *M. mazei* Gö1 (Deppenmeier *et al.*, 2002) and *M. thermautotrophicus* (Smith *et al.*, 1997) but revealed the A_1A_0 ATPase genes. Therefore, the question how the Na^+ gradient is converted to ATP synthesis is still open. Nevertheless, two related species, *Methanosarcina barkeri* (Accession No. NC_002724), and *Methanosarcina acetivorans* (Galagan *et al.*, 2002) indeed have both A_1A_0 and F_1F_0 ATPase clusters in their genomes, but an expression of the F_1F_0 ATPase genes of *M. barkeri* or the production of a functional F_1F_0 ATPase could not be demonstrated (Lemker and Müller, unpublished). Future studies using the genetically tractable *M. acetivorans* should shed light on the enigma of the F_1F_0 ATPase genes in some methanogens.

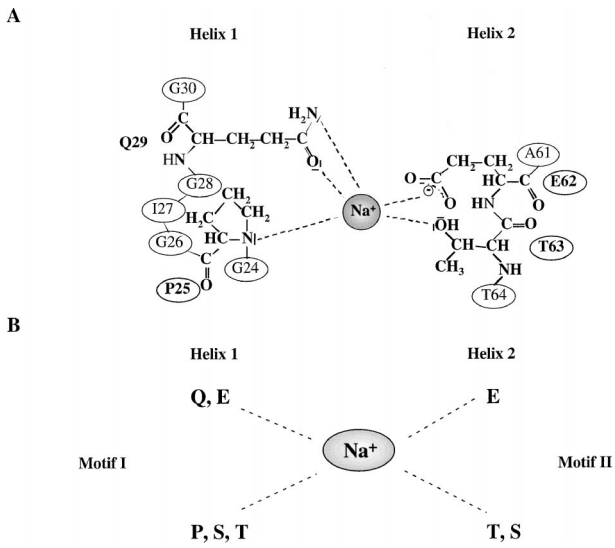

Fig. 4. *The Na^+-binding site in subunit c of ATPases*. Panel A depicts the proposed Na^+-binding site of subunit *c* of F_1F_0 ATPases, and Panel B depicts the proposed generalized Na^+-binding site in subunit *c* of F_1F_0, V_1V_0, and A_1A_0 ATPases. Motif I is located in helix one, motif II in helix two.

However, in silico analyses revealed the Na^+ binding motif in the proteolipid of some A_1A_0 ATPases and suggest that these enzymes use Na^+ as coupling ion. Motifs I and II are present in both hairpins of *M. thermautotrophicus*, in every hairpin of *M. kandleri*, but only in hairpins two and three of *M. jannaschii*. In the latter, the glutamine of helix one is changed to valine, and the glutamate of helix two is changed to glutamine. Although a glutamine could ligand a sodium ion, a valine could not, and therefore, this hairpin is unlikely to bind Na^+. In addition, the glutamate to glutamine change in helix two excludes H^+ translocation. In the 8-kDa proteolipids of *M. mazei*, *M. barkeri*, and *M. acetivorans* the glutamine of helix one is changed to glutamate, but this is considered a conservative change since glutamate is able to bind Na^+as well. Interestingly, the F-type proteolipids of *M. barkeri* and *M. acetivorans* also have an Na^+ binding motif. In both, the glutamine of motif I is also substituted by glutamate. The 16-kDa proteolipid of pyrococci have the Na^+ binding motif in hairpin two but not one. In the latter, the third residue is a valine and the fourth residue is a methionine. This configuration is able to transport neither Na^+ nor H^+.

Three of the Na^+ liganding residues are also conserved in *Thermoplasma volcanii* and *Thermoplasma acidophilum*. The first residue was changed to threonine, but this is a conservative change without effect on Na^+ transport.

Taken together, it is proposed that the Na^+ binding motif of subunit *c* of A_1A_0, F_1F_0, and V_1V_0 ATPases is

P/S/TXXXQ/E (motif I) and ET/S (motif II) (Fig. 4(B)). These in silico analyses suggest that the putative F_1F_0 ATPases of *M. barkeri* and *M. acetivorans* and the A_1A_0 ATPases of methanogenic archaea, pyrococci, *T. volcanii*, and *T. acidophilum* most likely use Na^+ as coupling ion. This assumption is, at least for methanogens, supported by their physiology and bioenergetics (Schäfer *et al.*, 1999). However, the biochemical verification of this hypothesis will be a challenging task for future experiments which has to await the purification on intact A_1A_0 ATPases.

