Minireview

The rotor in the membrane of the ATP synthase and relatives

Ignacio Arechaga*, Phil C. Jones[1]

The Medical Research Council Dunn Human Nutrition Unit, Wellcome Trust/MRC Building, Hills Road, Cambridge CB2 2XY, UK

Received 31 January 2001; revised 13 February 2001; accepted 8 March 2001

First published online 20 March 2001

Edited by Matti Saraste

Abstract In recent years, structural information on the F_1 sector of the ATP synthase has provided an insight into the molecular mechanism of ATP catalysis. The structure strongly supports the proposal that the ATP synthase works as a rotary molecular motor. Insights into the membrane domain have just started to emerge but more detailed structural information is needed if the molecular mechanism of proton translocation coupled to ATP synthesis is to be understood. This review will focus mainly on the ion translocating rotor in the membrane domain of the F-type ATPase, and the related vacuolar and archaeal relatives. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: ATP synthase; Subunit *c*; Proton stoichiometry; Sequence alignment

1. On the rotary mechanism of ATP synthase

Following the postulates of the chemiosmotic theory [1], the gradient of the electrochemical potential generated by the proton extrusion through the enzymes of the respiratory chain should be coupled to the generation of ATP. The enzyme that catalyses the synthesis reaction of ATP from ADP and Pi in mitochondria, chloroplast and bacteria is the F_1F_o ATP synthase. The enzyme is a multisubunit complex with distinct extramembranous and transmembrane domains, termed F_1 and F_o respectively. In *Escherichia coli* the F_1 sector, where ATP synthesis and hydrolysis occurs, is composed of five subunits in an $\alpha_3\beta_3\gamma\delta\varepsilon$ stoichiometry [2]. Homologous subunits are found in mitochondria and chloroplasts. Ion movement through F_o is coupled to ATP synthesis/hydrolysis at sites in F_1. The F_o domain in its simplest form (in *E. coli*) consists of three different polypeptides in the stoichiometry ab_2c_{10-12} [3] (see Fig. 1A).

The crystal structure of bovine mitochondrial F_1 ATPase [4] showed an alternating arrangement of three α subunits and three β subunits around the central helical coiled-coil γ subunit. The structure strongly supported the binding change mechanism proposed by P. Boyer [5], a mechanism involving cyclic alternance of nucleotide affinities of the catalytic β subunits. Rotation of the γ and ε subunits upon ATP hydrolysis has been directly observed in the optical microscope [6,7].

The structure of the central stalk in the context of an intact F_1 domain has been recently determined to high resolution [8]. The structure of yeast F_1 attached to a ring of 10 subunits *c* is also known [9]. The 3.9 Å resolution structure of the yeast F_1–c_{10} complex has provided the first insight into the interactions of the stalk subunits γ, δ and ε with the *c* ring. The close contact between these subunits and the *c* ring supports the idea that the central stalk and the *c* ring form the rotary ensemble of the ATPase motor (see Fig. 1A). Direct evidence for rotation of the *c* ring was recently presented [10,11], although the interpretation has been challenged [12]. Compelling evidence has come from cross-linking studies. The ε subunit can be cross-linked to the polar loops of subunit *c* with function retained [13]. The cross-link of subunit *c* with subunit γ resulted in an uncoupled enzyme, suggesting that these subunits may undergo conformational changes during rotation [13]. However, it has been recently shown that the linking of subunits *c*, γ and ε, via *c*–ε and ε–γ cross-links, resulted in no significant loss of enzyme activity during rotary catalysis [14]. Cross-linking of subunit *b* to subunit *c* indicates that the *c* rotor moves with respect to the subunit *b* stator [15].

