The Archaeal P-Type ATPases

Benoît De Hertogh,[1,2] Anne-Catherine Lantin,[1,2] Philippe V. Baret,[1] and André Goffeau[3,4]

A phylogenetic analysis was carried out of a total of 58 P-type ATPases encoded within the genomes of 20 archaea species. Members from six subfamilies were identified including: putative metal-, proton-, calcium-, sodium/potassium-, potassium-, and magnesium/nickel-transporting ATPases. Six novel putative proton-ATPases from archaea species growing under different temperature and pH conditions were shown to have shorter N- and C-termini than those of orthologous yeast or plant proton-ATPases. Moreover recent biochemical data are reviewed that report functional expression of putative archaea metal- or proton-ATPases in bacteria or yeast.

KEY WORDS: Archaea; ATPases; comparative genomics; functional expression; phylogeny; transport classification.

INTRODUCTION

The phylogenetic P-type ATPase superfamily (Pedersen and Carafoli, 1987) is one of the largest among the membrane protein families. Its members transport cations across membranes at the expense of ATP hydrolysis. They are characterized by the formation of an aspartyl-phosphate catalytic intermediate during ATP hydrolysis (Post and Kume, 1973) and by the transition of two conformations called E1 and E2 (de Meis and Vianna, 1979). The best known members of the P-type ATPase superfamily are the nerve Na/K ATPase (Skou, 1957), the sarcoplasmic Ca-ATPase (Hasselbach and Makinose, 1961), and the yeast H-ATPase (Dufour and Goffeau, 1978). The elucidation of the coupling mechanism of ATP hydrolysis and cation transport is one of the most challenging research field of contemporary biochemistry. The structure of the sarcoplasmic reticular Ca^{2+}-ATPase has been solved at 2.6 Å resolution for the protein to which two Ca^{2+} are bound, and at 3.1 Å resolution for the protein lacking Ca^{2+} (Toyoshima *et al.*, 2000; Toyoshima and Nomura, 2002).

The availability of the complete yeast genome sequence (Goffeau *et al.*, 1996) has allowed the establishment of the complete inventory of P-type ATPases in yeast (Catty *et al.*, 1997). Among the 16 yeast gene products identified as P-type ATPases, six phylogenetic subfamilies could be identified, including: the novel NAS (nonassigned substrate) and the PL (aminophospholipid transferase) subfamily. Further mining of genome data from bacteria, archaea, and Eukaryote species has identified a total of 10 phylogenetic subfamilies (Axelsen and Palmgren, 1998). Only three subfamilies (potassium, metal, and magnesium-ATPases) were found to have bacterial members, while the other subfamilies (H, Na, Na/K, H/K, Ca, NAS, and PL) were considered typical for eukaryote species. Some metal-ATPases are however also found in Eukaryotes (Argüello, in press). An exhaustive inventory of the P-type ATPases from archaea genomes has not been carried out yet, despite the prospect that the exploration of these species may offer access to novel P-type ATPase molecules of unusual stability under extreme pH or temperature conditions.

Over 20 archaea genomes have been completely or partially sequenced. This corresponds to near 50,000 genes among which we have detected 58 encoded P-type ATPases. We have in this paper made an *in silicio* analysis of all detected archaea P-type ATPases. We found that the archaea P-type ATPase genes contain members of phylogenetic clusters characteristic of both bacteria and

[1] Unité de Génétique (GENA), Université catholique de Louvain, Louvain-la-Neuve, Belgique.

[2] These two authors contributed equally to this work.

[3] Unité de Biochimie physiologique (FYSA), Université catholique de Louvain, Louvain-la-Neuve, Belgique.

[4] To whom correspondence should be addressed; e-mail: goffeau@fysa.ucl.ac.be.

Eukaryote kingdoms. We also have briefly reviewed the few biochemical data on archaea P-type ATPases reported so far.

IN SILICIO INVENTORY OF ARCHAEA P-TYPE ATPases

Table I lists the 58 archaea P-type ATPases that were detected in 17 archaea species by screening the databases from TIGR, NCBI, DOE, and TCDB. Only protein sequences of more than 600 aminoacyl residues were considered for further phylogenetic analyses. By blasting in TCDB, each archaea sequence was associated to an existing TC (Transport Classification) cluster characterized by a five-digit identification number as well as by a name reflecting substrate specificity.

Figure 1 shows a phylogenetic tree of the archaea P-type ATPases obtained by Clustal/Phylip analysis. Six subfamilies were distinguished: the metal-transporters, the Na/K-transporters, the magnesium-transporters, the calcium-transporters, the proton-transporters, and the potassium-transporters.

The Metal-ATPases

The largest subfamily comprised 29 archaea metal-ATPases. With one exception (*Methanococcus jannashii*), all analyzed archaea species contained 1–3 metal-ATPases. They belong to six different phylogenetic TC clusters (3.A.3.5.1/2/3/7 or 3.A.3.6.1/5) to which bacteria, fungi, plant, protozoa, human (Wilson disease protein), and archaea (CopA) members had been assigned. The proteins belonging to these clusters export (more rarely import), a variety of monovalent or divalent metals (copper, zinc, lead, cadmium, or silver). Only three out of the five transmembrane motifs for metal-transporting ATPases recently identified by Argüello (2003) were present in the archaea P-type ATPases. They correspond to the groupIB-1 (Cu^+/Ag^+), groupIB-2 ($Zn^{2+}/Cd^{2+}/Pb^{2+}$), and group IB-3 ($Cu^{2+}/Cu^+/Ag^+$) motifs. The motif IB-1 is present in the archaea members of the TC cluster 3.A.3.5.1., the motif IB-2 corresponds to the TC cluster 3.A.6.3.1, and the motif IB-B3 is observed in the TC cluster 3.A.3.5.2 (see Table I). The bacterial motifs detected by Argüello in group IB-4 (Co^{2+}) and groupIB-5 (unspecified metals) were not present in the analyzed archaea metal-ATPases. Detailed fitting of "Argüello motifs" remains to be carried out and may lead to identification of refined motifs for some archaea metal-ATPases.

