J. exp. Biol. **172**, 451-459 (1992)
Printed in Great Britain © The Company of Biologists Limited 1992

THE F_O COMPLEX OF THE PROTON-TRANSLOCATING F-TYPE ATPase OF *ESCHERICHIA COLI*

BY GABRIELE DECKERS-HEBESTREIT AND KARLHEINZ ALTENDORF

Universität Osnabrück, Fachbereich Biologie/Chemie, Arbeitsgruppe Mikrobiologie, Postfach 4469, D-4500 Osnabrück, FRG

Summary

The ATP synthase (F_1F_O) of *Escherichia coli* consists of two structurally and functionally distinct entities. The F_1 part is composed of five subunits α, β, γ, δ and ϵ (3:3:1:1:1) and carries the catalytic centres of the enzyme. The membrane-bound F_O complex functions as a proton channel and consists of the three subunits *a*, *b* and *c* (1:2:10±1).

Subunit *c* (8288 M_r) exhibits a hairpin-like structure within the membrane. A conserved acidic residue (Asp-61) in the C-terminal hydrophobic segment is absolutely required for proton translocation through F_O, whereas the hydrophilic loop region is necessary for F_1 binding. Expression of the chloroplast proteolipid together with subunits *a* and *b* of *E. coli* did not produce an active F_O hybrid complex. Therefore, the construction of hybrid *c* subunits consisting of parts of the proteolipid from both organisms is in progress to determine those parts of subunit *c* that are essential for a functional interplay with subunits *a* and *b*.

Subunit *a* (30276 M_r), which is also involved in proton translocation, is an extremely hydrophobic protein with 5–8 membrane-spanning helices. Studies with alkaline phosphatase fusion proteins resulted in controversial conclusions about the localization of the N and C termini of the protein. A foreign epitope (13 amino acids) has been inserted into the N- or C-terminal region of subunit *a* without affecting the function of F_O. Binding studies with a monoclonal antibody against this epitope are now under investigation to determine the orientation of subunit *a*.

Subunit *b* (17265 M_r) is anchored in the membrane by its apolar N-terminal region, whereas the hydrophilic part protrudes into the cytoplasm. Studies with proteases and truncated *b'* subunits revealed that the C-terminal part of subunit *b* is involved in binding of F_1 to F_O and is necessary for correct assembly of F_O.

ATP synthase

The membrane-bound ATP synthase (F-type ATPase) of *Escherichia coli* (EF_1F_O) catalyzes the synthesis of ATP in a reaction that is driven by an electrochemical gradient of protons generated by respiration. The enzyme can also function in the reverse direction, generating an electrochemical proton gradient by ATP hydrolysis. Other bacteria, mitochondria (MF_1F_O) and chloroplasts (CF_1F_O) contain similar enzyme complexes (Schneider and Altendorf, 1987; Fillingame, 1990; Senior, 1990). However,

Key words: F-type ATPase, F_O complex, *Escherichia coli*.

in *Propionigenium modestum* an F-type ATPase (PF_1F_o) is present, which utilizes an electrochemical gradient of sodium ions for the synthesis of ATP from ADP and inorganic phosphate (Laubinger and Dimroth, 1988). The enzyme complexes are composed of two structurally and functionally distinct entities, designated F_1 and F_o. The peripheral F_1 part carries the catalytic centres of the enzyme and can be easily dissociated from the membrane. It consists of five different subunits with a stoichiometry of α_3, β_3, γ_1, δ_1, ϵ_1. The membrane-integrated F_o complex functions as a proton channel. In *E. coli* the F_o complex (EF_o) is composed of the three subunits *a, b* and *c*, with relative molecular masses of 30276, 17265 and 8288, respectively, and a stoichiometry of 1:2:10±1 for *a*:*b*:*c*. The EF_o complex can be isolated and reconstituted into liposomes in a functional form. In addition, reconstitution is also possible starting from the three individual subunits. Furthermore, analyses of deletion strains and reconstitution experiments with purified EF_o subunits have revealed that all three subunits are necessary to obtain an EF_o complex functional in proton translocation and F_1 binding (Schneider and Altendorf, 1987; and references therein).

