Subunit Structure and Organization of the Genes of the A1A0 ATPase from the Archaeon Methanosarcina mazei Gö1*

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The proton-translocating A1A0 ATP synthase/hydrolase of Methanosarcina mazei Gö1 was purified and shown to consist of six subunits of molecular masses of 65, 49, 40, 36, 25, and 7 kDa. Electron microscopy revealed that this enzyme is organized in two domains, the hydrophilic A1 and the hydrophobic A0 domain, which are connected by a stalk. Genes coding for seven hydrophilic subunits were cloned and sequenced. From these data it is evident that the 65-, 49-, 40- and 25-kDa subunits are encoded by ahaA, ahaB, ahaC, and ahaD, respectively; they are part of the A1 domain or the stalk. In addition there are three more genes, ahaE, ahaF, and ahaG, encoding hydrophilic subunits, which were apparently lost during the purification of the protein. The A1 domain consists of at least the 7-kDa proteolipid and the 36-kDa subunit for which the genes have not yet been found. In summary, it is proposed that the A1A0 ATPase of Methanosarcina mazei Gö1 contains at least nine subunits, of which seven are located in A1 and/or the stalk and two in A0.

Methanogenesis from H2 + CO2 as catalyzed by the non-marine methanogenic Archaea Methanosarcina barkeri or Methanosarcina mazei Gö1 is obligatorily coupled to the generation of two primary ion gradients at the same time: the reduction of the heterodisulfide of coenzyme M1 and 7-mercaptoheptanoylthreoninephosphate is coupled to electric translocation of protons across the membrane; in addition the penultimate step of methanogenesis, the transfer of the methyl group from methyltetrahydromethanopterin to coenzyme M as catalyzed by the corrinoid-containing multi-subunit enzyme methyltetrahydromethanopterin:coenzyme M methyltransferase, is coupled to vectorial sodium ion translocation across the membrane (1). M. mazei Gö1 uses both gradients directly as driving force for ATP synthesis but employs two different enzymes for this purpose: an A1A0 ATP synthase couples ATP formation to Δψ41, whereas a F1F0 ATP synthase uses ΔϕNa1 as driving force (2). So far, methanogens are the only organisms known to contain two structurally different ATP synthases.

The archaeal A1A0 ATPase2 shares properties with both, bacterial F1F0 and eucaryal V1V0 ATPases (3, 4). It clearly functions as an ATP synthase, which is in accordance with F1F0 but in sharp contrast to V1V0 ATPases; the structure of the proteolipid (one of the subunits of the A0 domain), which is in the range of 6-8 kDa in A1A0 and F1F0 but 16 kDa in V1V0 ATPases, was suggested to be at least one of the reasons for this difference (5-8). On the other hand, the primary sequences of the subunits A and B of the catalytic A1 domain are clearly more closely related to vacuolar V1V0 ATPases (9-12). Therefore, the A1A0 ATPase is regarded as a chimeric protein in which the membrane domain is closely related to F1F0 but the catalytic domain closely to V1V0 ATPases.

Although A1A0 ATPase activity has been demonstrated in a number of Archaea, the subunit composition of this enzyme is far from being settled. It is generally accepted that the A1 domain contains at least two large subunits of 62-80 kDa (subunit A) and 49–55 kDa (subunit B) and the A0 domain contains a 7-kDa subunit, the so-called proteolipid. Minor subunits co-purified with the enzyme (5, 8, 13-17); but it has not been established whether these are genuine constituents of the ATPase. Furthermore, little is known about the genetic organization of the A1A0 ATPases. In a first step toward a better understanding of the structure and function of this interesting archaeal enzyme, we purified the A1A0 complex from membranes of M. mazei Gö1 and denon and characterized the 3' end of the A1A0 ATPase operon.