The Proton-ATPases

A surprisingly high number of archaea (all being Euryarchaeota) putative proton-ATPases gene products were detected. Until recently, this subfamily was considered to be restricted to fungi, protists, and plant species.

The alignment of Fig. 2 shows that the six full-sized putative proton-ATPases detected were made of 780 (Thermoplasma acidophylum) to 839 (Methanosarcina acetivorans) aminoacyl residues. Like all *bona fide* plant and yeast proton-ATPases, they exhibit 10 predicted transmembrane spans. As shown by Morsomme *et al.* (2002), the cation-binding transmembrane domains 4 and 6 are more similar to the corresponding plant proton-ATPase domains than to that of any other P-type-ATPase subfamily. The cytoplasmic amino and carboxy-terminals of the putative archaea proton-ATPases are unusually short (about 40 and 10 residues, respectively). The archaea cytoplasmic N (nucleotide-binding domains) is shorter than that of plant and yeast proton-ATPases, but Figure 2 shows that the sequence of the proton-ATPase from some archaea species contains specific inserts in their N domain. This may be related to the fact that the different host archaea species analyzed grow under very different conditions (Table II). Some members of this cluster belong to hyper-thermophilic (*Methanococcus jannaschii*), thermophilic (*Thermoplasma acidophilum*), mesophilic (*Ferroplasma acidarmanus*), or even cryophilic (*Methanococcoides burtonii*) species. This might lead to the identification of the aminoacyl residues involved in thermostability. Similarly, some of the host archaea are growing in extremely acid conditions (*Ferroplasma acidarmanus* and *Thermoplasma acidophilum* that both grow at pH near zero). One may expect that their proton pumping properties may be particularly efficient. In this view, transport measurements in reconstituted and purified proteolipid vesicles are expected to be particularly useful. However, it remains to demonstrate experimentally that the members of this putative proton-ATPase cluster do indeed transport protons.

The K- and Na/K-ATPases

The K influx-ATPases were detected so far only in Gram-negative bacteria. Presence of orthologous K influx in four archaea extremophiles should allow further studies of their function and mechanism.

The methanogenic species *Methanosarcina acetivorans, Methanosarcina mazei, Methanosarcina barkeri, Methanococcoides burtonii,* and *Methanobacterium thermoautotrophicum* contain an intriguing cluster of P-type