Previous studies with mutant strains (Cox *et al.* 1981) led to the proposal that subunits α and β of EF_1 are necessary for the assembly of EF_o. However, more recent experiments have revealed that plasmid-encoded EF_o subunits form a functional EF_o complex in the absence of coordinated synthesis of EF_1 (Fillingame *et al.* 1986), while a 'non-conducting' EF_o complex is assembled in the absence of any EF_1 subunit (Pati and Brusilow, 1989; Pati *et al.* 1991). In the latter case, addition of EF_1 brings about the 'education' of EF_o to translocate protons. These results indicate that, after binding of EF_1 to membranes and the subsequent depletion of those EF_1 complexes, the EF_o complex has been transformed from the closed state to an open proton-translocating channel (Pati and Brusilow, 1989). In addition, although reconstitution of EF_o sectors expressed in the presence or absence of EF_1 subunits revealed major differences in their ability to translocate protons, the rebinding of EF_1 is similar in both cases (Pati *et al.* 1991).

Subunit *c*

Subunit *c*, also referred to as proteolipid, is the most extensively studied subunit of the EF_o complex (Schneider and Altendorf, 1987; Fillingame, 1990; Senior, 1990). The protein has been sequenced from various sources and found to possess a number of common features. Subunit *c* consists of two hydrophobic stretches separated by a central polar loop (Fig. 1). Within the C-terminal hydrophobic region, a conserved acidic residue, Asp-61, exists; this reacts with dicyclohexylcarbodiimide (DCCD), resulting in an inhibition of H^+ translocation through F_o. The view that this carboxyl group plays an important role is supported by the observation that proton translocation is abolished by substitution of Asp-61 with glycine or asparagine (Schneider and Altendorf, 1987; Fillingame, 1990; Senior, 1990; and references therein). Furthermore, studies with a slow-growing revertant strain, in which in addition to the original Asp-61→Gly mutation Ala-24 has been replaced by aspartic acid, indicated that a carboxyl group in the middle of the membrane is absolutely required for H^+ translocation, but that it can be moved from the C-terminal hydrophobic helix to the N-terminal one (Miller *et al.* 1990).

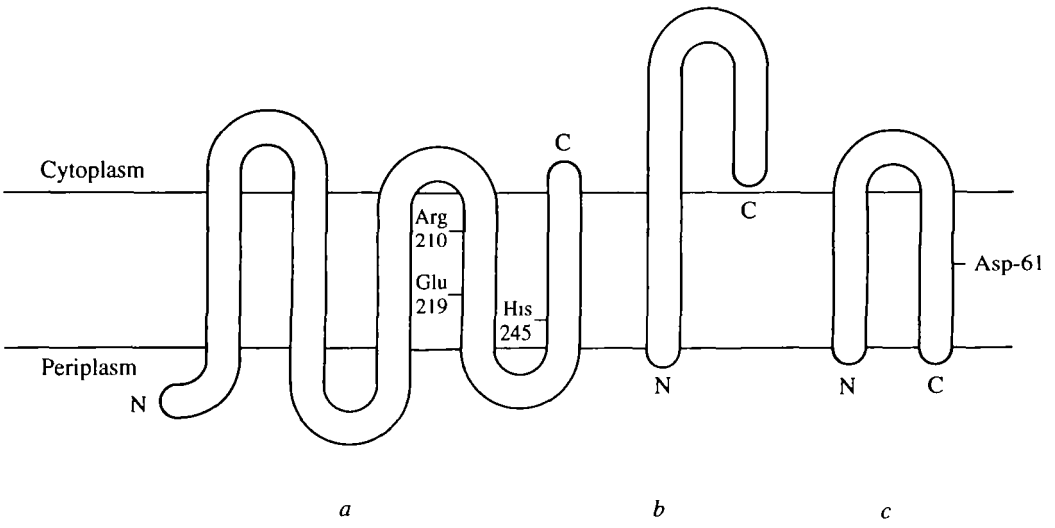

Fig. 1. Subunits *a*, *b* and *c* from *Escherichia coli* F_0 and their proposed orientation in the membrane (modified from Schneider and Altendorf, 1987). Amino acid residues involved in proton translocation are indicated.

Observations supporting a hairpin-like structure of the *E. coli* subunit *c* within the membrane (Fig. 1) are the following: (i) analyses of DCCD-resistant mutants indicate that Ile-28 and Ala-24 are close to Asp-61; (ii) analyses of revertant strains, which show partial suppression of the original mutations affecting proton translocation, indicate that the amino acid residues Asp-61/Ala-24 and Pro-64/Ala-20 are close to each other; (iii) the hydrophobic regions Leu-4 to Leu-19 and Phe-53 to Phe-76 are labelled with 3-trifluoromethyl-3-(*m*-[^{125}I]iodophenyl)diazirine (TID), suggesting that these regions are membrane-integrated; (iv) the C-terminal region is located at the periplasmic side of the membrane, as shown by chemical modification studies; and (v) studies with peptide-specific antibodies reveal that the polar loop region is exposed to the cytoplasm (Schneider and Altendorf, 1987; Fillingame, 1990; Senior, 1990; and references therein; Fillingame *et al.* 1991; Hensel *et al.* 1990; Miller *et al.* 1990).