EXPERIMENTAL PROCEDURES

Materials—All chemicals were reagent grade and were purchased from Merck AG, Darmstadt, Germany. K2-ATP, DES, N-ethylmaleimide, and NBD-Cl were from Sigma, Deisenhofen, Germany. DCCD was from Aldrich, Steinheim, Germany. Organisms and Plasmids—M. mazei Gö1 (DSM 3647) was obtained from the Deutsche Sammlung für Mikroorganismen und Zellkulturen, Braunschweig, Germany, and grown under strictly anaerobic conditions on methanol on the medium described by Hippe et al. (18) with the addition of sodium acetate (1 g/l). Escherichia coli DH5α (supE44 ΔlacI169F80lacZΔM15) hsdS17 recA1 endA1 gyrA96 thi1 relA1; 19) was grown on LB at 37°C. Plasmids used were pSE420 (20), pHSG772, pHSG399 (21). Purification of the A1A0 ATPase—Cells were washed once in buffer A (50 mM Tris-HCl, pH 6.9, 10 mM MgSO4, 40 mM KCl, 5 mM NaN3, 10% (v/v) glycerol) and disrupted in a French pressure cell at a pressure of 18843
138 MPa. Debris was removed by centrifugation (25,000 × g, 90 min) and washed twice with buffer A containing 1 mM phenylmethylsulfonyl fluoride. Membranes were resuspended for solubilization in 2 mM sodium pyrophosphate, 20 mM EDTA, and stirred on ice for 30 h. After ultracentrifugation (120,000 × g, 90 min) the supernatant was applied to a DEAE-Sephacel column (2.6 × 25 cm), equilibrated with 25 mM Tris-HCl, pH 8.0. Elution was performed with a salt gradient (0–0.6 M NaCl in 25 mM Tris-HCl, pH 6.9), and fractions with ATPase activity were pooled and applied to a DEAE-Sephacel column, equilibrated with 25 mM Tris-HCl, pH 8.0. Fractionation was performed by application of a linear gradient of 0–0.5 M NaCl in 25 mM Tris-HCl, pH 8.0. Finally, a gel filtration was carried out on a Sephacryl S-300 column (1.6 × 80 cm) at a flow rate of 15 ml/h in 50 mM Tris-HCl, pH 6.9. All steps were performed at 4 °C.

Alternatively, purification in small amounts in two days was achieved by EDTA/pyrophosphate treatment of membranes and DEAE-Sephacel. The membranes were pelleted by ultracentrifugation (120,000 × g, 90 min) and washed twice with buffer A containing 1 mM phenylmethylsulfonyl fluoride. Membranes were resuspended for solubilization in 2 mM sodium pyrophosphate, 20 mM EDTA and stirred on ice for 30 h. After ultracentrifugation (120,000 × g, 90 min) the supernatant was applied to a DEAE-Sephacel column (2.6 × 25 cm), equilibrated with 25 mM Tris-HCl, pH 8.0. Elution was performed with a salt gradient (0–0.6 M NaCl in 25 mM Tris-HCl, pH 6.9), and fractions with ATPase activity were pooled and applied to a DEAE-Sephacel column, equilibrated with 25 mM Tris-HCl, pH 8.0. Fractionation was performed by application of a linear gradient of 0–0.5 M NaCl in 25 mM Tris-HCl, pH 8.0. Finally, a gel filtration was carried out on a Sephacryl S-300 column (1.6 × 80 cm) at a flow rate of 15 ml/h in 50 mM Tris-HCl, pH 6.9. All steps were performed at 4 °C.

### Purification of the A1 ATPase

**TABLE I**

| Purification step | Protein (mg) | ATPase activity (milliunits) | Specific activity (milliunits/mg) | Enrichment (fold) | Yield (%) |
|-------------------|--------------|-----------------------------|---------------------------------|------------------|----------|
| membranes         | 3120         | 105,770                     | 33.9                            | 1.0              | 100.0    |
| EDTA depletion     | 960          | 72,384                      | 75.4                            | 2.2              | 68.4     |
| DEAE-Sepharose     | 240          | 27,840                      | 116.0                           | 3.4              | 26.3     |
| DEAE-Sephacel      | 37           | 16,502                      | 446.4                           | 13.2             | 15.6     |
| Sephacyl S-300     | 12           | 6805                        | 567.1                           | 16.7             | 6.4      |

**FIG. 1. SDS-PAGE of purified A1 ATPase.** A1 ATPase was purified by EDTA/pyrophosphate treatment of membranes and DEAE-Sephacel. Arrow indicates subunits of ATPase (left) or methyl-CoM-methylreductase (right). St, molecular mass standards.
### A1A0 ATPase of M. mazei