A sodium ion binding motif is apparently absent in the A_1A_0 ATPases from *Sulfolobus acidocaldarius*, *Sulfolobus solfataricus*, *Archaeoglobus fulgidus*, *Halobacterium* NRC-1, and *Halobacterium salinarium*. This is in line with the fact that Na^+ has not been reported to be involved in the physiology or bioenergetics of *Sulfolobus* and *Archaeoglobus*.

STRUCTURE AND FUNCTION OF THE A_0 DOMAIN

The rotor stoichiometry has not been solved for any A_1A_0 ATPase but two important conclusions regarding the structure of the rotor and the function of the enzyme can already be drawn.

For structural considerations, it is assumed for the sake of simplicity that the rotor contains 12 hairpins. This would accommodate 12 copies of the 8-kDa proteolipids from most archaea, six of the one from *M. thermautotrophicus* and pyrococci, and four of *M. jannaschii*. These rotors are multimeric but the number of subunits decreases in this order. A comparison to the optimal and maximal growth temperatures reveals a striking correlation of the number of rotor subunits to the optimal and maximal growth temperatures (Fig. 5). The higher the growth temperature of the organisms the lower is the number of subunits per rotor. The extreme is encountered in the monomeric rotor of *M. kandleri* that thrives at 110°C. It should be remembered that the proteolipids are embedded into the membrane and shielded from heat protective mechanisms present in the cytoplasm. Therefore, the proteolipids "sense" the heat directly and it is easily conceivable that the increase of covalently linked rotor subunits increases the stability and function of the rotor in the cytoplasmic membrane at high temperatures.

For the function of the enzymes, the number of ion-translocating residues per rotor unit are important. The capability to synthesize ATP is directly dependent on the number of ions translocated per ATP synthesized. According to $\Delta G_P = -n \cdot F \cdot \Delta p$, a phosphorylation potential (ΔG_P) of ~50 to 70 kJ/mol is sustained by the use of $n = 3$–4 ions/ATP at a physiological electrochemical ion potential of −180 mV (Δp). Assuming a rotor with 12 ion translocating groups and a catalytic domain with three $\alpha\beta$/AB pairs and thus three ATP binding sites, this gives exact the number of four ions required thermodynamically for ATP synthesis. This is apparently realized in most archaeal ATPases found to date. However, a special case is the rotor of *M. jannaschii* that has only eight ion binding sites (assuming 12 hairpin domains per rotor). Apparently, 2.6 carboxyl groups per catalytic center are already sufficient for ATP synthesis (Ruppert *et al.*, 1999).

A rotor with only six to seven ion binding sites as encountered in V_1V_0 ATPases (Fig. 6) gives a stoichiometry of only 2–2.3 ions/ATP which is too low to allow ATP synthesis at a ΔG_P of ~50 to 70 kJ/mol and a Δp of −180 mV. On the other hand, the reduced H^+/ATP stoichiometry makes the enzyme a better proton pump, because the same ΔG_P can account for a much higher Δp. A special case is the rotor of the pyrococcal A_1A_0 ATPases. The pyrococcal proteolipid is duplicated with only one ion binding site. Assuming six to seven monomers per rotor as in V_1V_0 ATPases, the enzyme has to be considered as an ATP-driven ion pump. However, recently it was shown that pyrococci produce ATP by ion gradient-driven phosphorylation in addition to substrate level phosphorylation (Sapra *et al.*,

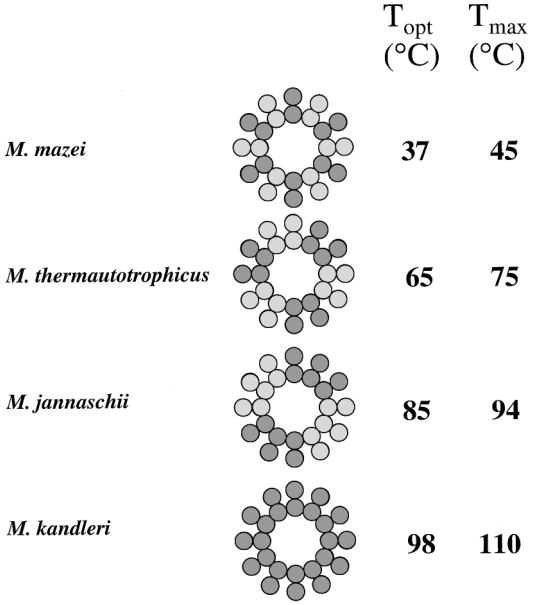

Fig. 5. The number of covalently linked hairpins in rotors of methanogenic archaea increases with increasing growth temperature. *M. mazei* is shown to have a rotor comprising 12 monomers, *M. thermautotrophicus* 6, *M. jannaschii* 4, and *M. kandleri* 1. Optimal (T_{opt}) and maximal (T_{max}) growth temperatures are indicated.