Analyses of mutant strains revealed that point mutations in the hydrophobic regions of subunit *c* produced effects on the assembly of EF_0 or affected proton translocation, whereas the hydrophilic loop region seemed to function in the coupling of proton translocation through EF_0 to ATP synthesis in EF_1 (Schneider and Altendorf, 1987; Fillingame, 1990; Senior, 1990; and references therein). Although the amino acid residues Ala-40, Arg-41, Gln/Asn-42 and Pro-43 of the polar loop region are highly conserved in bacteria, mitochondria and chloroplasts, it can be concluded from oligodeoxynucleotide-directed mutagenesis studies that Arg-41 is the only residue in this region that is absolutely required for function (Fraga and Fillingame, 1991).

Expression of chloroplast subunit *III* (homologous to subunit *c* of *E. coli*) together with subunits *a* and *b* of *E. coli* did not produce an active F_0 hybrid complex (Burkovski *et al.* 1990), although immunoprecipitations with antibodies against CF_0 *III* indicate that an F_0 complex is assembled (A. Burkovski, G. Deckers-Hebestreit and K. Altendorf,

unpublished results). We are therefore constructing hybrid *c* subunits, consisting of parts of both proteolipids, to determine those parts of subunit *c* that are essential for a functional interplay with subunits *a* and *b*.

Subunit *a*

It has been proposed that subunit *a*, which is an extremely hydrophobic protein, is involved in proton translocation together with subunit *c*. The analyses of missense mutants, which exhibit a reduction in H^+ translocation without a concomitant effect on the assembly of EF_o, revealed that the amino acid residues Arg-210, Glu-219 and His-245 are critical for proton translocation, whereas other residues, such as Asp-119, Glu-196, Gly-197, Ser-206 and Asn-214, are thought to affect proton translocation indirectly (Schneider and Altendorf, 1987; Fillingame, 1990; Senior, 1990; and references therein). In addition, characterization of the double mutant Glu-219→His/His-245→Glu, in which the function is less impaired than in each of the single mutants, has suggested a linked functioning of these two residues in subunit *a* (Cain and Simoni, 1988). Furthermore, the suggested involvement of Arg-210, Glu-219 and His-245 in proton translocation is strikingly similar to the 'proton relay' system proposed for the *lac* permease (Lee *et al.* 1989).

Subunit *a* exhibits structural homology to its counterpart in other F_1F_o complexes (Fig. 1) with strong amino acid homology in two regions towards the C-terminal part (Pro-190 to Leu-220 and Phe-244 to Tyr-263). Models containing 5–8 membrane-spanning helices have been predicted, but convincing evidence favouring one of those is still lacking (Fillingame, 1990; and references therein; Lewis *et al.* 1990; Bjorbaek *et al.* 1990). In addition, studies with alkaline phosphatase (PhoA) fusion proteins resulted in controversial conclusions about the localization of the N and the C termini of the protein with regard to the membrane (Lewis *et al.* 1990; Bjorbaek *et al.* 1990).

However, the following experimental evidence is useful in evaluating the different models: (i) Arg-210, Glu-219 and His-245, which are proposed to be critical for H^+ translocation, are located in transmembrane helices; (ii) analysis of the double mutant Glu-219→His/His-245→Glu indicated that both residues are close to each other in the native protein; (iii) a second-site suppressor mutation in subunit *a* (Pro-240→Ala or Leu) partially corrects defects of the Gly-9→Asp mutation in subunit *b*; (iv) analyses of subunit *a*/PhoA and subunit *a*/β-galactosidase fusion proteins revealed that the N terminus of subunit *a* is located in the periplasm; (v) subunit *a*/PhoA fusion proteins exhibiting high phosphatase activities have their fusion joints located in two regions of subunit *a*: between Val-110 and Leu-136 and between Leu-229 and Ile-246. Both regions are predicted by hydropathy analyses to be intramembranous. Therefore, from the theory of alkaline phosphatase fusions, both regions should be membrane-spanning, coming from the periplasm and going to the cytoplasm (Schneider and Altendorf, 1987; Fillingame, 1990; Senior, 1990; and references therein; Bjorbaek *et al.* 1990; Lewis *et al.* 1990; Lewis and Simoni, 1992).