Models for rotation of subunit *c* have been proposed where the ring of *c* subunits rotates past a fixed subunit *a*, with H^+ movement at this interface driving the rotation (Fig. 1A) [16,17]. This is supported by the findings that the ion binding cavity is found within the lipid bilayer [9,15], and models, based on the nuclear magnetic resonance structure of the protein in organic solvents [18], that imply access to it from the lipid milieu [19,20]. Hence, ion uptake and release can only be rationalised via an interaction with subunit *a*. Most models favour two half-channels within subunit *a*: one for proton release on one side of the membrane and the other for proton intake on the other (see Fig. 1A). Another plausible mechanistic model has been proposed for the related Na^+-translocating ATP synthase of *Propionigenium modestum* [21]. Here, there is good evidence for one channel within subunit *a*, and accessibility to the ion binding sites in the *c* ring directly from the cytoplasm, a divergence in the mechanism that may have occurred as a result of binding the larger Na^+ ion.

2. On the number of *c* subunits in the rotor and the H^+/ATP ratio

Recent structural data from yeast [9] and chloroplast [22] indicate that there are 10 and 14 *c* subunits per complex, respectively. This has come as a surprise as it has been widely anticipated in the field that the number would be fixed and likely to be 12. Earlier metabolic labelling studies suggested a

*Corresponding author. Fax: (44)-1223-252825.
E-mail: ia@mrc-dunn.cam.ac.uk

[1] Also corresponding author. E-mail: pcj@mrc-dunn.cam.ac.uk

0014-5793/01/$20.00 © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.
PII: S0014-5793(01)02300-6

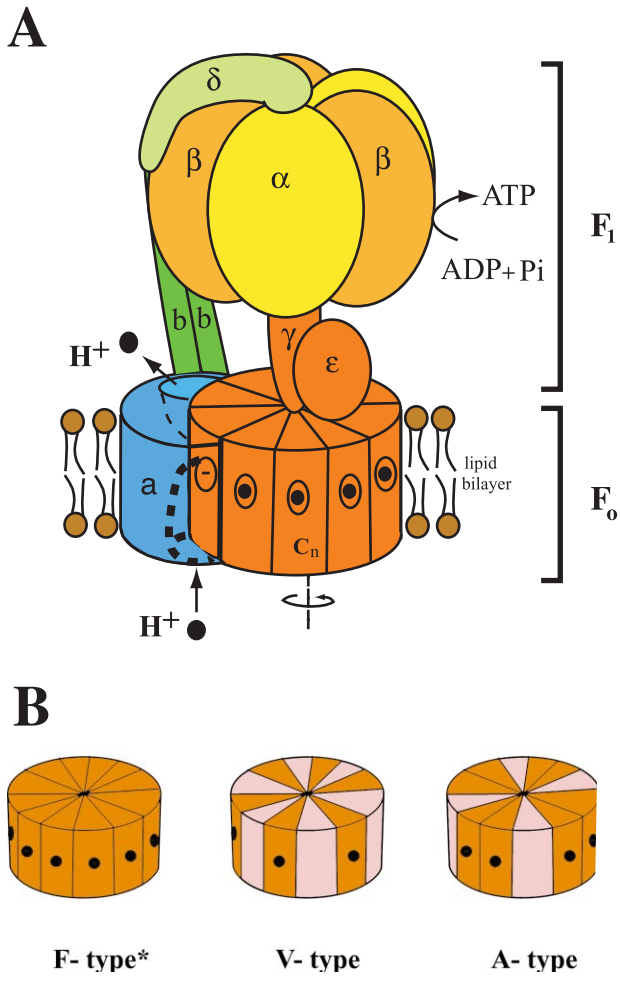

Fig. 1. A: *E. coli* F_1F_o ATP synthase. The F_1 domain is made up of five subunits with the stoichiometry $\alpha_3\beta_3\gamma\delta\varepsilon$. The F_o domain has three different subunits with the stoichiometry ab_2c_{10-12}. There is strong evidence to support a mechanism in which the central stalk of the soluble F_1 domain, together with the oligomeric ring of subunit *c* of the membrane sector, rotates as an ensemble (red), coupling ion movement with ATP synthesis/hydrolysis. B: Models of *c* rings of ATPases from different sources. Each segment corresponds to one helical hairpin. Subunit *c* is made up of two helical hairpins in V-type (vacuolar) enzymes and three in A-type (archaeal) enzymes. Red indicates helical hairpins containing a binding site. *In the Na^+-driven F-type enzymes the binding site is proposed to be in contact with the cytoplasm, rather than within the lipid bilayer.

value of 10 ± 1 but possibly 12 for the complex [23,24]. Recently, cross-linking and genetic experiments showed that c_2 and c_3 genetic fusions of subunit *c* are functional in *E. coli* giving cross-linked products corresponding to a c_{12} species [25].