Table I. Listing of the Analyzed P-Type ATPases

Code	Species	Id	AA	Subst	TC #	TC cluster name	TC typical organism
Ap1	Aeropyrum pernix	Q9Y8R2	621	Metal	3.A.3.6.1	Zn^{2+}-, Cd^{2+}-, Pb^{2+}-ATPase (efflux)	Bacteria; plants; fungi; protozoa
Ap2	Aeropyrum pernix	Q9YBZ6	835	Metal	3.A.3.6.1	Zn^{2+}-, Cd^{2+}-, Pb^{2+}-ATPase (efflux)	Bacteria; plants; fungi; protozoa
Af1	Archaeoglobus fulgidus	O29777	804	Metal	3.A.3.5.7	Cu^+-Ag^+-ATPase (efflux)	Archaea (CopA)
Af2	Archaeoglobus fulgidus	O30085	690	Metal	3.A.3.5.2	Cu^+-, Ag^+-ATPase (efflux)	Bacteria
Fa1	Ferroplasma acidarmanus	c157.218	783	Proton	3.A.3.3.4	Putative H^+-ATPase	Archaea
Fa2	Ferroplasma acidarmanus	c157.66	720	Potassium	3.A.3.7.1	K^+-ATPase (uptake)	Bacteria
Fa3	Ferroplasma acidarmanus	c130.3	880	Calcium	3.A.3.2.4	Ca^{2+}-ATPase (efflux)	Bacteria
Fa4	Ferroplasma acidarmanus	c131.6	676	Metal	3.A.3.5.1	Cu^{2+}-ATPase (uptake)	Bacteria
Hs1	Halobacterium sp.	P57699	719	Potassium	3.A.3.7.1	K^+-ATPase (uptake)	Bacteria
Hs2	Halobacterium sp.	Q9HN90	801	Metal	3.A.3.5.1	Cu^{2+}-ATPase (uptake)	Bacteria
Hs3	Halobacterium sp.	Q9HSN5	757	Metal	3.A.3.6.1	Zn^{2+}-, Cd^{2+}-, Pb^{2+}-ATPase (efflux)	Bacteria; plants; fungi; protozoa
Hs4	Halobacterium sp.	Q9HRH2	857	Metal	3.A.3.5.7	Cu^+-Ag^+-ATPase (efflux)	Archaea (CopA)
Ma1	Methanosarcina acetivorans	Q8TMZ3	947	Calcium	3.A.3.2.4	Ca^{2+}-ATPase (efflux)	Bacteria
Ma2	Methanosarcina acetivorans	Q8TR42	982	Metal	3.A.3.5.3	Cu^+-, Ag^+-ATPase (efflux)	Eukaryotes (Wilson's disease)
Ma3	Methanosarcina acetivorans	Q8TUA7	764	Metal	3.A.3.5.2	Cu^+-, Ag^+-ATPase (efflux)	Bacteria
Ma4	Methanosarcina acetivorans	Q8TJZ4	647	Metal	3.A.3.6.1	Zn^{2+}-, Cd^{2+}-, Pb^{2+}-ATPase (efflux)	Bacteria; plants; fungi; protozoa
Ma5	Methanosarcina acetivorans	Q8TIR0	909	Calcium	3.A.3.2.4	Ca^{2+}-ATPase (efflux)	Bacteria
Ma6	Methanosarcina acetivorans	Q8TQ74	839	Proton	3.A.3.3.4	Putative H^+-ATPase	Archaea
Ma7	Methanosarcina acetivorans	Q8TM37	819	Proton	3.A.3.3.4	Putative H^+-ATPase	Archaea
Ma8	Methanosarcina acetivorans	Q8THY0	929	Na/K	3.A.3.3.1	H^+-ATPase (efflux)	Plants; fungi; protozoa; archaea
Mba1	Methanosarcina barkeri	c1779.1134	842	Magnesium	3.A.3.4.1	Mg^{2+}/Ni^{2+}-ATPase (uptake)	Archaea
Mba2	Methanosarcina barkeri	c1882.2330	954	Metal	3.A.3.5.3	Cu^+-, Ag^+-ATPase (efflux)	Eukaryotes (Wilson's disease)
Mba3	Methanosarcina barkeri	c1889.2430	635	Metal	3.A.3.5.3	Cu^+-, Ag^+-ATPase (efflux)	Eukaryotes (Wilson's disease)
Mba4	Methanosarcina barkeri	c1951.3756	607	Metal	3.A.3.5.2	Cu^+-, Ag^+-ATPase (efflux)	Bacteria
Mba5	Methanosarcina barkeri	c1970.4500	645	Metal	3.A.3.6.1	Zn^{2+}-, Cd^{2+}-, Pb^{2+}-ATPase (efflux)	Bacteria; plants; fungi; protozoa
Mba6	Methanosarcina barkeri	c1980.4943	949	Na/K	3.A.3.3.1	H^+-ATPase (efflux)	Plants; fungi; protozoa; archaea
Mba7	Methanosarcina barkeri	c1985.5241	914	Calcium	3.A.3.2.4	Ca^{2+}-ATPase (efflux)	Bacteria
Mbu1	Methanococcoides burtonii	c160.610	942	Metal	3.A.3.5.3	Cu^+-, Ag^+-ATPase (efflux)	Eukaryotes (Wilson's disease)
Mbu2	Methanococcoides burtonii	c169.1090	887	Calcium	3.A.3.2.4	Ca^{2+}-ATPase (efflux)	Bacteria
Mbu3	Methanococcoides burtonii	c170.1141	815	Proton	3.A.3.3.4	Putative H^+-ATPase	Archaea
Mbu4	Methanococcoides burtonii	c179.2114	894	Calcium	3.A.3.2.4	Ca^{2+}-ATPase (efflux)	Bacteria
Mbu5	Methanococcoides burtonii	c183.3036	871	Na/K	3.A.3.3.1	H^+-ATPase (efflux)	Plants; fungi; protozoa; archaea
Mj1	Methanococcus jannaschii	Q58623	805	Proton	3.A.3.3.4	Putative H^+-ATPase	Archaea
Mm1	Methanosarcina mazei	Q8PU16	885	Calcium	3.A.3.2.3	Ca^{2+} or Mn^{2+}-ATPase (efflux)	Eukaryotes
Mm2	Methanosarcina mazei	Q8PSL0	942	Calcium	3.A.3.2.4	Ca^{2+}-ATPase (efflux)	Bacteria
Mm3	Methanosarcina mazei	Q8PWW5	939	Calcium	3.A.3.2.4	Ca^{2+}-ATPase (efflux)	Bacteria
Mm4	Methanosarcina mazei	Q8PYM6	910	Calcium	3.A.3.2.4	Ca^{2+}-ATPase (efflux)	Bacteria
Mm5	Methanosarcina mazei	Q8PYG1	945	Calcium	3.A.3.2.4	Ca^{2+}-ATPase (efflux)	Bacteria
Mm6	Methanosarcina mazei	Q8PXZ7	955	Na/K	3.A.3.3.1	H^+-ATPase (efflux)	Plants; fungi; protozoa; archaea
Mm7	Methanosarcina mazei	Q8PUK6	962	Metal	3.A.3.5.3	Cu^+-, Ag^+-ATPase (efflux)	Eukaryotes (Wilson's disease)
Mm8	Methanosarcina mazei	Q8PWW3	711	Metal	3.A.3.5.2	Cu^+-, Ag^+-ATPase (efflux)	Bacteria
Mt1	Methanobact. thermoautotrophicum	O27560	910	Na/K	3.A.3.3.1	H^+-ATPase (efflux)	Plants; fungi; protozoa; archaea
Mt2	Methanobact. thermoautotrophicum	O26849	675	Metal	3.A.3.5.2	Cu^+-, Ag^+-ATPase (efflux)	Bacteria
Mt3	Methanobact. thermoautotrophicum	O27578	790	Metal	3.A.3.5.7	Cu^+-Ag^+-ATPase (efflux)	Archaea (CopA)
Mt4	Methanobact. thermoautotrophicum	O26511	605	Metal	3.A.3.6.1	Zn^{2+}-, Cd^{2+}-, Pb^{2+}-ATPase (efflux)	Bacteria; plants; fungi; protozoa
Mt5	Methanobact. thermoautotrophicum	O27082	844	Calcium	3.A.3.2.3	Ca^{2+} or Mn^{2+}-ATPase (efflux)	Eukaryotes
Pab1	Pyrococcus abyssi	Q9V060	689	Metal	3.A.3.6.5	Mono- or divalent -ATPase	Bacteria (Bxa1)
Pae1	Pyrobaculum aerophilum	Q8ZUJ0	789	Metal	3.A.3.5.7	Cu^+-Ag^+-ATPase (efflux)	Archaea (CopA)
Pf1	Pyrococcus furiosus	Q8TH11	799	Metal	3.A.3.5.7	Cu^+-Ag^+-ATPase (efflux)	Archaea (CopA)
Ss1	Sulfolobus solfataricus	Q97VH4	755	Metal	3.A.3.5.7	Cu^+-Ag^+-ATPase (efflux)	Archaea (CopA)
Ss2	Sulfolobus solfataricus	Q97UU7	695	Metal	3.A.3.5.7	Cu^+-Ag^+-ATPase (efflux)	Archaea (CopA)
St1	Sulfolobus tokodaii	Q96ZX6	740	Metal	3.A.3.5.7	Cu^+-Ag^+-ATPase (efflux)	Archaea (CopA)
Ta1	Thermoplasma acidophilum	Q9HJ30	672	Metal	3.A.3.5.7	Cu^+-Ag^+-ATPase (efflux)	Archaea (CopA)
Ta2	Thermoplasma acidophilum	Q9HJC5	780	Proton	3.A.3.3.4	Putative H^+-ATPase	Archaea
Ta3	Thermoplasma acidophilum	P57700	665	Potassium	3.A.3.7.1	K^+-ATPase (uptake)	Bacteria
Tm1	Thermotoga maritima	Q9WYF3	726	Metal	3.A.3.5.7	Cu^+-Ag^+-ATPase (efflux)	Archaea (CopA)
Tv1	Thermoplasma volcanium	Q978Z8	678	Metal	3.A.3.5.7	Cu^+-Ag^+-ATPase (efflux)	Archaea (CopA)
Tv2	Thermoplasma volcanium	Q97BF6	668	Potassium	3.A.3.7.1	K^+-ATPase (uptake)	Bacteria
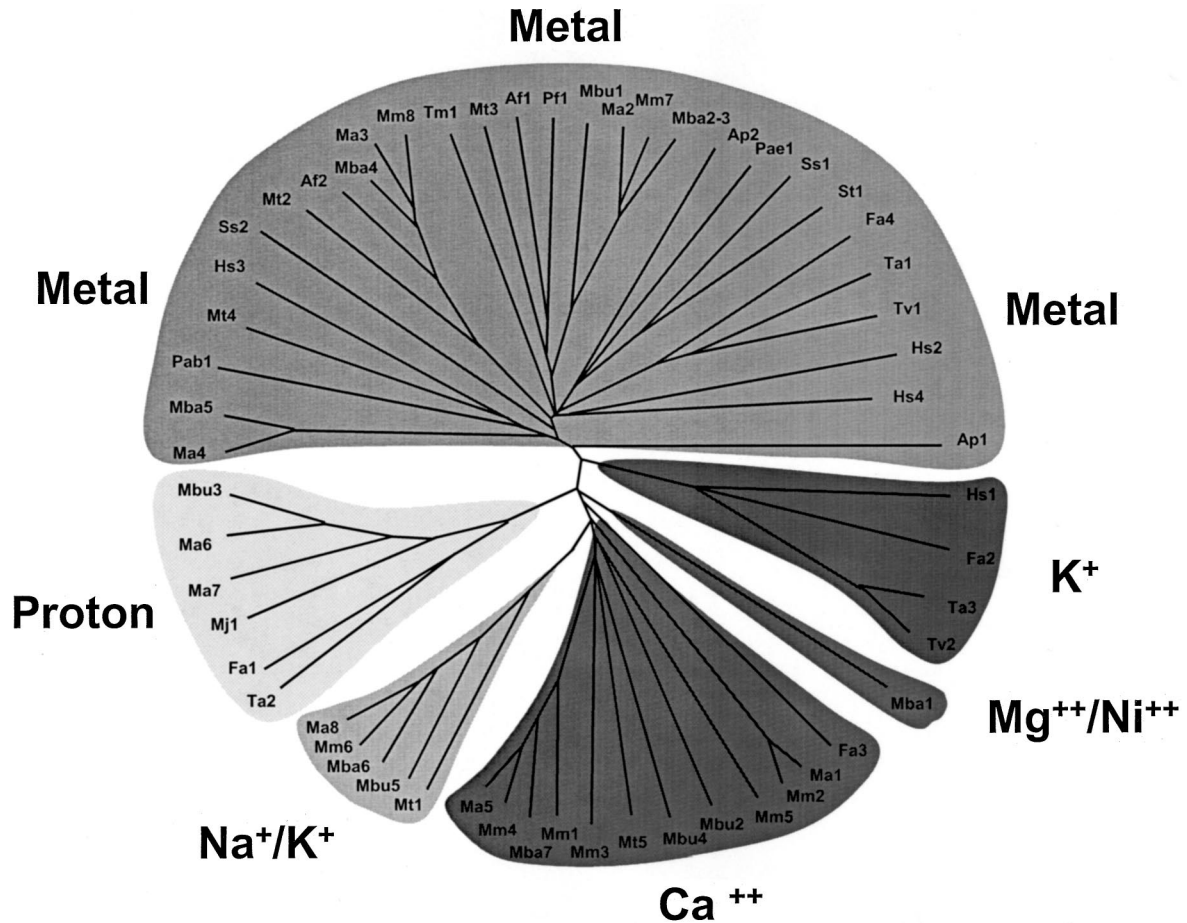
Fig. 1. Phylogenetic tree of the archaea P-type ATPases. The 52 archaea P-type ATPase proteins identified in Table I were aligned and clustered according to the ClustalX and Phylip softwares. The putative-transported substrates corresponding to the different tree branches are differentiated by arbitrary colors.