To determine the localization of both termini of subunit *a*, a small DNA fragment has now been inserted into the *Acy*I site at the 5′ or the 3′ end of the *uncB* gene producing

subunit *a* proteins, which carry at Thr-7 or at Ala-267 of the polypeptide chain an additional epitope of 11 amino acids (Asp-Asn-Pro-Ala-Ser-Thr-Thr-Asn-Lys-Asp-Lys; Charbit *et al.* 1986). These modified *uncB* genes are still able to complement an *uncB* deletion strain (H. Jäger, G. Deckers-Hebestreit and K. Altendorf, unpublished results). Binding studies with a monoclonal antibody against this epitope and with membrane vesicles of different orientation are now under way to disclose the location of this epitope.

Subunit *b*

The primary sequence of subunit *b* suggests that it is a hydrophilic protein, which is anchored in the cytoplasmic membrane by its apolar N-terminal region (about 25 amino acids), whereas the hydrophilic, highly charged part protrudes from the membrane in a predominantly α-helical structure. Labelling experiments with TID, hydrophobic nitrenes and a hydrophobic maleimide support this view. The TID labelling pattern and the analyses of mutants in which Cys-21 or Lys-23 have been substituted indicate that the region around Cys-21 is exposed to the lipid phase with little or no contact with other polypeptide chains. In contrast, the characterization of revertant strains, which partially suppress the Gly-9→Asp mutation of subunit *b*, as described above, supports the notion that the region around Gly-9 is in close contact with subunits *a* and *c*. In addition, the absence of TID labelling in the region Asn-2 to Gln-10 also suggests that these residues are part of a protein contact area. Studies with proteases and subunit-*b*-specific antibodies revealed that the hydrophilic part is exposed to the cytoplasm (Fig. 1). However, although subunit *b* is protected from proteolysis when EF_1 is bound to EF_o, polyclonal anti-*b* antibodies are able to bind to subunit *b* even in the presence of EF_1. The binding produced a partial removal of EF_1 from EF_o, whereas the residual membrane-bound ATPase activity is less DCCD-sensitive (Schneider and Altendorf, 1987; Fillingame, 1990; Senior, 1990; and references therein; Kauffer *et al.* 1991; Deckers-Hebestreit *et al.* 1992).

Treatment of F_1-stripped everted membrane vesicles or F_o liposomes with trypsin, which cleaves subunit *b* from the C terminus, resulted in impairment of F_1 binding, whereas the proton translocation remained unaffected. In contrast, if the degradation of subunit *b* with trypsin occurs prior to the isolation and reconstitution of 'F_o', H^+ translocation is also impaired. However, both functions can be restored by addition of intact subunit *b* (Steffens *et al.* 1987). In addition, cells carrying *amber* mutations within the *uncF* gene, which cause premature termination of subunit *b* biosynthesis, are not able to assemble a functional F_o complex even when only Glu-155 and Leu-156 are missing at the C terminus (Porter *et al.* 1985; Takeyama *et al.* 1988). These studies support the view that an intact subunit *b* is necessary for the correct assembly of F_o, but that, once F_o is formed, the C-terminal region is dispensable for proton translocation.

Arrangement of EF_o subunits

Very little information exists about the interplay of all three subunits (stoichiometry of $a_1b_2c_{10\pm1}$) in the formation of an F_o complex functional in proton translocation and F_1

binding. However, subunit *a* has a strong cooperative effect on the structure of the 10±1 copies of subunit *c* in the F_o complex, since the TID labelling pattern of subunit *c* is largely reduced when subunit *c* is reconstituted into liposomes together with subunit *a* or when it is present in the F_o complex compared to only subunit *c* in the membrane (Steffens *et al.* 1988). Two different models have been proposed for the arrangement of all three subunits in the EF_o complex. The first model (Fig. 2B,D) exhibits a ring of *c* subunits surrounding subunits *a* and *b* (Cox *et al.* 1986). However, this model does not fit very well with the TID labelling data. In contrast, Hoppe and Sebald (1986) and Schneider and Altendorf (1987) have suggested that the a_1b_2 complex is located outside a ring of *c* subunits and that it interacts only at one side with the subunit *c* oligomer (Fig. 2A,C). In addition, this model is much better at explaining the relative ease of extraction of subunit *b* from F_o (Schneider and Altendorf, 1984).