It has been widely accepted that the number of subunit *c* protomers in the F_1F_o ATPase complex and the number of ATP molecules produced per proton translocated should be directly correlated. Experimental determination of H^+/ATP ratios in intact mitochondria and bacteria has proved to be difficult. Nonetheless, approximate values of three protons per ATP have been reported for submitochondrial particles [26], although the total value of the H^+/ATP ratio in mitochondria is 4 for the combined ATP synthesis and export by the adenine nucleotide and phosphate carriers. A value of four H^+ per ATP synthesised has been obtained for chloroplast and cyanobacteria [27]. A comparison made of the energetics of ATP synthesis by submitochondrial particles and thylakoids suggests a relative proton translocation stoichiometry of approximately 3:4, which is not far from 10:14, the relative ratio of the number of *c* subunits in their respective F_o complexes [28].

The finding that the number of *c* subunits is not divisible by three implies that the number of protons translocated through the membrane is non-integral. This implication fits the structural mismatch of three- and ten-fold symmetries in the F_1 and F_o domains, respectively, of the ATP synthase from *Saccharomyces cerevisiae* [9]. Symmetry mismatch may prevent the ATP synthase from being trapped in an energy well that could occur during the continuous relative movement of the enzyme during ATP catalysis [9]. The principle of symmetry mismatch in molecular rotary engines has been discussed in relation to other macromolecular assemblies such as DNA helicases [29], bacteriophages [30] and the flagellar rotary motor [31]. It is worth noting that genetic fusions have shown a c_3 to result in a functional complex albeit with compromised activity [25]. This observation suggests that some plasticity in ring packing of *c* subunits can be accommodated and that mismatch of symmetries appears not to be essential, at least for a partially active enzyme.

Some studies suggest that the number of *c* subunits may vary depending on the prevalent metabolic conditions [32]. This may reflect a change in the lipid composition of the membrane over successive generations [33]. Since lipids are predicted to fill the interior of the oligomeric ring [9,19] optimal packing of the *c* ring may be affected by the lipid composition. In this respect, the lipid composition of the different energy transducing membranes is variable, as well as the subunit composition of the ATPases, factors that may influence the number of subunits *c*. The extent of interaction of the central stalk with the loop region could also be expected to influence the packing.

3. Is there any correlation between subunit *c* primary sequences and the variation in the number per complex in the yeast and chloroplast enzyme?

An alignment of the subunit *c* primary sequences from yeast mitochondria and spinach chloroplast as well as those of several bacteria is shown in Fig. 2. Also shown, for comparative purposes, is the highly conserved primary sequence of subunit *c* from mammalian mitochondria. Although it is difficult to draw conclusions from the alignments, there are some interesting variations between the sequences that are worth highlighting.

There is notable variation in the N- and C-termini between the subunit *c* sequences. The loop region is conserved, but the divergence of mitochondrial sequences from bacteria and chloroplast is evident. The latter appear to have more ionisable groups, but a lower overall charge (0 or +1), whereas the mitochondrial sequences have an overall charge of +1 to +3. Side chain interactions, or electrostatic repulsions, in the loop could restrict the number of subunits that can pack together. Electrostatic interactions appear to be important for connection with the central stalk [8].

The role of polar side chains towards stability and oligomerisation of transmembrane helices has recently been shown [34]. A serine residue is present in the bovine and human mitochondrial protein at a position equivalent to Ala24 in *E. coli* and there is a glutamine residue in the chloroplast protein at position equivalent to Ile28 in *E. coli*. The presence of these polar side chains may play a role in stability via hydrogen bonding. However, such polar side chains are not found in yeast and *E. coli*. It is possible that these polar side chains play a role in altering the pK_a of the essential carboxyl side chain, where they are predicted to pack, rather than affecting stoichiometry.

