Introduction of a Carboxyl Group in the First Transmembrane Helix of *Escherichia coli* F₁F_o ATPase Subunit *c* and Cytoplasmic pH Regulation

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The multicopy subunit *c* of the H⁺-transporting F₁F_o ATP synthase of *Escherichia coli* folds across the membrane as a hairpin of two hydrophobic α helices. The subunits interact in a front-to-back fashion, forming an oligomeric ring with helix 1 packing in the interior and helix 2 at the periphery. A conserved carboxyl, Asp⁶¹ in *E. coli*, centered in the second transmembrane helix is essential for H⁺ transport. A second carboxylic acid in the first transmembrane helix is found at a position equivalent to Ile²⁸ in several bacteria, some the cause of serious infectious disease. This side chain has been predicted to pack proximal to the essential carboxyl in helix 2. It appears that in some of these bacteria the primary function of the enzyme is H⁺ pumping for cytoplasmic pH regulation. In this study, Ile²⁸ was changed to Asp and Glu. Both mutants were functional. However, unlike the wild type, the mutants showed pH-dependent ATPase-coupled H⁺ pumping and passive H⁺ transport through F_o. The results indicate that the presence of a second carboxylate enables regulation of enzyme function in response to cytoplasmic pH and that the ion binding pocket is aqueous accessible. The presence of a single carboxyl at position 28, in mutants I28D/D61G and I28E/D61G, did not support growth on a succinate carbon source. However, I28E/D61G was functional in ATPase-coupled H⁺ transport. This result indicates that the side chain at position 28 is part of the ion binding pocket.

F₁F_o ATP synthases catalyze the formation of ATP by utilizing the energy of a transmembrane electrochemical gradient (reviewed in reference 3). H⁺ and Na⁺ translocating complexes exist. Closely related ATP synthases are found in the plasma membrane of eubacteria, the inner membrane of mitochondria, and the thylakoid membrane of chloroplasts. The enzyme is a multisubunit complex with distinct extramembranous and transmembrane domains, termed F₁ and F_o, respectively. In *Escherichia coli*, the F₁ sector is composed of five subunits in an $\alpha_3\beta_3\gamma\delta\epsilon$ stoichiometry. Homologous subunits are found in mitochondria and chloroplasts. A high-resolution structure of the $\alpha_3\beta_3\gamma$ portion of bovine F₁ shows the three α and three β subunits to alternate around the central γ subunit (1). Ion movement through F_o is coupled to ATP synthesis and hydrolysis at sites in F₁.

The *E. coli* F_o consists of three types of subunits with a stoichiometry of $a_1b_2c_{10-12}$ (12, 18). The *c* subunits of F_o are arranged in an oligomeric ring (7, 19). Subunit *c* folds in the membrane as a hairpin of two hydrophobic α helices connected by a polar loop on the F₁ binding side of the membrane (14). The arrangement and interaction of subunits in *E. coli* is supported by a 4-Å resolution X-ray diffraction density map of an F₁-c₁₀ subcomplex purified from yeast mitochondria (40). A conserved and essential Asp or Glu (Asp⁶¹ in *E. coli*) is centered in the second transmembrane helix of subunit *c*. The conserved carboxylate catalyzes ion movement via interaction with subunit *a*, a key residue being an arginine (Arg²¹⁰ in *E. coli*) in a transmembrane segment. Such data have supported

mechanistic models where ion movement occurs at the interface between subunits *a* and *c* through rotation of the subunit *c* oligomeric ring and subunits γ and ϵ within F₁ (reviewed in references 3, 10, and 41).

In the model of the oligomeric *c* ring, the Asp⁶¹ side chain is positioned within a four-helix bundle formed by the front and back faces of two adjacent monomers (see Fig. 5A) (7, 19). The ion binding pocket in the subunit *c* ring is predicted to be lined by side chains at positions equivalent to 24, 28, and 62, which surround the essential carboxyl at position 61, in *E. coli*. Interestingly, numerous sequences of subunit *c* from a wide variety of sources possess polar residues at all or some of these positions.