Application of the electron spectroscopic imaging mode in electron microscopy, which allows detection of small particles with optimal density and high resolution, made it possible to distinguish different regions of high density in EF_o complexes dissolved in

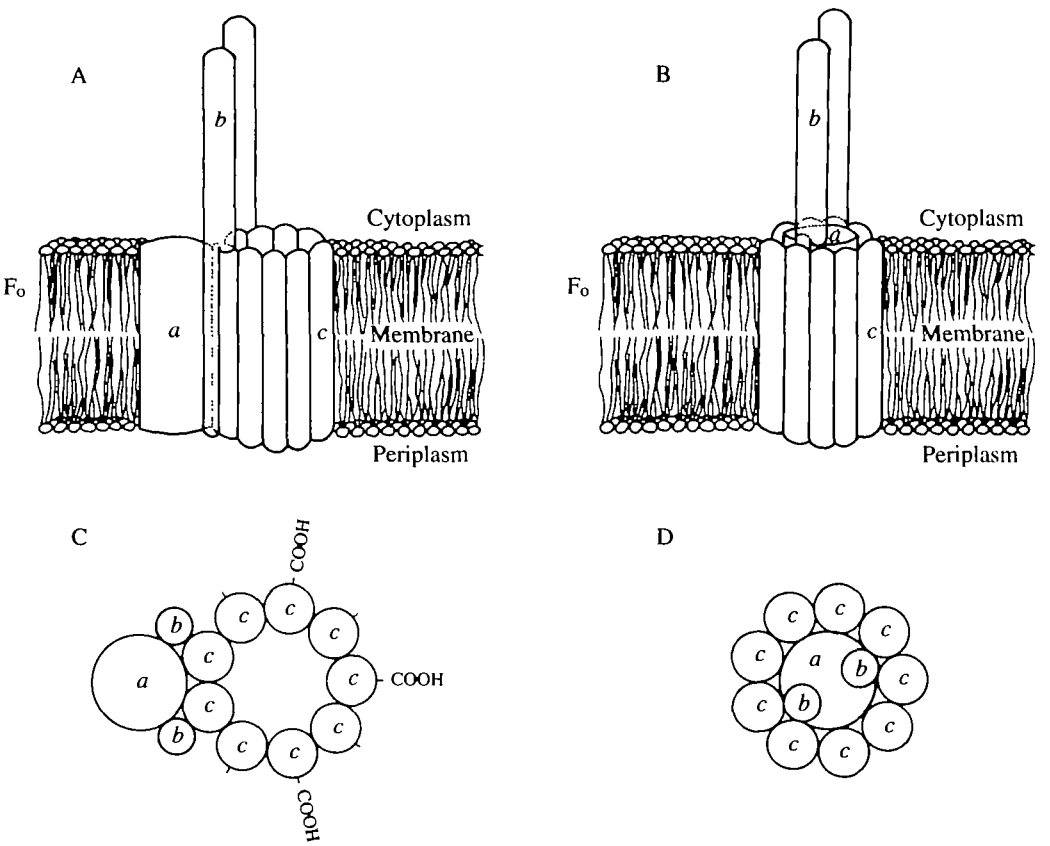

Fig. 2. Models for the arrangement of EF_o subunits. (A,B) Putative arrangements of subunits *a*, *b* and *c* of the EF_o complex in the membrane. (C,D) Top view of the EF_o complex. A and C are modified from Schneider and Altendorf (1987); D is modified from Cox *et al.* (1986).

cholate-containing buffer. Although single subunits of EF_o could not be identified, the overall shape of the single enzyme complexes fits much better with the F_o model proposed by Schneider and Altendorf (1987) and Sebald and Hoppe (1986) (Fig. 2A,C). In addition, electron micrographs of EF_o complexes immunodecorated with monoclonal antibodies against subunits *a, b* and *c* indicate a peripheral arrangement of all three subunits in an EF_o complex seen from the F_1 binding site (R. Birkenhäger, M. Hoppert, G. Deckers-Hebestreit, F. Mayer and K. Altendorf, unpublished results).