**Results**

**Characterization of the A1A0 ATPase in Inverted Membrane Vesicles**

Since M. mazei G01 was recently shown by determining rates of ATP synthesis under various conditions to contain a Na⁺-translocating F₁F₀ as well as a H⁺-translocating A₁A₀ ATP synthase (2), it was essential to analyze the membrane-bound ATPase activity in more detail. The ATPase activity in washed membranes of M. mazei G01 was optimal at pH 5.2; pH values lower than 5.2 decreased the activity very strongly (zero activity at pH 4.5) but at values higher than 5.2 the decrease was less pronounced (22% activity at pH 7.0). The enzyme was stimulated 3-fold by addition of sulfite (40 mM), 1.7-fold by ethane (18% v/v) and 2-fold by glycerol (20% v/v). Addition of various divalent cations resulted in different degrees of stimulation. Stimulation by Mg²⁺ was maximal at 10 mM, a value that corresponds to a Mg₆ATP ratio of 2:1. Zn²⁺ but not Mn²⁺ stimulated ATPase activity but the degree of stimulation was 1.3 times higher than that measured for Mg²⁺ stimulation. Other divalent cations such as Ca²⁺, Ni²⁺, Cu²⁺, and Fe²⁺ and the monovalent cations Na⁺ and K⁺ had only negligible effects. The ATPase activity of M. mazei G01 membranes was not inhibited by NBD-Cl (1 mM) or by azide (1 mM), typical inhibitors of F₁F₀ ATPases. Nitrate, a typical inhibitor of V₁V₀ ATPases, inhibited the enzyme with an I₅₀ of 30 mM. DCCD and DES both inhibited the enzyme with DES (I₅₀ 100 μM) being a more potent inhibitor than DCCD (I₅₀ 500 μM). Taken together, the catalytic properties and the inhibitor sensitivity of the ATPase activity at membranes of M. mazei G01 are in full accord with the action of an A₁A₀ ATPase. Apparently, the F₁F₀ type enzyme does not show ATP hydrolysis activity under these conditions. This is in accordance with our previous notion that the F₁F₀ type enzyme is active only in the presence of an electrochemical potential across the membrane (2).

#### Purification of the A₁ ATPase

The A₁ ATPase was released from the membranes by incubation in low ionic strength buffer (EDTA-pyrophosphate depletion) and purified by chromatography on DEAE-Sepharose, DEAE-Sephadex, and gel filtration with an enrichment factor of 16.7 and a yield of 6.4% (Table I). The purified A₁ ATPase had a molecular mass of 360 kDa as determined by gel filtration on Superose 6, and of 350 kDa as determined by native gradient gel electrophoresis (data not shown). When this sample was applied to SDS-PAGE, only two bands representing proteins with molecular masses of 65 (subunit A) and 49 kDa (subunit B) were detected. ATP hydrolysis by this preparation occurred with a V₅₀ of 90 milliunits/mg protein and a Kₘ for ATP of 4 m. Taken together, the catalytic properties and the inhibitor sensitivity of the ATPase activity at membranes of M. mazei G01 are in full accord with the action of an A₁A₀ ATPase. Apparently, the F₁F₀ type enzyme does not show ATP hydrolysis activity under these conditions. This is in accordance with our previous notion that the F₁F₀ type enzyme is active only in the presence of an electrochemical potential across the membrane (2).

### Table I

| Purification step | Protein | ATPase activity | Specific activity | Enrichment | Yield |
|------------------|---------|-----------------|------------------|------------|-------|
| Membranes        | 397     | 5722            | 14.4             | 1.0        | 100.0 |
| CHAPS supernatant| 310     | 6043            | 19.5             | 3.25       | 105.0 |
| DEAE-Sepharose   | 30      | 2312            | 77.4             | 5.37       | 41.0  |
| DEAE-Sephacel    | 7       | 416             | 59.4             | 4.13       | 7.2   |
| Gradient centrifug. | 1.5   | 239             | 159.3            | 11.1       | 4.1   |

**Fig. 2.** SDS-PAGE of purified A₁A₀ ATPase. Enzyme was purified as described under “Experimental Procedures” and applied to a SDS-PAGE. Lane 1, molecular mass standards; lane 2, A₁A₀ ATPase.
furtherevidencethattheATPasepurifiedfromM. mazeiof the A1A0 type.