A second carboxyl side chain at a position equivalent to Ile²⁸ is found in several bacteria (Fig. 1). Several of these bacteria, e.g., *Mycobacterium tuberculosis*, *Mycobacterium leprae*, and *Streptococcus pneumoniae*, are the cause of serious infectious disease. In addition, studies of *S. pneumoniae* indicate that some antimalarials target the ion binding site in subunit *c* and that this or the related vacuolar protein is a likely target in the etiological agent *Plasmodium falciparum* (9). In some streptococci and other lactic acid bacteria, the primary function of the enzyme is as an H⁺-pumping ATPase for cytoplasmic pH regulation, maintaining a transmembrane pH gradient with a cytoplasmic pH near neutrality when the extracellular pH is low (2, 6, 24; reviewed in references 23 and 30). In *Streptococcus mutans*, the activity of the F₁F_o ATPase appears to be regulated by intracellular pH (6).

In this study, replacement of Ile²⁸ of the *E. coli* F₁F_o ATP synthase subunit *c* with Asp and Glu was investigated. Both changes produced a pH-dependent function. The result suggests that the second carboxyl in some bacteria could be important for regulation of enzyme function in response to cyto-

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	Helix 1	Helix 2
<i>E. coli</i>	-AAIG I GILG-	-MGLV D AIPM-
<i>L. lactis</i>	-AAIG D GLIV-	-IAFV E GTFF-
<i>M. tuberculosis</i>	-AGIG D GVAG-	-VGLV E AAYF-
<i>M. leprae</i>	-AGIG D GMAG-	-VGLV E AAYF-
<i>S. pneumoniae</i> R6	-VSV G EGLLM-	-VAFI E GTFF-
<i>S. mutans</i>	-VSL G EGLIV-	-VAFI E GTFF-
<i>P. modestum</i>	-PGVG Q GYAA-	-QAIA E STGI-

FIG. 1. Some of the *c* subunits that have a carboxylic acid at a position equivalent to Ile²⁸ in *E. coli*. Sequence comparison around residues equivalent to Ile²⁸ and Asp⁶¹ in *E. coli* is shown. The sequences compared were from *E. coli* (42), *Lactococcus lactis* (25), *M. tuberculosis* (4), *M. leprae* (39), *S. pneumoniae* R6 (9), *S. mutans* (38), and *Propionigenium modestum* (8). *P. modestum* is included to show the polar residues at positions equivalent to 28, 61, and 62 that are essential for Na⁺ binding (21). Positions equivalent to 28 in helix 1 and position 61 in helix 2 of *E. coli* are in boldface.

plasmic pH. The presence of a single carboxyl in subunit *c* at position 28, in constructs I28D/D61G and I28E/D61G, did not allow growth on succinate as a carbon source. However, I28E/D61G was functional in ATPase-coupled H⁺ transport.

MATERIALS AND METHODS

Strains and plasmids. The plasmids used in this study are derivatives of plasmid pDF163, which contains the wild-type (WT) *uncBEFH* genes (bases 870 to 3216, encoding subunits *a*, *c*, *b*, and δ) cloned between the *Hind*III and *Sph*I sites of plasmid pBR322 (13). Plasmids pI28D and pI28E were constructed by a rapid site-directed mutagenesis procedure (26). An antisense oligonucleotide, 5'-ACCCCGAGGATGCCT/ATCACCGATCGCAGACC-3', corresponding to subunit *c* positions G23 to G33, was synthesized to incorporate base changes (boldface) to create an Asp or Glu codon at position 28 and to overlap the nearby *Ava*I restriction enzyme site (italics). PCR was then performed with this primer and a sense oligonucleotide primer designed to the coding strand (bases 1540 to 1560), upstream of the *Pst*I restriction enzyme site (1561 to 1566), using plasmid pDF163 DNA as the template. The PCR product was then digested with restriction enzymes *Pst*I and *Ava*I and ligated to the equivalent sites of plasmid pDF163 to generate plasmid pI28D or pI28E. The I28D/D61G and I28E/D61G double substitutions were generated by subcloning a *Pst*I/*Ava*I fragment from either plasmid pI28D or plasmid pI28E into the corresponding sites of plasmid pLH247 (32), a plasmid pDF163 derivative containing the subunit *c* D61G substitution. All constructs were confirmed by restriction mapping and sequencing. The chromosomal *uncBEFH* deletion strain, JWP109 (*pyrE41 entA403 argH1 rpsL109 supE44 Δ uncBEFH*) (17), was transformed with the plasmids. Complementation of the *unc* phenotype (lack of growth on succinate) was tested by transferring transformant, ampicillin-resistant colonies to minimal medium 63 plates containing 22 mM succinate, 2 mg of thiamine/liter, 0.2 mM uracil, 0.2 mM L-arginine, 20 μ M dihydroxybenzoic acid, and 100 mg of ampicillin/liter (31).