H^+ translocation through F_o

A 'proton relay' system or 'proton wire', in which protons cross the membrane by sequential interaction with a series of charged amino acids, has been proposed as a mechanism of proton translocation (Nagle and Tristam-Nagle, 1983; Cox *et al.* 1986). Amino acid residues involved include Arg-210, Glu-219 and His-245 of subunit *a* and Asp-61 of subunit *c*. However, one copy of subunit *a* must interact with an oligomer of 10±1 subunits *c*. Furthermore, one molecule of DCCD is able to abolish proton translocation through F_o by reaction with just one copy of subunit *c* (Hermolin and Fillingame, 1989). Rotational models were therefore suggested to account for these features (Cox *et al.* 1986; Schneider and Altendorf, 1987). However, only protons can be moved by such a mechanism. Alternatively, a transmembrane water-filled channel formed by subunits *a* and *c* could be envisaged, which would allow specific H^+ translocation, when equipped with an H^+-specific filter or gate. Studies with amphiphatic peptides, which have been constructed to mimic ion channels, indicate that differences in the number of polar groups have an influence on the number of water molecules bound in the channel and therefore also have an influence on the ion specificity (Lear *et al.* 1988). In addition, bacteriorhodopsin-bound water molecules are also involved in H^+ translocation (Henderson *et al.* 1990). For H^+ translocation through F_o such an ion transport mechanism has to be favoured, especially in the light of the recently discovered F-type ATPase of *Propionigenium modestum,* which uses an electrochemical gradient of sodium ions for the synthesis of ATP instead of a proton gradient (Laubinger and Dimroth, 1988). Furthermore, in the presence of low sodium concentrations, reconstituted PF_1F_o switches from a Na^+-translocating to a H^+-pumping mode (Laubinger and Dimroth, 1989).

The translocation rate for protons by EF_o reconstituted into liposomes was measured to be 20 H^+ per EF_o per second, which is in agreement with values obtained using F_1-stripped everted membrane vesicles from *E. coli.* In contrast, Schönknecht *et al.* (1986) and Lill *et al.* (1986) calculated the proton translocation rate through CF_o in thylakoid membranes from chloroplasts to be between 6.2×10^3 and 2×10^5 H^+ per CF_o per second measured by flash kinetic spectrophotometry. However, these high rates could only be observed for a fraction of the CF_o complexes available and only for a limited period after removal of CF_1 (Lill *et al.* 1986). In the case of *E. coli* F_o, the proton translocation rates could be increased up to 100–120 H^+ per EF_o per second by using, for the purification of EF_o, an *E. coli* strain that extensively overproduces the ATP synthase (Moriyama *et al.* 1991) and by applying the pH-indicating dye Phenol Red instead of using a pH electrode,

which allows proton translocation measurements on a very short time scale (in the millisecond range) (G. Deckers-Hebestreit, G. Althoff, W. Junge and K. Altendorf, unpublished results). Although the apparent proton translocation rate of EF_o has been increased at least by a factor of five, the discrepancy between the values obtained for CF_o and EF_o still exists. Therefore, the question remains as to whether the differences depend only on the experimental conditions used for the measurement of H^+ translocation or whether the translocation rate in everted membranes and in EF_o liposomes represents only some residual activity due to unrecognized structural changes within EF_o caused during preparation or due to structural changes upon removal of F_1 (Nelson, 1980). Alternatively, the enzyme in *E. coli* may work much slower than CF_1F_o.

References

BJORBAEK, C., FOERSOM, V. AND MICHELSEN, O. (1990). The transmembrane topology of the *a* subunit from the ATPase in *Escherichia coli* analyzed by PhoA protein fusions. *FEBS Lett.* **260**, 31–34.

BURKOVSKI, A., DECKERS-HEBESTREIT, G. AND ALTENDORF, K. (1990). Expression of subunit *III* of the ATP synthase from spinach chloroplasts in *Escherichia coli*. *FEBS Lett.* **271**, 227–230.

CAIN, B. D. AND SIMONI, R. D. (1988). Interaction between Glu-219 and His-245 within the *a* subunit of F_1F_o-ATPase in *Escherichia coli*. *J. biol. Chem.* **263**, 6606–6612.

CHARBIT, A., BOULAIN, J. C., RYTER, A. AND HOFNUNG, M. (1986). Probing the topology of a bacterial membrane protein by genetic insertion of a foreign epitope; expression at the cell surface. *EMBO J.* **5**, 3029–3037.

COX, G. B., DOWNIE, J. A., LANGMAN, L., SENIOR, A. E., ASH, G., FAYLE, D. R. H. AND GIBSON, F. (1981). Assembly of the adenosine triphosphatase complex in *Escherichia coli*: Assembly of F_o is dependent on the formation of specific F_1 subunits. *J. Bacteriol.* **148**, 30–42.