65, 49, 40, 36, 28, and 7 kDa were observed (Fig. 2). A SDS-PAGE polypeptides with apparent molecular masses of units/mg of protein (Table II). When this sample was applied to 400 precipitation and saccharose-glycerin-gradient centrifugation with a yield of 4.1% and a specific activity of 159 milli-

the A1A0 ATPase was solubilized from washed inverted vesicles by treatment with 0.6% (w/v) CHAPs at a protein concentration of 8–10 mg/ml corresponding to a protein:detergent ratio of 1:0.75 to 1:0.6. After ultracentrifugation 78% of the ATPase activity were recovered in the 39% identical and 61% homologous to HypF from E. coli. The start of ahaG is indicated by the bold arrow. Putative rho-independent transcription terminators are indicated by the bold arrows above the sequence. Tandem repeats and partial sequences thereof are boxed, and the first base is indicated by the arrow above. Putative promoter sequences (Box A and B) are boxed, and conserved residues are marked by asterisks. A putative SD sequence is underlined.

several A1A0 ATPases were aggregated in opposite orientation, brought about by interaction of their A0 parts. The isolated A1 parts of the enzyme showed a rotational symmetry and a size similar to that observed for the mitochondrial F1 ATPase and the A1 ATPase from S. acidocaldarius.

Nucleotide Sequence and Structure of the aha Operon

Coding for the A1 ATPase from M. mazei Go¨1. The direction of transcription from the lac promoter in the subclones is indicated by the arrows. B, BamHI;Bg, BglII; C, ClaI; E, EcoRI; H, HindIII; K, KpnI; S, Sall; X, XbaI.

Ultrastructure of the A1A0 and the A1 ATPase from M. mazei Go¨1, as Revealed by Electron Microscopy

In electron micrographs the A1A0 ATPase appeared to be composed of a base, a stalk, and a head part (Fig. 3). Often,
Ribosomal binding sites, stop and start codons of the aha genes and of hypF

Identified aha Genes

The program TESTCODE categorized the region from ahaE through G as a coding sequence above the 95% confidence level. The program CODONPREFERENCE also recognized all designated genes as authentic genes. Furthermore, the apparent molecular masses of the 65-, 49-, 40-, and 25-kDa subunit of the purified enzyme correspond well to the deduced molecular masses of AhaA, B, C, and D.

Properties of the Gene Products and Similarity to Polypeptides of V1V0 and F1F0 ATPases

Properties of the gene products are summarized in Table IV. Generally, ahaE through G code for hydrophilic polypeptides. Data base searches, primary sequence alignments, secondary structure predictions, and comparisons of the molecular masses and isoelectric points were used to identify homologous subunits in V1V0 and F1F0 ATPases.

AhaA—The deduced molecular mass of AhaD (66.106 kDa) corresponds well to the experimentally derived molecular weight of 65,000 of subunit A of the purified enzyme. The deduced gene product is 86% identical to the corresponding subunit from M. barkeri (11) and 63 and 52% of the residues are identical to subunit A of the A1A0 ATPase from Haloferax volcanii (32) and S. acidocaldarius (9), respectively (Fig. 6). Still 50–55% of the residues are identical to subunit A of the 39-kDa accessory protein (Vma6p) of V1V0 ATPases. On the other hand, only 27% of the residues are identical to subunit \( \beta \) of the F1F0 ATPase of E. coli (37). AhaA contains the Walker motifs A and B (38), which are part of the nucleotide binding domain, indicating that it represents the catalytic subunit. This is in agreement with the finding that anti-subunit A specific antibodies were 4 times more effective in inhibiting ATPase activity of the purified enzyme than M. mazei Go1 than anti-subunit B specific antibodies. From the multiple alignment, it is evident that all of the residues involved in nucleotide binding in the mitochondrial F1F0 ATPase (39) are conserved in the M. mazei Go1 A1A0 ATPase, and therefore this general scheme seems also valid for nucleotide binding in subunits B and A of A1A0 and V1V0 ATPases.

AhaB—ahaB codes for a polypeptide with a molecular mass of 50.348 kDa, which corresponds well to the experimentally derived value of 49 kDa of subunit B of the purified enzyme. Go1 than anti-subunit B-specific antibodies. From the multiple alignment, it is evident that all of the residues involved in nucleotide binding in the mitochondrial F1F0 ATPase (39) are conserved in the M. mazei Go1 A1A0 ATPase, and therefore this general scheme seems also valid for nucleotide binding in subunits B and A of A1A0 and V1V0 ATPases.