ATPases. These sequences are longer (from 839 to 940 aminoacyl residues) than the members of the archaea cluster of putative proton-ATPases. In TCDB they cluster within TC 3.A.3.3.1 which contains the classical proton-ATPase Pma1p from yeasts. However when blasted in Swissprot they are annotated as being homologous to the alpha subunit of sodium/potassium ATPases such as the human *ATN1* gene product. Alignment of the 44 residues of the transmembrane domains 4 and 6, known to be involved in cation-binding, shows that the P type-ATPase Mt2 from *Methanobacterium thermoautotrophicum* contains in these two transmembrane domains, a total of 25 residues identical to those of the similar domains of the human Na/K ATPase protein ATN1p. In contrast, only 12 residues from the same transmembrane domains of fungal proton ATPase Pma1p are detected. This indicates that, indeed, the archaea member of this cluster should not be considered as being homologous to the classical yeast

proton-ATPases. They are likely to represent an archeal cluster of Na$^+$/ K$^+$ ATPases.

The Ca^{2+}-ATPases

A surprisingly large number of 12 calcium-ATPases is present in methanogenic archaea (*Methanococcoides*, *Methanococcus*, and *Methanosarcina* species). Ten of them are similar to the bacterial efflux Ca^{2+}-ATPase of the TC cluster 3.A.3.2.4. Two others belong to the eukaryotic Ca^{2+} or Mn^{2+} efflux-ATPase TC cluster 3.A.3.2.3. This latter cluster comprises the Golgi Ca^{2+}-ATPase Pmr1p of Saccharomyces cerevisiae (Catty *et al.*, 1997).