4. The ion binding pocket

The ability to translocate sodium ions or protons appears to be reflected in the primary sequence of subunit *c* from different sources. The chloroplast and *P. modestum* proteins have a proline residue and a polar residue at positions equivalent to Ala24 and Ile28 in *E. coli*, respectively. These residues are predicted to make up the ion binding pocket around the essential carboxyl group in helix 2 (Asp61 in *E. coli*). In the model of the oligomeric *c* ring from *E. coli* the Ala24, Ile28 and Ala62 side chains surround Asp61 within a four helix bundle formed by the front and back face of two adjacent monomers [19]. Kaim et al. [35] have shown that residues Gln32, Glu65 and Ser66 are essential for Na^+ binding in *P. modestum*, equivalent to Ile28, Asp61 and Ala62 in *E. coli*.

The motif PXXEXXP, where E is the counterpart of the essential carboxyl of Asp61 in *E. coli*, has been identified in alkalophiles [36]. It is noteworthy that those that lack a Pro at a position equivalent to Gly58 in *E. coli* possess an Asn at a position equivalent to Ile28, including several thermophiles. An influence on the ion binding site is probable, based on their position, perhaps by altering the pK_a of the essential carboxyl group, another likely important factor depending on the energetics of the organism.

Presumably, the essential protonated carboxyl will form hydrogen bonds with side chains or, in the case of the yeast and *E. coli* enzymes, with a backbone carbonyl or amide, within the binding pocket. The breaking and reformation of these bonds will be expected to take place at the interface with subunit *a* as an H^+ is released and then rebound. An entire rotation of helix 2 was shown in the *E. coli* monomer upon ionisation of the essential carboxyl [20]. Whether such movements occur within the *c* oligomer of the intact enzyme is unknown. Proton binding and release may involve subtle movements in close proximity to the ion binding pocket. This is likely to include regions of subunit *a*, such as that

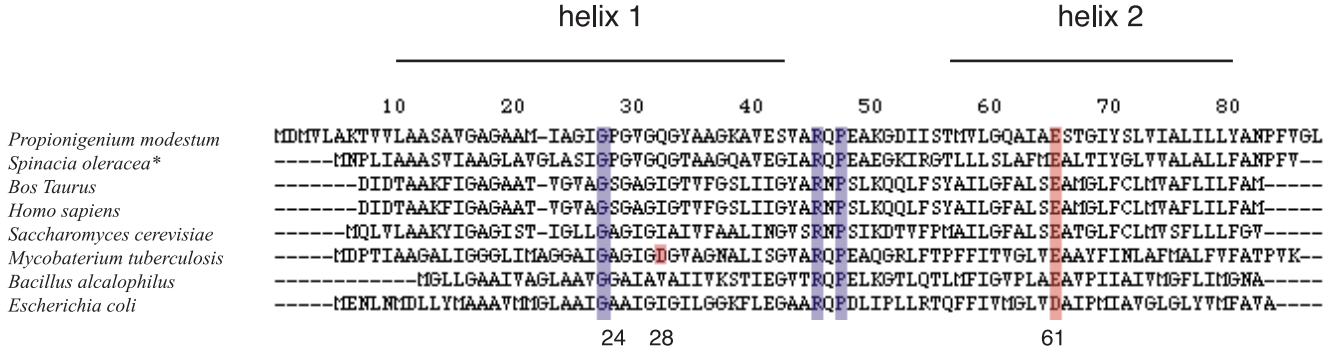

Fig. 2. Sequence alignment of subunit *c* from various species. Strictly conserved residues are purple and the essential carboxylic groups involved in proton (or sodium) translocation are red. An additional carboxyl is found in helix 1 of some bacteria, this is indicated in the *Mycobacterium tuberculosis* sequence. Numbers at the top indicate relative positions in *P. modestum* subunit *c*, whereas numbers at the bottom correspond to those relative to *E. coli* subunit *c*. Helix 1 (residues 3–38) and helix 2 (residues 50–77) as proposed by Dimitriev et al. [19]. In *P. modestum* four α-helical spans (residues 3–27, 32–45, 52–67 and 69–85) have been proposed per monomer [48]. *Chloroplast F_1F_o ATPase subunit *c*. The sequence shown is also conserved in *Zea mays* (maize) and *Oryza sativa* (rice).

one around Arg210 which may reposition between the two proposed half-channels depending on the direction of H^+ movement.