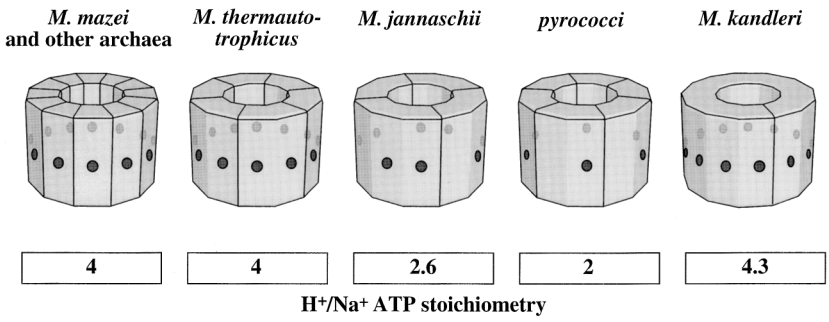

Fig. 6. *Diversity of rotors in archaea*. The number and size of monomers as well as the number of ion-binding sites per rotor are indicated. The *M. mazei*-like rotor is also present in other archaea (*M. acetivorans, M. barkeri* fusaro, *T. acidophilum, T. volcanii, A. fulgidus, Pyrobaculum aerophilum, S. acidocaldarius, S. solfataricus, Halobacterium* NRC-1, and *H. salinarium*). Because the exact stoichiometry of the proteolipids is unknown, 12 hairpin domains are assumed for every rotor. This would give the indicated H^+/Na^+ stoichiometry. The rotor shown for pyrococci supports ATP synthesis, but whether it also supports ATP synthesis is speculative. For further explanations, see the text.

2003). ATP synthesis could be achieved by a rotor that contains eight proteolipid monomers, giving an H^+/ATP stoichiometry of 2.6 which is apparently sufficient in *M. jannaschii* for ATP synthesis. On the other hand, an increased $\Delta\mu_{ion}$ could also account for ATP synthesis in a rotor with only six to seven ion binding sites. The elucidation of the structure and function of the pyrococcal A_1A_0 ATPase is a challenging task for future studies.

Methanogenic archaea are energy limited organisms, the conversion of $H_2 + CO_2$ to CH_4 is accompanied by a free energy change of -130 kJ/mol that allows, under standard conditions, only for the synthesis of two mol ATP. However, in their natural habitat the hydrogen partial pressure is much lower and only a fraction of an ATP can be synthesized (Schäfer *et al.*, 1999). One way to increase the phosphorylation potential at a given $\Delta\mu_{ion}$ is to increase the number of ions translocated, i.e., the ion/ATP stoichiometry. This could have been realized in *M. kandleri* that has 13 ion-translocating residues giving an ion/ATP stoichiometry of 4.3 (Fig. 6). An increase from 4 to 4.3 at a $\Delta\mu_{ion}$ of -180 mV will increase the phosphorylation potential already by 7%. In this context, it would be interesting to determine the subunit composition in multimeric rotors from methanogenic archaea.

EVOLUTION OF ATPases

Archaea are assumed to be early life forms that developed in the extreme environments encountered in early history, such as high temperatures, the presence of gaseous compounds that they learned to use as carbon and energy sources, high salt concentrations, low pH, and combinations thereof. The invention of chemiosmosis required the invention of ATP synthases, rotary enzymes able to operate without leakage at temperatures as high as 110°C. High temperatures might have favored the evolution of monomeric rotors. Decreasing temperatures eliminated the need for monomeric rotors and led to the generation of multimeric rotors with the number of monomers per ring decreasing with decreasing temperatures. Multimeric rotors have the advantage that the coupling efficiency and thus the physiological function of the ATPases can be changed by changing the number of (different) proteolipids in the rotor. That such a layer of regulation of ATPases exists is supported by some experiments (Schemidt *et al.*, 1998). However, its elucidation is still a challenging task for future studies.