A Mg^{2+}/Ni^{2+}-ATPase

The presence of a novel putative magnesium/nickel uptake-ATPase in *Methanosarcina barkeri* should provide
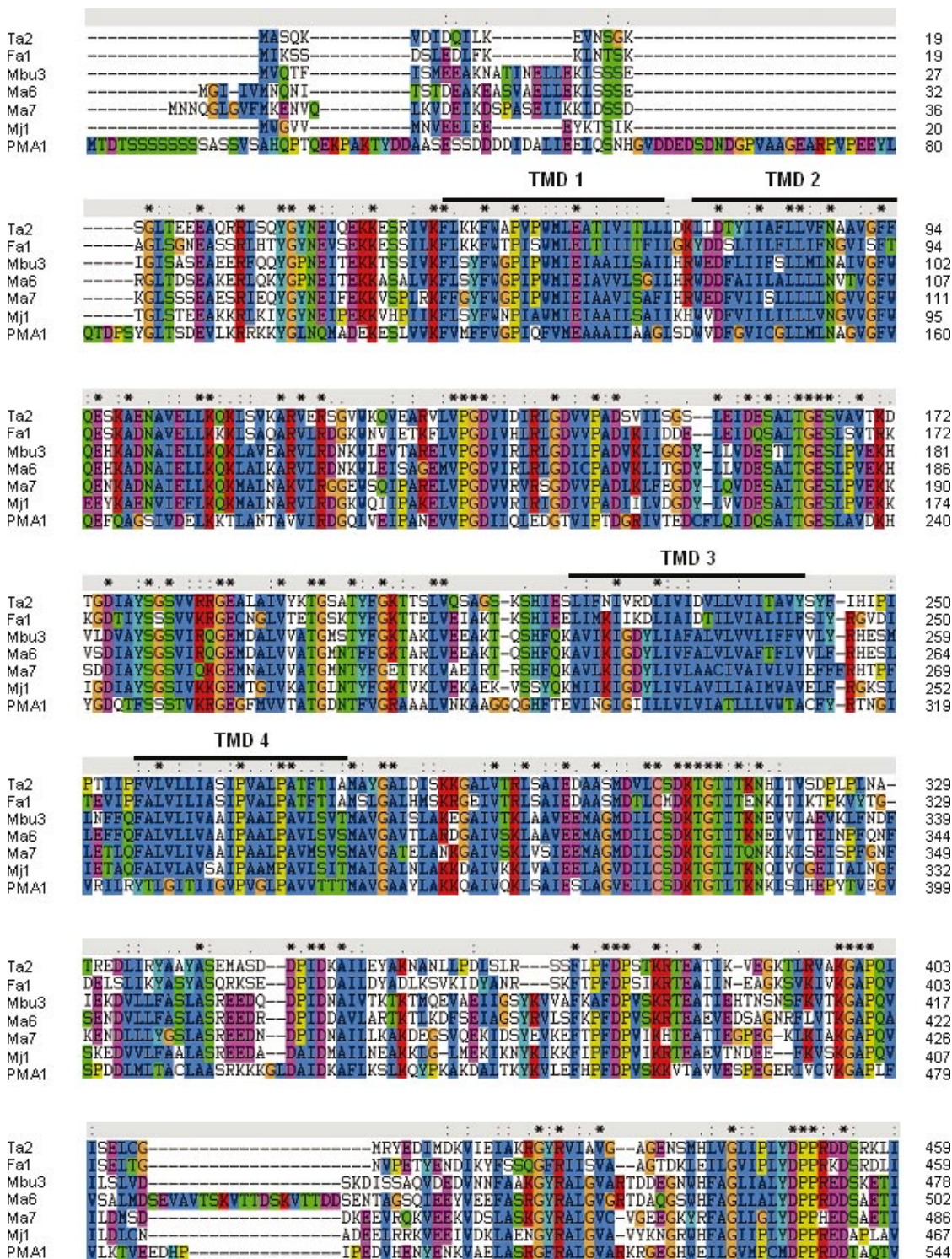
Fig. 2. Protein sequence alignment (ClustalX and Phylip/N-J) of archaea putative proton-ATPases and the *Saccharomyces cerevisiase* proton-ATPase Pma1p. The archaea proteins are identified according to the code of Table I. The transmembrane spans, identified consensually by HMMTOP ant TMHMM, are overlined by a black heavy line and a number on the top of the alignment. Conserved identical amino acids are marked with a star. Conserved similar aminoacids (according to Gonnet matrix) are indicated by double dots. The color code is the default ClustalX alignment colors consensus (residues are colored according to a consensus character assigned to their columns). Blue and cobalt blue correspond to hydrophobic regions.

```
                    * : ***      **: :                              :    : *: ****: *: : *
Ta2    SDIKNIGVSVKMVTGDNAPIAEEIANQVGIEGQVC-----SLHGNQKI----------SDECGIYAEVFPEDKFKIVRS    523
Fa1    TELKQISVSPVMITGDNSLIAEEVAGEIGLEKKLCNAE--NIKGNYAG----------ASDCSVFAEVFPEDKYYIVKA    526
Mbu3   KKAQSMGVNVKMVTGDHLAIAKEISKQVNLNPEIVLAT--SRIDMPDR-----KAQEVVETANGFAQVFPEHKYHIVEL    550
Ma6    RTAQDMGVDVKMITGDHLAIAKEISRQVNLKQDIMLPT--SFIDAPDR------NAEEIVETADGFAQVFPEHKYHIVEL    574
Ma7    KTANSLNVNVKMVTGDHIAIAKEIASQVGIGTNIITAD--DFVEKSDS------EAQELVEKADGFAQVFPEHKYRIVDI    558
Mj1    KKIKELGVIIKMVTGDHVAIAKNIARMIGIGDKIISIS--ELLKKLKRGEIKKEEKFDEIVHEADGFAEVFPEHKYKIVDS    545
PMA1   SEARHIGIRVKMITGDAVGIAKETCRQLGIGTNIYNAERLGLGGGGDMP---GSELADFVENADGFAEVFPQHKYRVVEI    621

                  **    ::   ::  ********** *:**  *: :**  ****  *:::*:*:        *: *  *: :::   * *:*:*  *  *
Ta2    IQEAGHVTGMTGDGVNDAPALKQAEVGIAVSNATDIAKASASIVLTHEGISDIVEAVKEGRKIFQRMLTYTHNKIVKTIQ    603
Fa1    LQKSGHIVGMTGDGVNDSPALKQAEFGVAVASATDIAKASASVVLTHSGLTDIVDGIKSGRRIYQRMLTYTINKIIKVIQ    606
Mbu3   LQRKGHIVGMTGDGVNDAPALKKADAGIAVAGATDAAKSAADIVITKPGLSVIIDAIKESRKIFQRMNNYSIYRIAETIR    630
Ma6    LQHRGHIIGMTGDGVNDAPALKKADAGIAVAGATDAAKSAADIVLTKPGLSTIVNALKESRKIFQRMNNYALYRITETIR    654
Ma7    LQKEEHIVGMTGDGVNDVPALKMADAGIAVAGATDAAKSAADIVFTISGLSIIINAIKESRKIFQRMKSYSIYRIAETVR    638
Mj1    LQKRGHIVAMTGDGVNDAPALKKADCGIAVSNATDAARAAADIVLLSPGISVIVDAIQEARRIFQRMESYVIYRITETIR    625
PMA1   LQNRGYLVAMTGDGVNDAPSLKKADTGIAVEGATDAARSAADIVFLAPGLSAIIDALKTSRQIFHRMYSYVVYRIAISLH    701
                                                                              TMD 5