AhaC—ahaC shows overall 33% identity (46), Vma1p of yeast (28% identity) (46), of V1V0 ATPase, and Vma8p of yeast (28% identity) (46). Vma8p of yeast (28% identity) (46) and Vma8p of yeast (28% identity) (46) are highly homologous to V1V0 ATPases. Furthermore, the apparent molecular masses of the 65-, 49-, 40-, and 25-kDa subunit of the purified enzyme correspond well to the deduced molecular masses of AhaA, B, C, and D.

AhaD—ahaD codes for a polypeptide with a molecular mass of 50.348 kDa, which corresponds well to the experimentally derived value of 49 kDa of subunit B of the purified enzyme. Go1 than anti-subunit B-specific antibodies. From the multiple alignment, it is evident that all of the residues involved in nucleotide binding in the mitochondrial F1F0 ATPase (39) are conserved in the M. mazei Go1 A1A0 ATPase, and therefore this general scheme seems also valid for nucleotide binding in subunits B and A of A1A0 and V1V0 ATPases.

AhaE—ahaE codes for a polypeptide with a molecular mass of 50.348 kDa, which corresponds well to the experimentally derived value of 49 kDa of subunit B of the purified enzyme. Go1 than anti-subunit B-specific antibodies. From the multiple alignment, it is evident that all of the residues involved in nucleotide binding in the mitochondrial F1F0 ATPase (39) are conserved in the M. mazei Go1 A1A0 ATPase, and therefore this general scheme seems also valid for nucleotide binding in subunits B and A of A1A0 and V1V0 ATPases.

AhaF—ahaF codes for a polypeptide with a molecular mass of 50.348 kDa, which corresponds well to the experimentally derived value of 49 kDa of subunit B of the purified enzyme. Go1 than anti-subunit B-specific antibodies. From the multiple alignment, it is evident that all of the residues involved in nucleotide binding in the mitochondrial F1F0 ATPase (39) are conserved in the M. mazei Go1 A1A0 ATPase, and therefore this general scheme seems also valid for nucleotide binding in subunits B and A of A1A0 and V1V0 ATPases.

AhaG—ahaG codes for a polypeptide with a molecular mass of 50.348 kDa, which corresponds well to the experimentally derived value of 49 kDa of subunit B of the purified enzyme. Go1 than anti-subunit B-specific antibodies. From the multiple alignment, it is evident that all of the residues involved in nucleotide binding in the mitochondrial F1F0 ATPase (39) are conserved in the M. mazei Go1 A1A0 ATPase, and therefore this general scheme seems also valid for nucleotide binding in subunits B and A of A1A0 and V1V0 ATPases.

Human (53%) (41), and to subunit \( \alpha \) from the F1F0 ATPase of E. coli (24%) (37). AhaC—ahaC codes for a polypeptide with a molecular mass of 41.486 kDa, which corresponds well to the experimentally derived value of 40 kDa of subunit C of the purified enzyme. By data base searches, NtpC of E. hirae V1V0 ATPase (22% identity; 44% homology) (42) and AtpC of H. volcanii A1A0 ATPase (GenBank accession no. X79516) (35% identity, 58% homology) were found to be homologous. A multiple alignment of these polypeptides to the 39-kDa accessory protein (Vma6p) of V1V0 ATPases (43, 44) revealed a number of homologous residues indicating that these proteins are homologous (Fig. 8). Nine residues seem to be invariant, five of these are located in the C-terminal region, which in addition has several conserved substitutions. Of these five residues, three are charged and positioned in a predicted \( \beta \)-sheet structure. Apparently, there is no homologous polypeptide in F1F0 ATPases.

AhaD—ahaD codes for a polypeptide with a molecular mass of 50.348 kDa, which corresponds well to the experimentally derived value of 49 kDa of subunit B of the purified enzyme. Go1 than anti-subunit B-specific antibodies. From the multiple alignment, it is evident that all of the residues involved in nucleotide binding in the mitochondrial F1F0 ATPase (39) are conserved in the M. mazei Go1 A1A0 ATPase, and therefore this general scheme seems also valid for nucleotide binding in subunits B and A of A1A0 and V1V0 ATPases.