There was always the hypothesis that the diversion of the A_1A_0 and V_1V_0 ATPases took place by a duplication of the proteolipids accompanied by a loss of one ion binding site (Nelson and Taiz, 1989). However, duplicated proteolipids with only one ion binding site are also found in ATPases of archaea, the pyrococci. It should be mentioned in this context that it was speculated that the switch from a A_1A_0 to a V_1V_0 ATPase occurred after an archaeon had picked up a bacterial symbiont on the way to becoming an eukaryote (Cross and Taiz, 1990). However, in the light of the finding of V_1V_0-like proteolipids in *Pyrococcus*, this switch must have occurred earlier and could have been driven by a loss of the ability of ion gradient-driven phosphorylation. *Pyrococcus* or its ancestors might be descendents of the archaeal host that gave rise to eukaryotes.

The bacteria *Thermus thermophilus* and *E. hirae* contain ATPases that are always referred to in the literature as V_1V_0 ATPases. However, phylogenetic analyses clearly

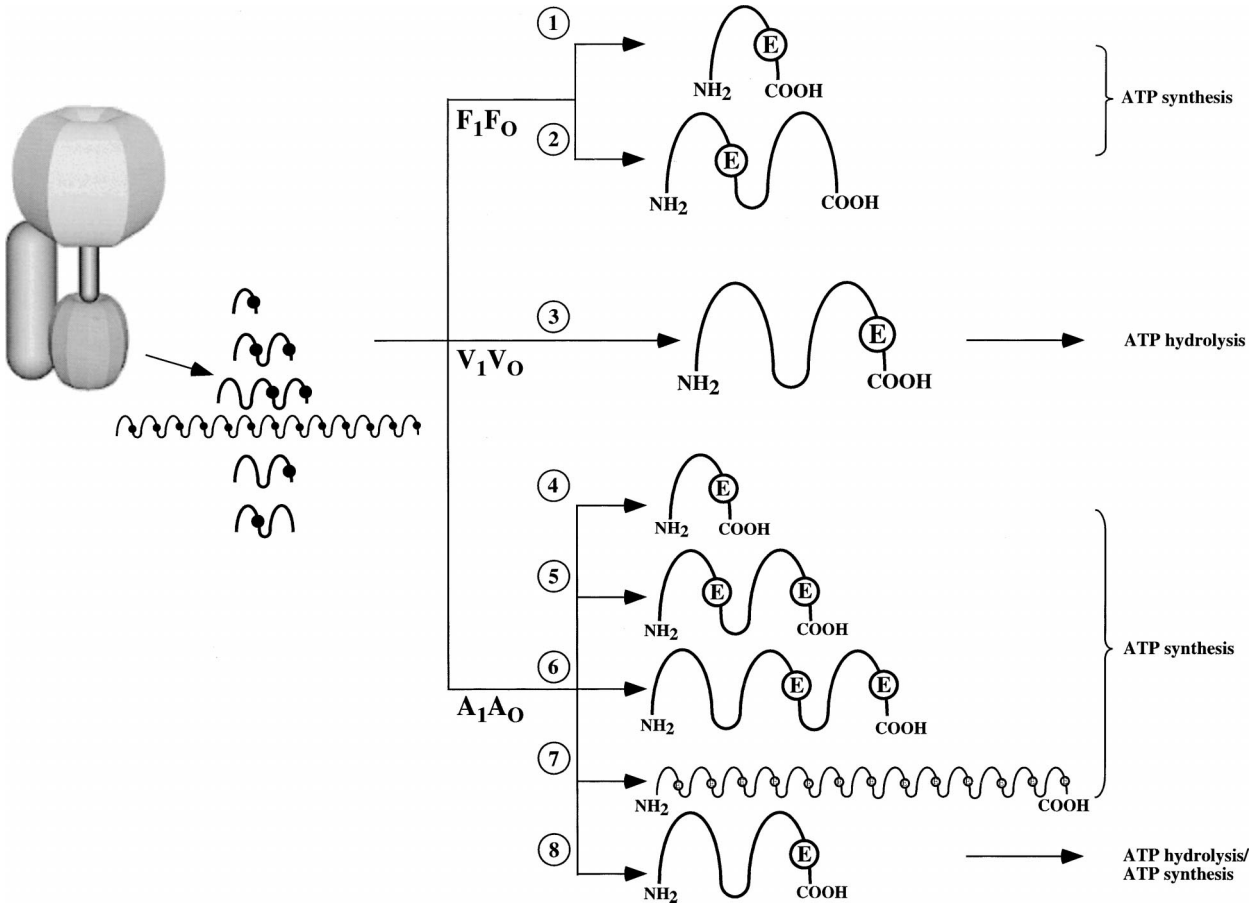

Fig. 7. *Evolution of structure and function of ATPases*. The different proteolipids encountered in the ATPases were already invented before the separation of the ATPases and are conserved in the domain archaea. Please note that only the active carboxylate is indicated, but not the Na^+ binding sites. For explanations, see the text. (1) bacteria (including *A. woodii*), mitochondria, chloroplasts. (2) *A. woodii*. (3) eukarya. (4) *M. mazei, M. acetivorans, M. barkeri* fusaro, *T. acidophilum, T. volcanii, A. fulgidus, P. aerophilum, S. acidocaldarius, S. solfataricus, Halobacterium* NRC-1, *H. salinarium*, and the bacterium *T. thermophilus*. (5) *M. thermoautotrophicus*. (6) *M. jannaschii*. (7) *M. kandleri*. (8) *P. abyssi, P. furiosus, P. horikoshii*, and bacterium *E. hirac*.

demonstrate that they are of archaeal origin translocated by horizontal gene transfer (Olendzenski *et al.*, 1998) and therefore, they should be considered as A_1A_0 ATPases. The same could be true for other so-called V-type ATPases of bacteria. Horizontal gene transfer turns out to have been very massive in the early history of life and has also crossed kingdom barriers, as deduced from genome analyses.