                                                            _____

               **     ::                 *  :  ::*  :: *:**  ****  *::: *:*      *: *: *  :::     *:*: ::* *  :*
Ta2    VVIFILTASFFAVRYFVTTPFDIILLLFANDFVTMSIATDNVKYSNRPEKWNVKALIVTSGLIAAILVVEGFIII---YIG    680
Fa1    IVFFILTLSFFFVQGFVTTAFDVILLIFANDFVTMAIATDNVGYSIKPERWNVNSLISSSVIIAAFLVVESFIFL---YIG    683
Mbu3   ILLFITLSIIVEQFYPVTALMIVLLALLNDAPIMTIAYDNVKYSDMPEKWDMRNLISMATILGIIGVITSFGIL---YIG    707
Ma6    VLLFITSSILAEKFYPVTSLMIVLLALLNDAPIMTIAYDNVKYSDLPEKWDMRILISMATLGIVIGVISSFGIL---YIG    731
Ma7    VLFFIATSIIVENFYPITALMIVLLAILNDAPITRGHFVSPPEKWNMREVVRVSTFLGILGVIASFLIV---YIG    715
Mj1    ILFFVEICILIIGGIYPITALMIVLLAILNDIPILAIAYDNVVEPKSPVRURMFEIIMLSTAIGISGVVSSFLIF---YIS    702
PMA1   LEIFIGIWIAIIDNSIDIDI-IVFIAIFADVATLAIAYDNAPYSPKPVKWNLPRLGWHSIILGIVIAIGSWITITTMFIP    780
        _____           _____
                    TMD 6                                   TMD 7

Ta2    -IYLHFSKDMIHTLIFDMLVFSGLENVFEIRRFWHSR-PSRYLLVSIAGDIVGISLISTFG---------------II    744
Fa1    -IYAGLRISQIHTFIFDMLVFSGQFTVYMVRERKRFSNSM-PSKILLSSIVDIIITISFISYYG---------------II    747
Mbu3   LHIFQLDHEVLQSFIYLKISVAGHLTLFVARTKGPFWSVK-PALPLFIAVITTQLIATIITVYG---------------IL    772
Ma6    LHIFQLSHEVLQSFIYLKISVAGHLTIFVARTSYFWSVK-PAKTLFAAVIITQIIATLITVYG---------------FL    796
Ma7    ARVIYLSPGVLQSFIFLKLAVAGHLTIFVARTRGHFWSPP-PGKILLFVGVMGTNIIGTIVAAEG---------------IY    781
Mj1    DVFIHLTIAELQSFVFLKIILAGHATIFVTRIHDRLWKKPYPSKLLFWGVMGTNIIGTIVAAEG---------------IF    768
PMA1   KGGIIQNFGAHNGIMFLQISLTENWLIFITRAAGPFWSSI-PSWQLAGAVFAVDIIATMFTLRGWWSENWTDIVTVVRVVU    859

                                                *                                *   *             *
Ta2    -IYLHFSKDMIHTLIFDMLVFSGLENVFEIRRFWHSR    744
```
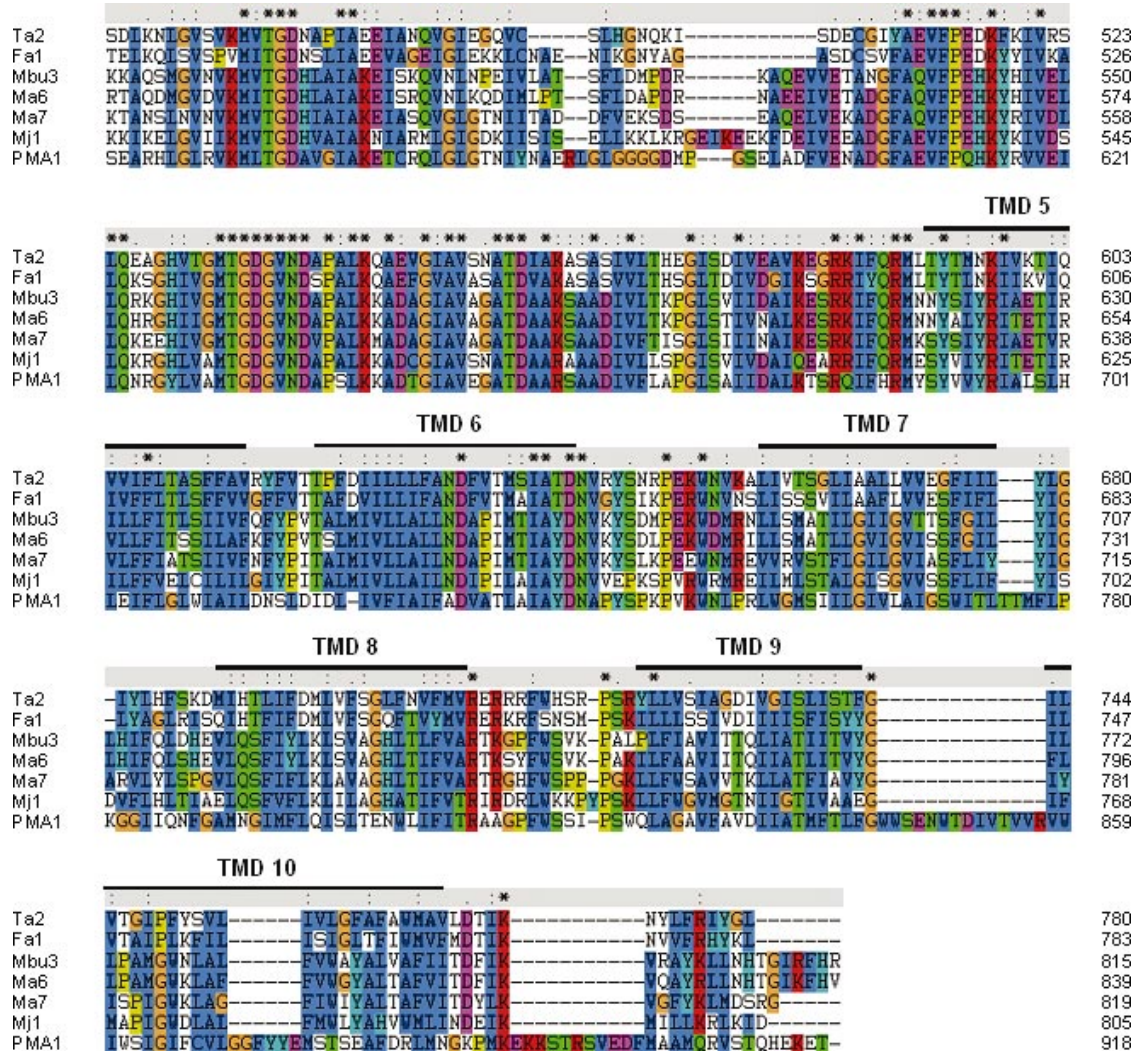
```
                                 TMD 10
                          _____
                                           :*
Ta2    VTGIPFYSVL-------IVLGFAFAWMAVLDTIK------------NYLFRIYGL    780
Fa1    VTAIPLKFIL-------ISIGITRIWMVFMDTIK------------NVVFRHYKL    783
Mbu3   IPAMGWNLAI-------FVWAYALVAFIITDFIK------------VRAYKLINHTGIRFHR    815
Ma6    LPAMGWKLAF-------FVWGYAITAFVITDFIK------------VQAYRLINHTGIKFHV    839
Ma7    ISPIGWKLAG-------FIWIYAITAFVITDYLK------------VGFYKLMDSRG    819
Mj1    MAPIGWDLAI-------FMWLYAHVWMLINDEIK------------MIILKRIKID------    805
PMA1   IWSLGIFCVIGGFVYENSTSEAFDRLMNGKPMKEKKSTRSVEDFHAAMQRVSTQHEKET-    918
```