AhaF—ahaF codes for a polypeptide with a molecular mass of 50.348 kDa, which corresponds well to the experimentally derived value of 49 kDa of subunit B of the purified enzyme. Go1 than anti-subunit B-specific antibodies. From the multiple alignment, it is evident that all of the residues involved in nucleotide binding in the mitochondrial F1F0 ATPase (39) are conserved in the M. mazei Go1 A1A0 ATPase, and therefore this general scheme seems also valid for nucleotide binding in subunits B and A of A1A0 and V1V0 ATPases.
were identified by BLAST as the homologues of AhaE. Secondary structure analysis predicts that AhaE and its homologues are largely $\alpha$-helical. A multiple alignment shows that the region Gly-145 to Leu-149 is well conserved between prokaryotic and eukaryotic subunits (Fig. 10). According to its size, AhaE could be the homologue of subunit $d$ of $F_1F_0$ ATPases. This is in agreement with the observation that the aforementioned residues are also conserved in subunit $d$ of $E. coli$ F1F0 (37).

AhaF—Data base searches identified only AtpE of $H. volcanii$ A1A0 ATPase (GenBank accession no. X79516) to be homologous (11.582 kDa; 50% identity). However, its physical localization within the operon and its size suggest that AhaF is the homologue of NtpG of $E. hirae$ V1V0 (42), the 14-kDa subunit of V1V0 ATPases of tobacco hornworm (51), $Drosophila$ melanogaster (GenBank accession no. Z26918), Vma7p of $S. cerevisiae$ (52), and subunit $e$ of $F_1F_0$ ATPases (37). Multiple alignments showed that the N terminus is fairly well conserved (Fig. 11), and the polypeptide is predicted to have a small $\alpha$-helical region at its N terminus and a $\beta$-sheet region at its C terminus.

AhaG—Of the polypeptides tested, AhaG is the only one which did not reveal a homologous polypeptide by data base search. However, according to its size (6135 kDa) and its location within the operon we suggest that AhaG, NtpH of $E. hirae$ (37), is the homologue of NtpH of $E. hirae$ V1V0 ATPase of $M. mazei$. Identical and homologous residues are indicated by asterisks and circles, respectively.

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**FIG. 6. Alignment of the deduced amino acid sequence of AhaA of $M. mazei$ $A_1A_0$ ATPase (Mma) with subunit A of $A_1A_0$ ATPases of $M. barkeri$ (Mba), $H. volcanii$ (Hvo), and $S. acidocaldarius$ (Sac), with subunit A or Vma1p of $V_1V_0$ ATPases of $E. hirae$ (Ehi), bovine (Bta), and $S. cerevisiae$ (Sce), and with subunit $b$ of $F_1F_0$ ATPases of bovine (BtaUncD) and $E. coli$ (EcoUncD).**

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The alignment is shown with identical and homologous residues indicated by asterisks and circles, respectively.
**DISCUSSION**

Structure of the $A_{1A0}$ ATPase and Possible Function of Subunits—It is apparent from the electron micrographs that the $A_{1A0}$ ATPase consists, like the $F_{1}F_{0}$ and the $V_{1}V_{0}$ ATPase, of a hydrophilic (A1) and a hydrophobic domain (A0), which are connected by a stalk. From the combined biochemical and molecular results, we suggest that this structure is built up by at least 9 non-identical subunits used in different stoichiometry (Fig. 13). In view of the apparent similarities, the discussion of the structure/function prediction of individual subunits of the $A_{1A0}$ ATPase will be done on the basis of the known features of $F_{1}F_{0}$ and $V_{1}V_{0}$ ATPases (53–56). Subunits A and B were both present in the purified enzyme; they share extensive homologies with Vma1p/subunit A and Vma2p/subunit B of $V_{1}V_{0}$ ATPases and with $\beta$ and $\alpha$ of $F_{1}F_{0}$ ATPases. According to the apparent homology, it is suggested that subunits A and B of the $A_{1A0}$ ATPase are present in triplicate each and are arranged