COX, G. B., FIMMEL, A. L., GIBSON, F. AND HATCH, L. (1986). The mechanism of ATP synthase: A reassessment of the functions of the *b* and *a* subunits. *Biochim. biophys. Acta* **849**, 62–69.

DECKERS-HEBESTREIT, G., SIMONI, R. D. AND ALTENDORF, K. (1992). Influence of subunit-specific antibodies on the activity of the F_o complex of the ATP synthase of *Escherichia coli*. I. Effects of subunit *b*-specific polyclonal antibodies. *J. biol. Chem.* **267**, 12364–12369.

FILLINGAME, R. H. (1990). Molecular mechanics of ATP synthesis by F_1F_o-type H^+-transporting ATP synthases. In *The Bacteria: A Treatise on Structure and Function,* vol. XII (ed. T. A. Krulwich), pp. 345–391. New York: Academic Press.

FILLINGAME, R. H., OLDENBURG, M. AND FRAGA, D. (1991). Mutation of alanine 24 to serine in subunit *c* of the *Escherichia coli* F_1F_o-ATP synthase reduces reactivity of aspartyl 61 with dicyclohexylcarbodiimide. *J. biol. Chem.* **266**, 20934–20939.

FILLINGAME, R. H., PORTER, B., HERMOLIN, J. AND WHITE, L. K. (1986). Synthesis of a functional F_o sector of the *Escherichia coli* H^+-ATPase does not require synthesis of the alpha or beta subunits of F_1. *J. Bacteriol.* **165**, 244–251.

FRAGA, D. AND FILLINGAME, R. H. (1991). Essential residues in the polar loop region of subunit *c* of *Escherichia coli* F_1F_o ATP synthase defined by random oligonucleotide-primed mutagenesis. *J. Bacteriol.* **173**, 2639–2643.

HENDERSON, R., BALDWIN, J. M., CESKA, T. A., ZEMLIN, F., BECKMANN, E. AND DOWNING, K. H. (1990). Model for the structure of bacteriorhodopsin based on high-resolution electron cryo-microscopy. *J. molec. Biol.* **213**, 899–929.

HENSEL, M., DECKERS-HEBESTREIT, G., SCHMID, R. AND ALTENDORF, K. (1990). Orientation of subunit *c* of the ATP synthase of *Escherichia coli* – a study with peptide-specific antibodies. *Biochim. biophys. Acta* **1016**, 63–70.

HERMOLIN, J. AND FILLINGAME, R. H. (1989). H^+-ATPase activity of *Escherichia coli* F_1F_o is blocked after reaction of dicyclohexylcarbodiimide with a single proteolipid (subunit *c*) of the F_o complex. *J. biol. Chem.* **264**, 3896–3903.

HOPPE, J. AND SEBALD, W. (1986). Topological studies suggest that the pathway of the protons through F_o is provided by amino acid residues accessible from the lipid phase. *Biochimie* **68**, 427–434.

KAUFFER, S., DECKERS-HEBESTREIT, G. AND ALTENDORF, K. (1991). Substitution of the cysteinyl residue (Cys-21) of subunit *b* of the ATP synthase from *Escherichia coli*. *Eur. J. Biochem.* **202**, 1307–1312.

LAUBINGER, W. AND DIMROTH, P. (1988). Characterization of the ATP synthase of *Propionigenium modestum* as a primary sodium pump. *Biochemistry, N.Y.* **27**, 7531–7537.

LAUBINGER, W. AND DIMROTH, P. (1989). The sodium ion translocating ATPase of *Propionigenium modestum* pumps protons at low sodium ion concentrations. *Biochemistry, N.Y.* **28**, 7194–7198.

LEAR, J. D., WASSERMAN, Z. R. AND DEGRADO, W. F. (1988). Synthetic amphiphilic peptide models for protein ion channels. *Science* **240**, 1177–1181.

LEE, J. A., PUTTNER, I. B. AND KABACK, H. R. (1989). Effect of distance and orientation between arginine-302, histidine-322, and glutamate-325 on the activity of *lac* permease from *Escherichia coli*. *Biochemistry, N.Y.* **28**, 2540–2544.

LEWIS, M. J., CHANG, J. A. AND SIMONI, R. D. (1990). A topological analysis of subunit *a* from *Escherichia coli* F_1F_o-ATP synthase predicts eight transmembrane segments. *J. biol. Chem.* **265**, 10541–10550.