It is apparent that the rotors seen today in the three classes of ATPases were already invented in early evolution in archaea. Life at high temperatures with energy-limited substrates may have driven the formation of monomeric rotors, chemiosmosis rotors allowing ATP synthesis, and a fermentative metabolism may have driven the formation of rotors with only six ion binding sites. Out of this pool of combinations the modern-day V_1V_0 and F_1F_0 ATPases recruited their rotors. Therefore, the major changes in the evolution of ATPases did not occur in the motor domains but rather in the hydrophilic domains including subunit composition, primary sequences, regulatory events, and assembly factors (Fig. 7).

CONCLUSIONS

In summary, the rotors of A_1A_0 ATPases may be monomeric or multimeric, but with different subunit stoichiometry. They have an extraordinary variability with respect to the number of ion binding sites that can be 13, 12, 8, or 6, an extraordinary finding. The different motor domains seen today in A_1A_0, F_1F_0, and V_1V_0 were already present in early history of evolution of ATPases before the A_1A_0, F_1F_0, and V_1V_0 ATPases developed from

a universal gene pool. F_1F_0 and V_1V_0 ATPases developed only one major kind of motor as an adaptation to either chemiosmotic energy generation or membrane energization by ATP hydrolysis. Archaea are early life forms that have a fascinating and diverse metabolism which is either chemiosmotic or fermentative. Therefore, they developed the different motors as an adaptation to their cellular energy metabolism needs and maintained them during evolution. The motors of archaeal A_1A_0 ATPases can be seen as reminiscents of early evolution; they clearly deserve more attention.

ACKNOWLEDGMENTS

I am grateful to my coworkers for their excellent work and the Deutsche Forschungsgemeinschaft for continous and generous support.