It is worth noting that in *P. modestum* there appears to be an insertion of four amino acids or one turn of an α-helix in the first transmembrane helix based on alignments by Blair et al. [37]. This would be expected to place the ion binding site closer to the cytoplasmic face in the oligomer. Positioning the essential carboxyl group closer to the cytoplasmic surface has been suggested to be an important feature for the mechanism of this enzyme [21]. The effect of deleting some or all these residues remains to be elucidated.

5. Subunit *c* stoichiometry in the V-ATPase

Is the subunit *c* stoichiometry fixed, having a presumed value of 6, or is a value of 5 or 7, consistent with symmetry mismatch, more likely? The V-type ATPase has a homologous subunit *c*, often referred to as the proteolipid, composed of four transmembrane helices, that appears to have evolved by duplication of a progenitor gene [38]. It seems likely that the subunit will be arranged similar to that in the F-type enzyme. Each V-type subunit *c* has only a single essential carboxylate in one of the two helical hairpins (see Fig. 1B), lowering the H^+/ATP ratio to presumably enable ATP-driven H^+ pumping to generate greater electrochemical gradients whilst preserving structural features of the complex [39]. Several homologues, Vma11p and Vma16p or subunit *c′* and *c″* respectively, have been found and shown in yeast to be essential for activity and present within the oligomeric complex [40]. The Vma16p subunit *c* has two carboxylates, one in helix 3 and the other in helix 5. These helices are equivalent to helix 2 in the F-type subunit *c*. The second carboxylate (E188) appears not to be essential for activity [40].

Stoichiometric studies on the coated vesicle V-ATPase estimated 5.55 copies of the 17 kDa protein (subunit *c*/*c′*) and 0.82 copy of a 19 kDa protein which is likely to be subunit *c″* based on the amino acid composition [41]. Unfortunately, these data would suggest that there are a total of six or seven proteolipids per complex. Reassessment of the total content of 17 kDa protein is warranted.

Does the H^+/ATP ratio offer any insights? A ratio of 1.71 was determined for V-ATPase of the chromaffin granule [42], consistent with five copies of subunit *c* per complex. However, such studies may be hampered by the fact that there appears to be a variable ratio of H^+ to ATP coupling or 'slip' which is proposed to be important for regulation of acidification [43]. Analysis of V-ATPases from plant vacuoles suggest that the value can vary from 1.75 to 3.28 [44]. The higher value is difficult to reconcile if one H^+ is translocated per subunit *c* as 10 carboxyl groups and 20 helical hairpins would be required per complex. In another study, it was shown that lemon fruit V-ATPase had half as much 'slip' as the epicotyl V-ATPase [45]. The fruit enzyme appeared to be a modified V-ATPase [46]. The presence of two carboxyls in subunit *c″* could be responsible for 'slip', pH sensitivity or a greater H^+/ATP ratio if there is more than one copy per complex. Another possibility is that the stoichiometry of subunit *c* may vary in V-ATPases. A lower subunit *c* stoichiometry would be in accord with the ability of plant vacuoles to reach such low pH, as low as pH 2.2. Here it is predicted that such values can only be attained by H^+/ATP ratios of <2 [44]. Previous studies may reflect analyses of a mixed population of V-ATPases. Like the variation seen in the number of subunits *c* per F-type complex it should be considered that the number could also vary for V-ATPases and that this variation provides a way to respond or, as in this case, to regulate the energetics of the system.