**FIG. 7.** Alignment of the deduced amino acid sequence of AhaB of *M. mazei* Göl $A_{1A0}$ ATPase with subunit B of $A_{1A0}$ ATPases of *E. hirae* and bovine, with Vma2p of *S. cerevisiae*, and with subunit $\beta$ of $F_{1}F_{0}$ ATPases of bovine and *E. coli*. Identical and homologous residues are indicated by asterisks and circles, respectively. For abbreviations, see Fig. 6.
alternately in an orange-like structure. This is supported by the rotational symmetry determined in the electron micrographs. AhaA carries the catalytic nucleotide binding sites, whereas the non-catalytic sites are located on AhaB. All of the residues important for nucleotide binding in $\alpha$ and $\beta$ of F1F0 (39) are conserved in A and B of A1AO. The proline-rich hydrophobic sleeves suggested to be involved in $\gamma$-$\beta$ interaction in F1F0 are also conserved in AhaA (residues 134–199). The N-terminal region including Gln-270, Thr-274, Glu-276, and Glu-279, which were shown by mutagenesis studies to be important for function in E. coli F1F0 (58), is not present in the counterpart of prokaryotic V1V0 and A1A0 ATPases, indicating that not particular residues but rather a secondary structure is important for function. The fact that this subunit is only present in the A1A0 rather than in the A1 preparation might suggest a structural (and functional?) interaction also with the membrane domain. On the other hand, it is conceivable that its non-appearance in the A1 preparation might be due to preparation artifacts. However, the available data indicate that the general structure and function of subunits A, B, and D of A1AO and V1V0 ATPases are comparable to subunits $\beta$, $\alpha$, and $\gamma$ of F1F0 ATPases.

AhaC is identical to the 40-kDa subunit of the purified enzyme; it has no counterpart in F1F0 ATPases. The eukaryotic homologue of AhaC (Vma6p) of S. cerevisiae is hydrophilic as is AhaC. However, since Vma6p co-purifies with the V0 domain, it is assumed to bind to the membrane domain. This is supported by the rotational symmetry determined in the electron micrographs. AhaA carries the catalytic nucleotide binding sites, whereas the non-catalytic sites are located on AhaB. All of the residues important for nucleotide binding in $\alpha$ and $\beta$ of F1F0 (39) are conserved in A and B of A1AO. The proline-rich hydrophobic sleeves suggested to be involved in $\gamma$-$\beta$ interaction in F1F0 are also conserved in AhaA (residues 134–199). The N-terminal region including Gln-270, Thr-274, Glu-276, and Glu-279, which were shown by mutagenesis studies to be important for function in E. coli F1F0 (58), is not present in the counterpart of prokaryotic V1V0 and A1A0 ATPases, indicating that not particular residues but rather a secondary structure is important for function. The fact that this subunit is only present in the A1A0 rather than in the A1 preparation might suggest a structural (and functional?) interaction also with the membrane domain. On the other hand, it is conceivable that its non-appearance in the A1 preparation might be due to preparation artifacts. However, the available data indicate that the general structure and function of subunits A, B, and D of A1AO and V1V0 ATPases are comparable to subunits $\beta$, $\alpha$, and $\gamma$ of F1F0 ATPases.

AhaD is identical to the 28-kDa subunit of the purified protein; it is homologous to subunit $\gamma$ of F1F0 ATPases, which plays a crucial role in transmitting energy from the F0 to the F1 domain (57). The X-ray structure revealed that subunit $\gamma$ is highly $\alpha$-helical at its N and C termini, which both protrude in the orange-like head assembly of the catalytic and non-catalytic subunits (39). A secondary structure analysis strongly predicts $\alpha$-helical N and C termini also in AhaD. A multiple alignment did not reveal conserved residues (Fig. 8). Further alignment did not reveal conserved residues (Fig. 9).
membrane via interaction with a membrane-bound subunit (44) and probably is part of the stalk. Since AhaC was found in the A1 preparation detached from membranes of M. mazei, we regard this subunit as part of the A1 domain or the stalk.

AhaE through G were apparently not present in the purified A1A0 ATPase, although it cannot be excluded that they did not stain well. From the multiple alignment, it is suggested that AhaE is the homologue of Vma4p or the 31-kDa subunit of eukaryotic V1V0 and subunit d of F1F0 ATPases, and AhaF and its homologues are suggested to be the homologues of subunit e of F1F0 ATPases. However, in both cases the structure and function of the subunits are not well understood. Whether AhaG plays a structural role in the A1A0 ATPase remains to be established. AhaG has no counterpart in eukaryotic V1V0 ATPases. In this context it is interesting to note that there is a small gene (uncI) as the first gene in the unc operon, which codes for the F1F0 ATPase. The function of the gene product is obscure but it is not essential for an active F1F0 ATP synthase complex. Based on size, AhaG and its bacterial homologues could very well be the homologues of the uncI gene product.