Membrane preparations and biochemical assays. Inside-out membrane vesicles, F₁ head group on the outer face, were prepared and stored in TMDG buffer (50 mM Tris-HCl [pH 7.5], 5 mM MgCl₂, 1 mM dithiothreitol, 10% [vol/vol] glycerol) at 20 mg/ml after passage of cells through a French press (43). F₁-lacking stripped membranes were prepared by centrifugation and incubation of whole membranes at 5 mg/ml in TEDG buffer (1 mM Tris-HCl [pH 8], 0.5 mM Na₂EDTA, 1 mM dithiothreitol, 10% [vol/vol] glycerol) for 30 min at 30°C, followed by centrifugation and one wash with TEDG buffer. Protein concentration was determined by using a Pierce bicinchoninic acid assay kit with the addition of 0.01% sodium dodecyl sulfate. ATPase activity was assayed in HMK buffer (10 mM HEPES-KOH [pH as indicated], 5 mM MgCl₂, 300 mM KCl) using 25 μ g (total protein) of membranes and 5 mM ATP by measuring inorganic phosphate release (28). ATP-driven 9-amino-6-chloro-2-methoxyacridine (ACMA) quenching and NADH-driven quinacrine quenching assays were carried out in HMK assay buffer as described previously (43). Membranes were incubated in HMK assay buffer for 15 min in the presence of 45 μ M *N,N'*-dicyclohexylcarbodiimide (DCCD) prior to addition of NADH to test its effect on NADH-driven quenching. The rate of NADH oxidation was determined by absorbance

change at a wavelength of 340 nm, using 100 μ M NADH and 50 μ g of whole membranes/ml in HMK buffer (pH 7.0). ATP synthesis was carried out as described by Schulenberg and Capaldi (37), with the pH of the Tris buffer and reaction buffer varied accordingly. All reactions were carried out at room temperature. The values reported are averages of triplicate assays, and quenching curves are representative of several independent experiments.

RESULTS

Effect of I28X substitutions on function. Asp and Glu were introduced at position 28 in subunit *c* and into a D61G mutant. The modified *c* subunits were expressed from a plasmid carrying the *uncBEFH* genes, encoding subunits *a*, *c*, *b*, and δ of F₁F₀, respectively, and transformed into the Δ *uncBEFH* strain JWP109. Growth of transformants was tested on succinate minimal medium, where growth depends on a functional oxidative phosphorylation system. The growth properties, ATPase activities, and rates of NADH oxidation of isolated inside-out membranes are shown in Table 1. Both I28x mutants grew on succinate. The I28E mutant grew similarly to the wild type, whereas the I28D mutant grew less well. The I28D/D61G and I28E/D61G mutants did not grow on succinate minimal medium. The levels of expression of subunit *c* as determined by immunoblot analysis from each mutant were similar (data not shown).

All membranes showed an NADH-driven quenching of quinacrine fluorescence equivalent to that of WT membranes (data not shown). The reduced ATP-driven quenching response therefore cannot be a result of enhanced H⁺ permeability of these membranes. The ATPase activity of WT membranes at pH 7.0 was only two- to threefold greater than that of the mutant membranes (Table 1). There appears to be little correlation between ATPase activity at these levels and H⁺ pumping. Greater differences between WT and mutant subunit *c* membranes have been reported previously and at lower levels of activity are capable of maximal ATP-driven quenching (43). At pH 8.0, the ATPase activities of all membranes were lower but similar, with the WT membranes still reaching a near-maximal ATP-driven H⁺ pumping response.

All mutants showed a reduced or inhibited ATP-driven ACMA quenching response. The response of the I28E mutant membranes was the least affected but was still reduced to approximately 35% of the WT membrane level (Fig. 2). This is somewhat surprising, as this mutant grew as robustly as the wild type on a succinate carbon source. The rates of ATP

TABLE 1. Growth properties and activity assays

Construct ^a	Growth on succinate ^b	ATPase activity (μ mol min ⁻¹ mg ⁻¹) ^c		Rate of NADH oxidation ^c (μ mol min ⁻¹ mg ⁻¹)
		pH 7.0	pH 8.0	
WT	2.0	0.412	0.134	1.03
I28D	0.5	0.122	0.101	1.21
I28E	2.0	0.161	0.100	0.98
I28D/D61G	0	0.135	0.089	0.88
I28E/D61G	0	0.192	0.126	1.07
D61G	0	0.165	0.074	0.76

^a See text for descriptions.