REFERENCES

Abrahams, J. P., Leslie, A. G. W., Lutter, R., and Walker, J. E. (1994). *Nature* **370**, 621–628.
Adams, M. W. W. (1994). *FEMS Microbiol. Rev.* **15**, 261–277.
Aufurth, S., Schägger, H., and Müller, V. (2000). *J. Biol. Chem.* **275**, 33297–33301.
Becher, B., and Müller, V. (1994). *J. Bacteriol.* **176**, 2543–2550.
Bickel-Sandkötter, S., Gärtner, W., and Dane, M. (1996). *Arch. Microbiol.* **166**, 1–11.
Boyer, P. D. (1997). *Annu. Rev. Biochem.* **66**, 717–749.
Coskun, Ü., Grüber, G., Koch, M. H., Godovac-Zimmermann, J., Lemker, T., and Müller, V. (2002). *J. Biol. Chem.* **277**, 17327–17333.
Cross, R. L., and Taiz, L. (1990). *FEBS Lett.* **259**, 22227–22229.
Danson, M. J. (1993). In *The Biochemistry of Archaea* (Kates, M., Kushner, D. J., and Matheson, A. T., eds.), Elsevier, Amsterdam, pp. 1–24.
Deckers-Hebestreit, G., and Altendorf, K. (1996). *Annu. Rev. Microbiol.* **50**, 791–824.
Deppenmeier, U., Johann, A., Hartsch, T., Merkl, R., Schmitz, R. A., Martinez-Arias, R., Henne, A., Wiezer, A., Bäumer, S., Jacobi, C., Brüggemann, H., Lienard, T., Christmann, A., Bömeke, M., Stec kel, S., Bhattacharyya, A., Lykidis, A., Overbeek, R., Klenk, H.-P., Gunsalus, R. P., Fritz, H.-J., and Gottschalk, G. (2002). *J. Mol. Microbiol. Biotechnol.* **4**, 453–461.
Deppenmeier, U., Müller, V., and Gottschalk, G. (1996). *Arch. Microbiol.* **165**, 149–163.
Dimroth, P. (1997). *Biochim. Biophys. Acta* **1318**, 11–51.
Dimroth, P., Kaim, G., and Matthey, U. (1998). *Biochim. Biophys. Acta* **1365**, 87–92.
Dimroth, P., Matthey, U., and Kaim, G. (2000). *Biochim. Biophys. Acta* **1459**, 506–513.
Dimroth, P., Wang, H., Grabe, M., and Oster, G. (1999). *Proc. Natl. Acad. Sci. U.S.A.* **96**, 4924–4929.
Fillingame, R. H., Jiang, W., Dmitriev, O. Y., and Jones, P. C. (2000). *Biochim. Biophys. Acta* **1458**, 387–403.
Galagan, J. E., Nusbaum, C., Roy, A., Endrizzi, M. G., Macdonald, P., FitzHugh, W., Calvo, S., Engels, R., Smirnov, S., Atnoor, D., Brown, A., Allen, N., Naylor, J., Stange-Thomann, N., DeArellano, K., Johnson, R., Linton, L., McEwan, P., McKernan, K., Talamas, J., Tirrell, A., Ye, W., Zimmer, A., Barber, R. D., Cann, I., Graham, D. E., Grahame, D. A., Guss, A. M., Hedderich, R., Ingram-Smith, C., Kuettner, H. C., Krzycki, J. A., Leigh, J. A., Li, W., Liu, J., Mukhopadhyay, B., Reeve, J. N., Smith, K., Springer, T. A., Umayam, L. A., White, O., White, R. H., Conway de Macario, E., Ferry, J. G., Jarrell, K. F., Jing, H., Macario, A. J., Paulsen, I., Pritchett, M., Sowers, K. R., Swanson, R. V., Zinder, S. H., Lander, E., Metcalf, W. W., and Birren, B. (2002). *Genome Res.* **12**, 532–542.
Groth, G., and Walker, J. E. (1997). *FEBS Lett.* **410**, 117–123.
Grüber, G., Svergun, D. I., Coskun, Ü., Lemker, T., Koch, M. H., Schägger, H., and Müller, V. (2001). *Biochemistry* **40**, 1890–1896.
Grüber, G., Wieczorek, H., Harvey, W. R., and Müller, V. (2001). *J. Exp. Biol.* **204**, 2597–2605.
Hilario, E., and Gogarten, J. P. (1998). *J. Mol. Evol.* **46**, 703–715.