LEWIS, M. J. AND SIMONI, R. D. (1992). Deletions in hydrophobic domains of subunit *a* from the *Escherichia coli* F_1F_o-ATP synthase interfere with membrane insertion or F_o assembly. *J. biol. Chem.* **267**, 3482–3489.

LILL, H., ENGELBRECHT, S., SCHÖNKNECHT, G. AND JUNGE, W. (1986). The proton channel, CF_o, in thylakoid membranes. Only a low proportion of CF_1-lacking CF_o is active with a high unit conductance (169 fS). *Eur. J. Biochem.* **160**, 627–634.

MILLER, M. J., OLDENBURG, M. AND FILLINGAME, R. H. (1990). The essential carboxyl group in subunit *c* of the F_1F_o ATP synthase can be moved and H^+-translocating function retained. *Proc. natn. Acad. Sci. U.S.A.* **87**, 4900–4904.

MORIYAMA, Y., IWAMOTO, A., HANADA, H., MAEDA, M. AND FUTAI, M. (1991). One-step purification of *Escherichia coli* H^+-ATPase (F_oF_1) and its reconstitution into liposomes with neurotransmitter transporters. *J. biol. Chem.* **266**, 22141–22146.

NAGLE, J. F. AND TRISTAM-NAGLE, S. (1983). Hydrogen bonded chain mechanisms for proton conduction and proton pumping. *J. Membr. Biol.* **74**, 1–14.

NELSON, N. (1980). Proton channels in chloroplast membranes. *Ann. N. Y. Acad. Sci.* **358**, 25–36.

PATI, S. AND BRUSILOW, W. S. A. (1989). The roles of the α and γ subunits in proton conduction through the F_o sector of the proton-translocating ATPase of *Escherichia coli*. *J. biol. Chem.* **264**, 2640–2644.

PATI, S., BRUSILOW, W. S. A., DECKERS-HEBESTREIT, G. AND ALTENDORF, K. (1991). Assembly of the F_o proton channel of the *Escherichia coli* F_1F_o ATPase: Low conductance of reconstituted F_o sectors synthesized and assembled in the absence of F_1. *Biochemistry, N.Y.* **30**, 4711–4714.

PORTER, A. C. G., KUMAMOTO, C., ALDAPE, K. AND SIMONI, R. D. (1985). Role of the *b* subunit of the *Escherichia coli* proton-translocating ATPase. A mutagenic analysis. *J. biol. Chem.* **260**, 8182–8187.

SCHNEIDER, E. AND ALTENDORF, K. (1984). Subunit *b* of the membrane moiety (F_o) of ATP synthase (F_1F_o) from *Escherichia coli* is indispensable for H^+ translocation and binding of the water-soluble F_1 moiety. *Proc. natn. Acad. Sci. U.S.A.* **81**, 7279–7283.

SCHNEIDER, E. AND ALTENDORF, K. (1987). Bacterial adenosine 5′-triphosphate synthase (F_1F_o): Purification and reconstitution of F_o complexes and biochemical and functional characterization of their subunits. *Microbiol. Rev.* **51**, 477–497.

SCHÖNKNECHT, G., JUNGE, W., LILL, H. AND ENGELBRECHT, S. (1986). Complete tracking of proton flow in thylakoids – the unit conductance of CF_o is greater than 10 fS. *FEBS Lett.* **203**, 289–294.

SENIOR, A. E. (1990). The proton-translocating ATPase of *Escherichia coli*. *A. Rev. Biophys. biophys. Chem.* **19**, 7–41.

STEFFENS, K., HOPPE, J. AND ALTENDORF, K. (1988). F_o part of the ATP synthase from *Escherichia coli*. Influence of subunits *a* and *b* on the structure of subunit *c*. *Eur. J. Biochem.* **170**, 627–630.

STEFFENS, K., SCHNEIDER, E., DECKERS-HEBESTREIT, G. AND ALTENDORF, K. (1987). F_o portion of *Escherichia coli* ATP synthase. Further resolution of trypsin-generated fragments of subunit *b*. *J. biol. Chem.* **262**, 5866–5869.

TAKEYAMA, M., NOUMI, T., MAEDA, M. AND FUTAI, M. (1988). F_o portion of *Escherichia coli* H^+-ATPase. Carboxyl-terminal region of the *b* subunit is essential for assembly of functional F_o. *J. biol. Chem.* **263**, 16106–16112.