^b Colony size after 72 h of incubation at 37°C on minimal medium plates containing 22 mM succinate.

^c Average of triplicate assays. The percentage difference between individual assays and the average was less than 5% in all cases.

FIG. 2. Comparison of ATP-driven quenching of ACMA fluorescence by WT and mutant membranes. WT and I28E mutant membranes were diluted to 0.25 mg/ml, and the others were diluted to 0.5 mg/ml, in HMK buffer (pH 7.0) containing 0.3 μ g of ACMA/ml. At the times indicated, ATP was added to 0.94 mM and uncoupler SF6847 was added to 0.3 μ M. WT denotes I28/D61.

synthesis were also equivalent at both pH 7.0 and pH 8.0. The rates for WT and I28E mutant membranes at pH 7.0 were 40.0 and 40.5 $\text{nmol min}^{-1} \text{mg}^{-1}$, respectively, and at pH 8.0 were 39.1 and 39.4 $\text{nmol min}^{-1} \text{mg}^{-1}$, respectively. The ATP-driven ACMA quenching response of the I28D and I28E/D61G mutants was greatly reduced, the I28D mutant having a response approximately 20 to 30% of that of the I28E mutant at an equivalent amount of membranes used. To aid investigation, twice as much of these membranes as of the WT and I28E mutant membranes was analyzed, thus obtaining an approximately twofold increase in the response. The I28E/D61G mutant membranes showed a significant quenching response even though the activity was insufficient to support growth by oxidative phosphorylation. The cell culture from which these membranes were derived was devoid of succinate-positive revertants.

H^+ pumping by the wild type was relatively insensitive to the pH of the buffer (Fig. 3A). In fact, the response was slightly higher at pH 7.5 than at pH 7.0; at pH 8.0, there was a slight decrease. In contrast, the I28D, I28E, and I28E/D61G mutants showed a pH dependence of ATP-driven H^+ pumping (Fig. 3). The ATP-driven quenching response decreased with increasing pH. A striking difference was observed between pH 7.0 and 7.5 for the I28E mutant (Fig. 3C). The response at pH 6.75 was equivalent to that at pH 7.0 for all membranes. The effect at pH 8.0 was fully reversible (data not shown).

The passive H^+ permeability of stripped membranes, where F_1 is removed from the complex, was examined at pH 7.0 (Fig. 4A and B) and pH 8 (Fig. 4C). WT stripped membranes

showed reduced NADH-driven quenching responses at pH 7.0 and 8.0 in comparison to the controls, indicating that the membranes were H^+ permeable and contained an F_0 functional in H^+ transport. A much larger quenching response was observed for the control stripped DCCD-inhibited WT and D61G mutant membranes due to the inability of F_0 to transport H^+ (Fig. 4A and B). A large quenching response was observed with I28D, I28E/D61G, and I28D/D61G (not shown) stripped membranes at pH 7.0, indicating little H^+ movement through F_0 . DCCD treatment of these membranes had no observable effect (data not shown). The stripped I28E mutant membranes produced a reduced quenching response at pH 7.0. DCCD treatment of the stripped I28E mutant membranes enhanced the quenching response, confirming that H^+ movement occurred through F_0 (Fig. 4B). In contrast, all stripped mutant membranes showed close to a maximal NADH-driven quenching response at pH 8.0 (Fig. 4C). The effect on the I28E mutant clearly shows that H^+ transport through F_0 is pH dependent. The impaired H^+ transport through F_0 of the mutant membranes correlates well with the effects on ATP-driven H^+ pumping discussed above.