Hirata, R., Graham, L. A., Takatsuki, A., Stevens, T. H., and Anraku, Y. (1997). *J. Biol. Chem.* **272**, 4795–4803.
Ihara, K., Watanabe, S., Sugimura, K., and Mukohata, Y. (1997). *Arch. Biochem. Biophys.* **341**, 267–272.
Inatomi, K. I., Maeda, M., and Futai, M. (1989). *Biochem. Biophys. Res. Commun.* **162**, 1585–1590.
Jones, P. C., Jiang, W., and Fillingame, R. H. (1998). *J. Biol. Chem.* **273**, 17178–17185.
Junge, W., Lill, H., and Engelbrecht, S. (1997). *Trends Biochem. Sci.* **22**, 420–423.
Kaim, G., and Dimroth, P. (1995). *J. Mol. Biol.* **253**, 726–738.
Kaim, G., Matthey, U., and Dimroth, P. (1998). *EMBO J.* **17**, 688–695.
Kakinuma, Y., and Igarashi, K. (1995). *FEBS Lett.* **359**, 255–258.
Kakinuma, Y., Kakinuma, S., Takase, K., Konishi, K., Igarashi, K., and Yamato, I. (1993). *Biochem. Biophys. Res. Commun.* **195**, 1063–1069.
Kawarabayasi, Y., Sawada, M., Horikawa, H., Haikawa, Y., Hino, Y., Yamamoto, S., Sekine, M., Baba, S., Kosugi, H., Hosoyama, A., Nagai, Y., Sakai, M., Ogura, K., Otsuka, R., Nakazawa, H., Takamiya, M., Ohfuku, Y., Funahashi, T., Tanaka, T., Kudoh, Y., Yamazaki, J., Kushida, N., Oguchi, A., Aoki, K., and Kikuchi, H. (1998). *DNA Res.* **5**, 147–155.
Kawasaki-Nishi, S., Nishi, T., and Forgac, M. (2001). *Proc. Natl. Acad. Sci. U.S.A.* **98**, 12397–12402.
Laubinger, W., and Dimroth, P. (1988). *Biochemistry* **27**, 7531–7537.
Lemker, T., Grüber, G., Schmid, R., and Müller, V. (2003). *FEBS Lett.* **544**, 206–209.
Lübben, M., Lünsdorf, H., and Schäfer, G. (1988). *Biol. Chem. Hoppe Seyler* **369**, 1259–1266.
Lübben, M., and Schäfer, G. (1989). *J. Bacteriol.* **171**, 6106–6116.
Mandel, M., Moriyama, Y., Hulmes, J. D., Pan, Y.-C. E., Nelson, H., and Nelson, N. (1988). *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5521–5524.
Müller, V., Aufurth, S., and Rahlfs, S. (2001). *Biochim. Biophys. Acta* **1505**, 108–120.
Müller, V., Blaut, M., and Gottschalk, G. (1993). In *Methanogenesis* (Ferry, J. G., ed.), Chapman & Hall, New York, pp. 360–406.
Müller, V., and Grüber, G. (2003). *Cell. Mol. Life Sci.* **60**, 474–494.
Müller, V., Ruppert, C., and Lemker, T. (1999). *J. Bioenerg. Biomembr.* **31**, 15–28.
Murata, T., Arechaga, I., Fearnley, I. M., Kakinuma, Y., Yamato, I., and Walker, J. E. (2003). *J. Biol. Chem.* **278**, 21162–21167.
Nelson, N. (1992). *Biochim. Biophys. Acta* **1100**, 109–124.
Nelson, N., and Taiz, L. (1989). *Trends Biochem. Sci.* **14**, 113–116.
Nishi, T., and Forgac, M. (2002). *Nat. Rev. Mol. Cell. Biol.* **3**, 94–103.
Olendzenski, L., Hilario, E., and Gogarten, J. (1998). In *Horizontal Gene Transfer* (Syvanen, M., and Kado, C. I., eds.) Chapman & Hall, New York, pp. 349–362.
Pänke, O., Gumbiowski, K., Junge, W., and Engelbrecht, S. (2000). *FEBS Lett.* **472**, 34–38.
Pedersen, P. L., Ko, Y. H., and Hong, S. (2000). *J. Bioenerg. Biomembr.* **32**, 325–332.
Rahlfs, S., Aufurth, S., and Müller, V. (1999). *J. Biol. Chem.* **274**, 33999–34004.
Rahlfs, S., and Müller, V. (1997). *FEBS Lett.* **404**, 269–271.