The fact that the 36-kDa subunit co-purifies with the complex only after solubilization of the membrane is indicative for its membrane localization. Therefore, we suggest that the A0 domain consists of at least two subunits of Mr 7,000 (the proteolipid) and 36,000. In yeast V1V0 three subunits (excluding Vma6p) are present in the membrane domain: the 16-kDa proteolipid (59), a recently described 13-kDa protein with homologies to subunit b of F1F0 ATPases (60), and a 115-kDa protein (61).

Structure of the aha Operon—There is a striking homology between the aha operon of M. mazei Göl, the ntp operon of E. hirae VV0, Vma4p of S. cerevisiae, 31-kDa subunit of bovine VV0, and with subunit δ of E. coli F1F0 ATPase. Identiﬁcation and homologous residues are indicated by asterisks and circles, respectively. Residues that are homologous or identical only in A1A0 and V1V0 ATPases are boxed. For abbreviations, see Fig. 6.

Fig. 11. Alignment of deduced amino acid sequence of AhaE of M. mazei Göl A1A0 ATPase with AtpE of H. volcanii A1A0, NtpE of E. hirae V1V0, Vma4p of S. cerevisiae, and subunit d of E. coli F1F0 ATPase. Identical and homologous residues are indicated by asterisks and circles, respectively. For abbreviations, see Fig. 6.

Fig. 12. Alignment of deduced amino acid sequence of AhaG of M. mazei Göl A1A0 ATPase with AtpE of S. acidocaldarius A1A0, and NtpB of E. hirae VV0. Identical and homologous residues are indicated by asterisks and circles, respectively. For abbreviations, see Fig. 6.

Göl, we regard this subunit as part of the A1 domain or the stalk.

AhaE through G were apparently not present in the purified A1A0 ATPase, although it cannot be excluded that they did not stain well. From the multiple alignment, it is suggested that AhaE is the homologue of Vma4p or the 31-kDa subunit of eukaryotic V1V0 and subunit δ of F1F0 ATPases, and AhaF and its homologues are suggested to be the homologues of subunit e of F1F0 ATPases. However, in both cases the structure and function of the subunits are not well understood. Whether AhaG plays a structural role in the A1A0 ATPase remains to be established. AhaG has no counterpart in eukaryotic V1V0 ATPases. In this context it is interesting to note that there is a small gene (uncI) as the first gene in the unc operon, which codes for the F1F0 ATPase. The function of the gene product is obscure but it is not essential for an active F1F0 ATP synthase complex. Based on size, AhaG and its bacterial homologues could very well be the homologues of the uncI gene product.

The fact that the 36-kDa subunit co-purifies with the complex only after solubilization of the membrane is indicative for its membrane localization. Therefore, we suggest that the A0 domain consists of at least two subunits of Mr 7,000 (the proteolipid) and 36,000. In yeast V1V0 three subunits (excluding Vma6p) are present in the membrane domain: the 16-kDa proteolipid (59), a recently described 13-kDa protein with homologies to subunit b of F1F0 ATPases (60), and a 115-kDa protein (61).

Structure of the aha Operon—There is a striking homology between the aha operon of M. mazei Göl, the ntp operon of E. hirae (42) and the atp operon of S. acidocaldarius (45) and H. volcanii (GenBank accession no. X79516) (Fig. 14). Regarding the hydropilic subunits, the arrangements of ahaE through G of M. mazei Göl is identical to the corresponding genes in the eubacterium E. hirae and the homologues of ahaE through B are also conserved in the same order in H. volcanii. However, the organization of the genes coding for the hydropilic sub-
units is different. A NtpF homologue is not present in M. mazei G61. This is expected since the ATPase of E. hirae is believed to function as a K+/H+ antiporter (62) and NtpF is believed by sequence similarities to play a role in this exchange (42). There is no more aha gene downstream of ahaG, but preliminary expression studies indicate that there are more genes in the aha operon upstream of ahaE; sequencing of this region is currently under way.

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Fig. 14. Arrangement of genes in known bacterial and archaean V,V,E, and A,A,A,A, ATPase operons. Genes encoding hydrophobic subunits are marked by asterisks. Homologous genes are depicted by the same pattern.