Fig. 2. (*Continued.*)

new possibilities for studying the properties and mechanism of this magnesium/nickel-ATPase recently discovered in bacteria (Smith and Maguire, 1998).

IN VITRO ANALYSES OF ARCHAEA P-TYPE ATPases

The molecular and structural properties of P-type ATPases are difficult to study unless large quantities of purified proteins are available. As archaea cells are often slow growers, biochemical analysis of archaeal gene products requires their overexpression in a fast growing host such as *Escherichia coli* or *Saccharomyces cerevisiae*. This has been achieved only for a few archaeal ATPases.

The first biochemical identification of an archaea ATPase activity was carried out in the plasma membranes of the thermoacidophilic *Acidianus ambivalens* (Hinrichs *et al.*, 1999). The enzyme activated by sulfite and acid pH, was inhibited by high concentration of vanadate. Even though no demonstration of aspartyl-phosphate intermediate was provided, the inhibitor sensitivity pattern of this ATPase activity is in agreement with that of P-type ATPases.

A soluble gene product MJ0968 from *Methanococcus jannaschii* was overexpressed in *Escherichia coli* and purified (Ogawa *et al.*, 2000). As the recombinant protein shows vanadate-sensitive ATPase activity, and phosphorylation by gamma-labelled ATP, it was concluded that MJ0968 is a soluble P-type ATPase, which could be the ancestor of the core catalytic domain of membrane-bound P-type ATPases.

However it was recently shown that a similar phosphorylation pattern is provided by alpha-labelled ATP

Table II. Growth Conditions of Archaea-Containing Proton P-Type ATPase Genes

Species	Growth conditions	Habitat
Ferroplasma acidarmanus	Very acid; 35–36°C; aerobiosis	Mine drainage biofilms
Methanococcoides burtonii	Neutral to alcaline; −2.5°C to 30°C; anaerobiosis	Lake water at 24-m depth, Antarctica
Methanococcus jannaschii	Acid; 75°C; high pressure; anaerobiosis	Deep sea fumes
Methanosarcina acetivorans	Extensive diversity	Water, soil, oil wells, wastes, faeces, rumen…
Thermoplasma acidophilum	Very acid; 55–60°C; aerobiose and anaerobiose	Self-heating coal refuse piles and solfatara fields

and that the labelled product has not the properties of a catalytic aspartyl-phosphate intermediate (Bramkamp *et al.*, 2003). It was concluded that MJ0968 is a mere phosphatase.