DISCUSSION

Effects and implications of a second carboxyl group. In some bacteria, a second carboxylic acid is found at a position equivalent to Ile²⁸ in *E. coli* (Fig. 1 and 5A). In this study, alteration of Ile²⁸ to Asp or Glu resulted in functional enzymes that show pH-dependent function (Fig. 3 and 4). The I28E mutant grew similarly to the wild type and had a greater ATP-driven H^+ pumping response than the I28D mutant. The pH dependence of function is similar to observations seen on an A24D mutant by Zhang and Fillingame (43). As these authors suggest, the effect of pH on the rate of H^+ movement through F_0 is likely to limit ATPase-coupled H^+ transport. The results here suggest that the presence of the second carboxyl, at a position equivalent to 28 in *E. coli*, may be important for regulation of enzyme function in response to cytoplasmic pH. The presence of a second carboxyl reduces H^+ pumping activity when the intracellular pH is above neutrality. In *S. mutans*, which has this additional carboxyl group, the F_1F_0 ATPase appears to regulate intracellular pH but not above pH 7.5 (6). The effect of replacing the carboxylic acid at the equivalent position in *S. mutans* and other bacteria requires investigation.

The effect of pH on the cytoplasmic facing side on H^+ movement has been mimicked. The pH sensitivity suggests that the carboxyl group, or ion binding cavity, is accessible to the cytoplasm. The ion cavity is positioned within the lipid bilayer (20, 40). Hence, the pH sensitivity of the mutants support the idea of aqueous exit/entry channels within subunit *a* and/or at the interface between it and subunit *c*. Deprotonation of the second carboxyl group at higher pH may inhibit movement at the subunit *a* and *c* interface. The lack of effect of pH on ATP synthesis, and growth equivalent to WT growth on succinate, in the I28E mutant is interesting. This could be because the events at the ion binding site, as a result of pH change, are not rate limiting during synthesis. However, it may correlate with a model where the pK_a of the essential carboxyl group in each half channel switches between a high and low value according to the mode of the enzyme.

FIG. 3. Comparison of ATP-driven quenching of ACMA fluorescence by WT and mutant membranes at various pHs. Conditions were as described for Fig. 2, apart from the pH of the buffer. Note that twice as much I28D and I28E/D61G mutant membranes as other membranes were used.

Analysis of subunit *a* has given some insights into likely regions of interaction with subunit *c* (10, 41). In the proposed transmembrane span 2, there is the potential for a net negative charge (-2) from the residues that fall on the half facing the periplasm, and it has been suggested that they may make up part of an aqueous channel (10, 41). However, in *S. mutans*, where the primary role appears to be cytoplasmic H^+ extrusion, the residues at equivalent positions appear to have been replaced so that there is a net positive charge (e.g., D119 $\rightarrow$ G, insertion between 122/123 $\rightarrow$ K, D124 $\rightarrow$ Q, L126 $\rightarrow$ K, and P127 $\rightarrow$ D). This region incorporates a repeat of residues DLLP in *E. coli*, which is altered in *S. mu-*

tans by the aforementioned changes, and has been linked with functional importance (27). Intriguingly, the sequence DAIP is found at the H^+ binding site in *E. coli* subunit *c*, and there also appears to be homology in the flanking sequence. These changes may be rationalized if this region is part of the periplasmic facing channel, where the primary role in *S. mutans* is H^+ release.

Switching the essential carboxyl group to helix 1. The I28E/D61G mutant, but not the I28D/D61G mutant, showed H^+ pumping activity despite the inability of both to support growth by oxidative phosphorylation (Fig. 2). The function of the

FIG. 4. Effect of pH on H^+ transport through F_0 by NADH-driven quenching of quinacrine fluorescence. Stripped membranes were diluted to 0.1 mg/ml in HMK buffer (pH as indicated) containing 0.375 μ g of quinacrine/ml. NADH was added to a final concentration of 200 μ M at the time indicated. The reversal of quenching is due to consumption of NADH in the cuvette. DCCD-treated membranes are labeled (+DCCD).

I28E/D61G mutant may relate to the greater side chain length placing the carboxyl group at a more optimal position toward the center of the pocket or a slight variation in the pK_a of side chain carboxyl. The reduced H^+ pumping activity appears to be pH

FIG. 5. Models of positions of the carboxyl groups and neighboring residues in the subunit c oligomer of the F- and V-type ATPases. (A) Schematic representation of a section of the arrangement of the oligomeric ring of subunit c of *E. coli*, modified from Jones et al. (19). Helices from each subunit are labeled 1 and 2, and neighboring individual subunits and their residues are indicated by a single or double primes. The relative positions of residues surrounding the essential Asp⁶¹ are shown. (B) Hypothetical arrangement of the V-type ATPase Vma16p and relative positions of the two carboxyl groups based on the model of the *E. coli* oligomeric ring as shown in panel A. The packing of helices 2 and 3 with respect to helices 4 and 5 is based on the *E. coli* data (19) and cross-linking studies by Harrison et al. (15). Two of several possible arrangements and their functional implications are shown. (C) Schematic model of the V-type subunit c oligomeric ring, with the position of Vma16p indicated. Formation of the F-type like face is depicted. E and e represent the essential carboxyl group and the carboxyl group at position 188, respectively.

sensitive in this mutant, which implies that the effects seen on the double-carboxyl mutants are a direct result of changes in the ionization of this side chain. The mutation causes a lack of growth on succinate and a large reduction in H^+ pumping activity, and so the significance is difficult to assess.