The related A_1A_o ATPase of *Methanococcus jannaschii* has six predicted transmembrane helices in its *c* subunit and is a triplicated version of the F-type subunit *c*. However, it contains only two conserved carboxyl groups with the first helical hairpin lacking one [47]. Here another evolutionary adaptation for lowering the H^+/ATP ratio may have taken place. If there are four copies of subunit *c* per complex the H^+/ATP ratio is expected to be 2.66. Hence, symmetry mismatch in A_1A_o ATP synthases could be achieved by a variation in the number of carboxyl groups (see Fig. 1B).

Note added in proof

Since submission Stahlberg et al. [49] have shown by atomic force and electron microscopy that there are 11 protomers in the *c* ring of the Na^+ driven F-type ATPase from *Ilyobacter tartaricus*.

Acknowledgements: We thank John Walker for comments on the manuscript and for his continuous support and advice.

References

[1] Mitchell, P. (1961) Nature 191, 144–148.
[2] Walker, J.E. (1998) Angew. Chem. 37, 2309–2319.
[3] Fillingame, R.H., Jiang, W., Dimitriev, O.Y. and Jones, P.C. (2000) Biochim. Biophys. Acta 1458, 387–403.
[4] Abrahams, J.P., Leslie, A.G.W., Lutter, R. and Walker, J.E. (1994) Nature 370, 621–628.
[5] Boyer, P.D. (1997) Annu. Rev. Biochem. 66, 717–749.
[6] Noji, H., Yasuda, R., Yoshida, M. and Kinosita, K. (1997) Nature 386, 299–302.
[7] Hisabori, T., Kondoh, A. and Yoshida, M. (1999) FEBS Lett. 463, 35–38.
[8] Gibbons, C., Montgomery, M.G., Leslie, A.G.W. and Walker, J.E. (2000) Nature Struct. Biol. 7, 1055–1061.
[9] Stock, D., Leslie, A.G.W. and Walker, J.E. (1999) Science 286, 36–40.
[10] Sambongi, Y., Iko, Y., Tanabe, M., Omote, H., Iwamotokihara, A., Ueda, I., Yanagida, T., Wada, Y. and Futai, M. (1999) Science 28, 1722–1724.
[11] Panke, O., Gumbiowski, K., Junge, W. and Engelbrecht, S. (2000) FEBS Lett. 472, 34–38.
[12] Tsunoda, S.P., Aggeler, R., Noji, H., Kinosita, K., Yoshida, M. and Capaldi, R.A. (2000) FEBS Lett. 470, 244–248.
[13] Schulenberg, B., Aggeler, R., Murray, J. and Capaldi, R.A. (1999) J. Biol. Chem. 274, 34233–34237.
[14] Tsunoda, S.P., Aggeler, R., Yoshida, M. and Capaldi, R.A. (2001) Proc. Natl. Acad. Sci. USA 98, 898–902.
[15] Jones, P.C., Hermolin, J., Jiang, W. and Fillingame, R.H. (2000) J. Biol. Chem. 275, 31340–31346.
[16] Engelbrecht, S. and Junge, W. (1997) FEBS Lett. 414, 485–491.
[17] Elston, T., Wang, H.Y. and Oster, G. (1998) Nature 391, 510–513.
[18] Girvin, M.E., Rastogi, V.K., Abildgaard, F., Markley, J.L. and Fillingame, R.H. (1998) Biochemistry 37, 8817–8824.
[19] Dimitriev, O.Y., Jones, P.C. and Fillingame, R.H. (1999) Proc. Natl. Acad. Sci. USA 96, 7785–7790.
[20] Rastogi, V.K. and Girvin, M.E. (1999) Nature 402, 263–268.
[21] Dimroth, P., Wang, H., Grabe, M. and Oster, G. (1999) Proc. Natl. Acad. Sci. USA 96, 4924–4929.
[22] Seelert, H., Poetsch, A., Dencher, N.A., Engel, A., Stahlberg, H. and Muller, D.J. (2000) Nature 405, 418–419.