We have detected homologues of the soluble phosphatase MJ0968 in *Methanobacterium thermoautotrophicum, Methanococcus maripaludis, Methanosarcina acetivorans, Methanosarcina mazei, Methanosarcina barkeri,* and *Sulfolobus acidocaldarius*. These sequences exhibit significant homology to the large soluble domain of Cu^{2+}-ATPase, but do not include the conserved DK-TGT phosphorylation motif. However, conservation of other motives such as GD and MVGDXND, detected in metal-ATPases (see the P-type transport ATPase data base of Axelsen), suggests the existence of evolutionary relations between metal P-type ATPase and these soluble phosphatase domains that do not include the DKTGT motif.

Two metal-transporting ATPase genes *CopA* and *CopB* from the thermophilic archae *Archaeoglobus fulgidus* were cloned in *E. coli,* purified, and their ATPase activity were biochemically characterized (Mana-Capelli *et al.*, 2003; Mandal *et al.*, 2002). The thermophilic ATPase activity of CopA was best activated by the monovalent metals Ag^+ and Cu^+ while CopB was activated by the divalent Cu^{2+}. These studies demonstrate the possibility to apply mutagenesis to study transmembrane amino acid motifs presumed to be involved in metal binding selectivity (Arguëllo *et al.*, 2003). The ATPase activities so obtained were of the order of 10–30 micromol/h/mg at 75°C.

More than hundred times higher specific activity was obtained by the expression in *Saccharomyces cerevisiae* of the putative proton-ATPase *MJ1226* gene from the thermophilic archaea *Methanococcus janashii* (now called *Methanocaldococcus jannaschii*). The MJ1226 protein was labelled by six histidines at its N-terminus. The total membranes were solubilized with n-dodecyl beta-d-maltoside. A three-step purification protocol, including an heat-step at 95° for 5 min, a Ni-NTA chromatography, and a Superose 6 HPLC step provided an homogeneous detergent-solubilized fraction protein. The three-dimensional structure of the purified detergent-solubilized protein obtained at 2.4 nm resolution by electron microscopy showed a dimeric organization in which the size and the shape of each monomer was compatible with the reported structures of the SERCA calcium-ATPases. The purified MJ1226p ATPase was inactive at 40°C and was active at elevated temperature only. High specific activity, up to 180 micromol/min/mg, were obtained at 95°C. Maximum ATPase activity was observed at pH 4.2 and required the addition of up to 200 mM monovalent salts. The ATPase activity was stable for several days upon storage at 65°C and was highly resistant to urea and guanidine hydrochloride. The protein formed catalytic phosphoenzyme intermediates from MgATP or Pi, a characteristic trait of P-type ATPases.

CONCLUSION

These studies validate the approach of heterologous expression of archaea genes in yeast and bacteria for biochemical and structural studies of P-type ATPases. Preliminary studies have been already performed for the biochemical and structural studies of archaea metal-ATPases and proton-ATPases, which have been expressed in bacteria or yeast respectively. The function and mechanism of the other archaea subfamilies (potassium, magnesium/nickel, sodium/potassium, and calcium-ATPases) may also be investigated in heterologous systems. It remains however to demonstrate that successful heterologous overexpression of archaea membrane proteins can be obtained from other species than *M. janaschii* (in *S. cerevisiae*) and *A. fulgidus* (in *E. coli*). Another bottleneck is the lack of an appropriate reconstitution system of purified archaea ATPases in thermoresistant lipid vesicles to study the transport properties of thermophilic membrane proteins.

REFERENCES

Arguëllo, J. M. (in press). *J. Mol. Microbiol. Biotechnol.*

Arguëllo, J. M., Mandal, A. K., and Mana-Capelli, S. (2003). *Ann. N.Y. Acad. Sci.* **986**, 212–218.

Axelsen, K. B., and Palmgren, M. G. (1998). *J. Mol. Evol.* **46**, 84–101.

Bramkamp, M., Gassel, M., Herkenhoff-Hesselmann, B., Bertrand, J., and Altendorf, K. (2003). *FEBS Lett.* **543**, 31–36.

Catty, P., d'Exaerde, A. D., and Goffeau, A. (1997). *FEBS Lett.* **409**, 325–332.

de Meis, L., and Vianna, A.L. (1979). *Annu. Rev. Biochem.* **48**, 275–292.

Dufour, J. P., and Goffeau, A. (1978). *J. Biol. Chem.* **253**, 7026–7032.

Goffeau, A., Barrell, B. G., Bussey, H., Davis, R. W., Dujon, B., Feldmann, H., Galibert, F., Hoheisel, J. D., Jacq, C., Johnston, M., Louis, E. J., Mewes, H. W., Murakami, Y., Philippsen, P., Tettelin, H., and Oliver, S. G. (1996). *Science* **274**, 546–567.

Hasselbach, W., and Makinose, M. (1961). *Biochem. Zoal.* **333**, 518–528.

Hinrichs, K.-U., Hayes, J. M., Sylva S. P., Brewer, P. G., and DeLong, E. F. (1999). *Nature*, **398**, 802–805.

Mana-Capelli, S., Mandal, A. K., and Arguëllo, J. M. (2003). *J. Biol. Chem.* **278**, 40534–40544.

Mandal, A. K., Cheung, W. D., and Argüello, J. M. (2002). *J. Biol. Chem.* **277**, 7201–7208.

Morsomme, P., Chami, M., Marco, S., Nader, J., Ketchum, K. A., Goffeau, A., and Rigaud, J.-L. (2002). *J. Biol. Chem.* **277**, 29608–29616.

Ogawa, H., Haga, T., and Toyoshima, C. (2000). *FEBS Lett.* **471**, 99–102.

Pedersen, P. L., and Carafoli, E. (1987). *Trends in Biochem. Sci.* **12**, 146–150.

Post, R. L., and Kume, S. (1973). *J. Biol. Chem.* **248**, 6993–7000.

Skou, J. R. (1957). *Biochim. Biophys. Acta* **23**, 394–401.

Smith, R. L., and Maguire, M. E. (1998). *Mol. Microbiol.* **28**, 217–226

Toyoshima, C., and Nomura, H. (2002). *Nature* **418**, 598–599.

Toyoshima, C., Nakasako, M., Nomura, H., and Ogawa, H. (2000). *Nature* **405**, 633–634.