The H⁺ pumping ability of the I28E/D61G mutant leads to the possibility that in the I28E and I28D mutants, both carboxyls undergo protonation and deprotonation during catalytic turnover. The switching of the essential carboxyl group to the inner helix, at position 24, with retention of function has been previously described (32). The ability of a single carboxyl group present on helix 1 to retain function seems to be at odds with mechanisms involving entire rotations of helix 2 (17, 35). Ion binding and release between subunits *a* and *c* may occur through only subtle local side chain and backbone movement.

Two carboxyl groups in transmembrane helices of subunit *c* are found in several pathogenic bacteria and in the related vacuolar ATPase. A second carboxylic acid, at a position equivalent to Ile²⁸ in *E. coli*, is found in several pathogenic bacteria (Fig. 1). Studies of *S. pneumoniae* indicate that the antimalarial agents quinine and optochin target subunit *c* (9, 34). Quinine- and optochin-resistant mutants were found to have single replacements at positions equivalent to 29, 32, 57, 58, and 59 in *E. coli*. No mutants with replacement of either of the carboxylic acids have been identified. In the models of the *E. coli* oligomeric subunit *c* ring, these positions all fall around Asp⁶¹ and Ile²⁸, i.e., the ion binding pocket. Positions 57 and 58 are near positions 28 and 61 (Fig. 5A). As several other pathogenic bacterial species, such as *M. tuberculosis*, *M. leprae*, and pathogenic oral bacteria, possess these two carboxylic acids, it is tempting to speculate that the ion binding cavity of subunit *c* could be a valuable target for future therapeutic drugs.

Studies of *S. pneumoniae* have led to the suggestion that the primary target for quinine in *P. falciparum* could be the subunit *c* of either the mitochondrial F₁F₀ ATP synthase or the V₁V₀ ATPase (34). This is germane to the data presented here, as recent studies indicate that *P. falciparum* has a plasma membrane V₁V₀ ATPase that is responsible for regulation of cytoplasmic pH (36).

The V-type ATPase has a homologous subunit *c* composed of four transmembrane helices that seem to have evolved by duplication of a progenitor gene (29). Each V-type subunit *c* has only a single essential carboxylate in one of the two helical hairpins, lowering the H⁺/ATP ratio to presumably enable ATP-driven H⁺ pumping to generate greater electrochemical gradients while preserving structural features of the complex (5). Two homologues, Vma11p and Vma16p, have been found and shown to be essential for activity in yeast (16). The Vma16p subunit *c* has two carboxylates in helices 3 and 5 that are equivalent to helix 2 in the F-type subunit *c*. The second carboxylate (E188) appears not to be essential for activity (16). It falls in the last helix at a position similar to that of Ile²⁸ in the *E. coli* subunit *c*. The carboxylate may lie in the ion binding cavity proximal to the essential carboxylate; hence, protonation and deprotonation could play a role in pH sensitivity as described above (Fig. 5B). V₁ and V₀ reassembly has been shown to be pH dependent (22). Deprotonation of E188 at alkaline pH could prevent reassembly, possibly via long-range perturbations through the cytoplasmic loops. As the cytoplasmic pH falls, protonation of this carboxylate could then allow reassembly and activity. The carboxylates in Vma16p could also be positioned so as to generate neighboring ion binding sites that will form an F-type subunit *c*-like face if the subunits are arranged similarly to the F₀ *c* ring (Fig. 5C) (19). In this arrangement, the potential for H⁺ movement is evident via a

limited forward-and-backward idling motion at this face or inhibition via a variation in the pK_a of the carboxylates (see above). The models can provide a mechanistic explanation for the "slip" mechanism (33), variable H⁺-ATP coupling modulation, by allowing an uncoupling between H⁺ movement and ATP hydrolysis.

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