[23] Foster, D.L. and Fillingame, R.H. (1982) J. Biol. Chem. 257, 2009–2015.
[24] Von Meyenburg, K., Jorgensen, B.B., Nielsen, J., Nansen, F.G. and Michelsen, O. (1982) Tokai J. Exp. Clin. Med. 7 (Suppl.), 23–31.
[25] Jones, P.C. and Fillingame, R.H. (1998) J. Biol. Chem. 273, 29071–29075.
[26] Ferguson, S.J. and Sorgato, M.C. (1982) Annu. Rev. Biochem. 51, 185–217.
[27] Van Walraven, H., Strotmann, H.S., Schwarz, O. and Rumberg, B. (1996) FEBS Lett. 379, 309–313.
[28] Ferguson, S.J. (2000) Curr. Biol. 10, R804–R808.
[29] Hendrix, R.W. (1998) Cell, 147–150.
[30] Valpuesta, J.M., Fernandez, J.J., Carazo, J.M. and Carrascosa, J.L. (1999) Structure 7, 289–296.
[31] Thomas, D.R., Morgan, D.G. and DeRosier, D.J. (1999) Proc. Natl. Acad. Sci. USA 96, 10134–10139.
[32] Schemidt, R.A., Qu, J., Williams, J.R. and Brusilow, W.S. (1998) J. Bacteriol. 180, 3205–3208.
[33] DiRusso, C.C. and Nystrom, T. (1998) Mol. Microbiol. 27, 1–8.
[34] Choma, C., Gratowski, H., Lear, J.D. and DeGrado, W.F. (2000) Nature Struct. Biol. 7, 161–166.
[35] Kaim, G., Wehrle, F., Gerike, U. and Dimroth, P. (1997) Biochemistry 36, 9185–9194.
[36] Ivey, D.M. and Krulwich, T.A. (1992) Res. Microbiol. 143, 467–470.
[37] Blair, A., Ngo, L., Park, J., Paulsen, I.T. and Saier, M.H. (1996) Microbiology 142, 17–32.
[38] Mandel, M., Moriyama, Y., Hulmes, J.D., Pan, Y.C., Nelson, H. and Nelson, N. (1988) Proc. Natl. Acad. Sci. USA 85, 5521–5524.
[39] Cross, R.J. and Taiz, L. (1990) FEBS Lett. 259, 227–229.
[40] Hirata, R., Graham, L.A., Takatsuki, A., Stevens, T.H. and Anraku, Y. (1997) J. Biol. Chem. 272, 4795–4803.
[41] Arai, H., Terres, G., Pink, S. and Forgac, M. (1988) J. Biol. Chem. 263, 8796–8802.
[42] Johnson, R.G., Beers, M.F. and Scarpa, A. (1982) J. Biol. Chem. 257, 10701–10707.
[43] Moriyama, Y. and Nelson, N. (1988) in: The Ion Pumps, Structure, Function and Regulation (Stein, W.D., Ed.), pp. 387–394, Alan R. Liss, New York.
[44] Davies, J.M., Hunt, I. and Sanders, D. (1994) Proc. Natl. Acad. Sci. USA 91, 8547–8551.
[45] Muller, M.L., Irkens-Kiesecker, U., Rubinstein, B. and Taiz, L. (1996) J. Biol. Chem. 271, 1916–1924.
[46] Muller, M.L., Irkens-Kiesecker, U., Kramer, D. and Taiz, L. (1997) J. Biol. Chem. 272, 12762–12770.
[47] Ruppert, C., Kavermann, H., Wimmers, S., Schmid, R., Kellermann, J., Lottspeich, F., Huber, H., Stetter, K.O. and Muller, V. (1999) J. Biol. Chem. 274, 25281–25284.
[48] Matthey, U., Kaim, G., Braun, D., Wüthrich, K. and Dimroth, P. (1999) Eur. J. Biochem. 261, 459–467.
[49] Stahlberg, H., Müller, D., Suda, K., Fotiadis, D., Engel, A., Meier, T., Matthey, U. and Dimroth, P. (2001) EMBO reports 2, 229–233.