Reidlinger, J., and Müller, V. (1994). *Eur. J. Biochem.* **223**, 275–283.
Robb, F. T., Maeder, D. L., Brown, J. R., DiRuggiero, J., Stump, M. D., Yeh, R. K., Weiss, R. B., and Dunn, D. M. (2001). *Methods Enzymol.* **330**, 134–157.
Ruppert, C., Kavermann, H., Wimmers, S., Schmid, R., Kellermann, J., Lottspeich, F., Huber, H., Stetter, K. O., and Müller, V. (1999). *J. Biol. Chem.* **274**, 25281–25284.
Ruppert, C., Wimmers, S., Lemker, T., and Müller, V. (1998). *J. Bacteriol.* **180**, 3448–3452.
Sambongi, Y., Iko, Y., Tanabe, M., Omote, H., Iwamoto-Kihara, A., Ueda, I., Yanagida, T., Wada, Y., and Futai, M. (1999). *Science* **286**, 1722–1724.
Sapra, R., Bagramyan, K., and Adams M. W. W. (2003). *Proc. Natl. Acad. Sci. U.S.A.* **100**, 7545–7550
Schäfer, G., Engelhard, M., and Müller, V. (1999). *Microbiol. Mol. Biol. Rev.* **63**, 570–620.
Schemidt, R. A., Qu, J., Williams, J. R., and Brusilow, W. S. (1998). *J. Bacteriol.* **180**, 3205–3208.
Schnick, C., Forrest, L. R., Sansom, M. S., and Groth, G. (2000). *Biochim. Biophys. Acta* **1459**, 49–60.
Seelert, H., Poetsch, A., Dencher, N. A., Engel, A., Stahlberg, H., and Müller, D. J. (2000). *Nature* **405**, 418–419.
Senior, A. E., Weber, J., and Alshawi, M. K. (1995). *Biochem. Soc. Trans.* **23**, 747–752.
Slesarev, A. I., Mezhevaya, K. V., Makarova, K. S., Polushin, N. N., Shcherbinina, O. V., Shakhova, V. V., Belova, G. I., Aravind, L., Natale, D. A., Rogozin, I. B., Tatusov, R. L., Wolf, Y. I., Stetter, K. O., Malykh, A. G., Koonin, E. V., and Kozyavkin, S. A. (2002). *Proc. Natl. Acad. Sci. U.S.A.* **99**, 4644–4649.
Smigan, P., Majernik, A., and Greksak, M. (1994). *FEBS Lett.* **347**, 190–194.
Smigan, P., Majernik, A., Polak, P., Hapala, I., and Greksak, M. (1995). *FEBS Lett.* **371**, 119–122.
Smigan, P., Rusnak, P., Greksak, M., Zhilina, T. N., and Zavarzin, G. A. (1992). *FEBS Lett.* **300**, 193–196.
Smith, D. R., Doucette-Stamm, L. A., Deloughery, C., Lee, H., Dubois, J., Aldredge, T., Bashirzadeh, R., Blakely, D., Cook, R., Gilbert, K., Harrison, D., Hoang, L., Keagle, P., Lumm, W., Pothier, B., Qiu, D., Spadafora, R., Vicaire, R., Wang, Y., Wierzbowski, J., Gibson, R., Jiwani, N., Caruso, A., Bush, D., Safer, H., Patwell, D., Prabhakar, S., McDougall, S., Shimer, G., Goyal, A., Pietrokovski, S., Church, G. M., Daniels, C. J., Mao, J.-I., Rice, P., Nölling, J., and Reeve, J. N. (1997). *J. Bacteriol.* **179**, 7135–7155.
Speelmans, G., Poolman, B., Abee, T., and Konings, W. N. (1994). *J. Bacteriol.* **176**, 5160–5162.
Stahlberg, H., Müller, D. J., Suda, K., Fotiadis, D., Engel, A., Meier, T., Matthey, U., and Dimroth, P. (2001). *EMBO Rep.* **2**, 229–233.
Steinert, K., Wagner, V., Kroth-Pancic, P. G., and Bickel-Sandkötter, S. (1997). *J. Biol. Chem.* **272**, 6261–6269.
Stevens, T. H., and Forgac, M. (1997). *Annu. Rev. Cell Dev. Biol.* **13**, 779–808.
Stock, D., Gibbons, C., Arechaga, I., Leslie, A. G., and Walker, J. E. (2000). *Curr. Opin. Struct. Biol.* **10**, 672–679.
Stock, D., Leslie, A. G., and Walker, J. E. (1999). *Science* **286**, 1700–1705.
Vonck, J., von Nidda, T. K., Meier, T., Matthey, U., Mills, D. J., Kühlbrandt, W., and Dimroth, P. (2002). *J. Mol. Biol.* **321**, 307–316.
Wilkens, S., and Forgac, M. (2001). *J. Biol. Chem.* **276**, 44064–44068.
Wilms, R., Freiberg, C., Wegerle, E., Meier, I., Mayer, F., and Müller, V. (1996). *J. Biol. Chem.* **271**, 18843–18852.
Woese, C. R., Kandler, O., and Wheelis, M. L. (1990). *Proc. Natl. Acad. Sci. U.S.A.* **87**, 4576–4579.
Yoshida, M., Muneyuki, E., and Hisabori, T. (2001). *Nat. Rev. Mol. Cell Biol.* **2**, 669–677.
Zhang, Y., and Fillingame, R. H. (1995). *J. Biol. Chem.* **270**, 87–93.