On the Role of Arg-210 and Glu-219 of Subunit a in Proton Translocation by the <i>Escherichia coli</i> F<sub>0</sub>F<sub>1</sub>-ATP Synthase*  

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A strain of <i>Escherichia coli</i> was constructed which had a complete deletion of the chromosomal uncB gene encoding subunit a of the F<sub>0</sub>F<sub>1</sub>-ATP synthase. Gene replacement was facilitated by a selection protocol that utilized the sacB gene of <i>Bacillus subtilis</i> cloned in a kanamycin resistance cartridge (Ried, J. L., and Collmer, A. (1987) <i>Gene</i> (Amst.) 57, 239–246). F<sub>0</sub> subunits b and c inserted normally into the membrane in the ΔuncB strain. This observation confirms a previous report (Hermolin, J., and Fillingame, R. H. (1995) <i>J. Biol. Chem.</i> 270, 2815–2817) that subunit a is not required for the insertion of subunits b and c. The ΔuncB strain has been used to characterize mutations in Arg-210 and Glu-219 of subunit a, residues previously postulated to be essential in proton translocation. The aE219G and aE219K mutants grew on a succinate carbon source via oxidative phosphorylation and membranes from these mutants exhibited ATPase-coupled proton translocation (i.e. ATP driven 9-amino-6-chloromethoxyacridine quenching responses that were 60–80% of wild type membranes). We conclude that the aGlu-219 residue cannot play a critical role in proton translocation. The aR210A mutant did not grow on succinate and membranes exhibited no ATPase-coupled proton translocation. However, on removal of F<sub>1</sub> from membrane, the aR210A mutant F<sub>0</sub> was active in passive proton translocation, i.e. in dissipating the ΔpH normally established by NADH oxidation with these membrane vesicles. aR210A membranes with F<sub>1</sub> bound were also proton permeable. Arg-210 of subunit a may play a critical role in active H<sup>+</sup> transport that is coupled to ATP synthesis or hydrolysis, but is not essential for the translocation of protons across the membranes.

F<sub>0</sub>F<sub>1</sub>-ATP synthases catalyze the synthesis of ATP during oxidative phosphorylation utilizing the energy of an electrochemical proton gradient generated by H<sup>+</sup> pumping electron transport complexes (1). Structurally similar F<sub>0</sub>F<sub>1</sub>-ATP synthases are also present in mitochondria, chloroplasts, and most eubacteria. The enzymes are composed of two structurally and functionally distinct sectors termed F<sub>1</sub> and F<sub>0</sub>. The F<sub>1</sub> sector is responsible for binding of F<sub>1</sub> to F<sub>0</sub> and is responsible for coupling of proton translocation to structural changes in F<sub>1</sub> leading to ATP synthesis. The bulk of the b subunit extends from the membrane surface and is responsible for binding of F<sub>1</sub> to F<sub>0</sub>. The role of subunit a in F<sub>0</sub> function has not been clearly defined. It is not thought to play a major role in F<sub>1</sub> binding but has been postulated to play a key role in proton translocation (2–5).

All genetic studies on the function of subunit a have been carried out by complementation with uncB (subunit a) genes expressed from either plasmids, F<sup>+</sup> episomes or λ-transducing phage in a background strain carrying a chromosomal unc<sub>B</sub> truncation mutation, e.g. strains like CP242 (W231stop) (6) or RH305 (V239A, P240W, and W241stop) (7). The presence of the truncated subunit a in the complementation systems used might introduce complications into studies of function or assembly. For example, Hermolin and Fillingame (8) studied the insertion of subunits b and c in a strain carrying a W231stop truncation of subunit a and concluded that these subunits inserted into the membrane independently of subunit a. However, they were unable to rule out a transient role for the truncated subunit a in insertion of subunits b and c. To avoid complications that can arise due to the presence of a truncated subunit, we have constructed a strain that has a complete deletion of the chromosomal uncB gene encoding subunit a. We have re-examined the dependence of subunit insertion in the ΔuncB background and conclude that subunits b and c do insert independently of subunit a.

The ΔuncB strain constructed here also provides an improved recipient background for study of plasmid-borne uncB mutations and was used here to study substitutions in Arg-210 and Glu-219, residues previously concluded to play key roles in function. Glu-219 can be mutated to Asp with full retention of function. Glu-219 can be mutated to Asp with full retention of function while most other substitutions tested have resulted in enzymes with very little function (9, 10). Vik and Antonio (11) suggested an interaction between Glu-219 and Gln-252 based upon the function of the three mutants with double substitutions in subunit a, i.e. E219G/Q252E, E219D/Q252E, and E219K/Q252E. However, they did not test the phenotype of the E219G and the E219K mutations by themselves. We show here that mutants with the E219G and E219K single substitutions are by themselves functional. These results severely limit the possible function of Glu-219. Arg-210 is thought to be essential as all substitutions for this residue result in loss of function. The R210A mutation, previously studied by Hatch et al. (12), is re-examined here and found to be unusual in that ATP hydrolysis-coupled proton translocation is completely abolished.
Arg-210 and Glu-219 of Subunit α

whereas passive proton translocation through F₀ is unaffected. Arg-210 may be an important component for coupled proton translocation but is not an essential component of the passive proton conductance pathway through F₀.

EXPERIMENTAL PROCEDURES

Construction of the uncB deletion using PCR—A PCR

1 The abbreviations used are: PCR, polymerase chain reaction; DCCD, dicyclohexylcarbodiimide; ACMA, 9-amino-6-chloro-2-methoxyacridine; LDAO, lauryldimethylamine oxide; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

2 The unc DNA numbering system corresponds to that used by Walker et al. (13).

FIG. 1. Construction of the uncB deletion using PCR. Primer 2 (TGAGTGTTGGTGATAAATCCCATTTTT) and primer 3 (CTGCTTTGGCAGAGG) are wild type unc primers corresponding to nucleotides 45–59 and 2694–2714 according to the numbering system of Walker et al. (13). The PCR product obtained in the first PCR reaction was used along with plasmid pDF163 as a template in the second PCR reaction. Plasmid pDF163 carries an unc DNA fragment from bases 870–3216.

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FIG. 2. Plasmids used in construction of the uncB deletion strain. Key restriction sites are shown. The unc fragments in the plasmids are shaded. The darkened BamHI site in plasmid pAP55 corresponds to the region of unc DNA replaced by the sacRB-nptI cartridge. The vector portion of the plasmids are numbered according to the pACYC184 sequence (16) and unc DNA numbered in italics based upon the sequence of Walker et al. (13).
CAGTTGTTTCGCTTCTGTTCGGTAAC, initiating DNA synthesis at unc nucleotides 943 and 1879, respectively. The product strain, Sac-14, was concluded to have the sacR::nptI cartridge inserted between the BamHI sites in the chromosomal uncB gene.

**Cartridge Excision**—The presence of the sacR::nptI cartridge in the strain Sac-14 makes the cells sensitive to sucrose (14). Loss of the cartridge by a double recombination event with uncB sequences present on a plasmid should make the cells resistant to sucrose and sensitive to kanamycin. Strain Sac-14 was transformed with the plasmid pVF208 (described above) and chloramphenicol-resistant transformants were isolated. A sample of a pVF208/Sac-14 transformant grown overnight in LB medium (19) was plated on LB medium agar containing 5% sucrose. Sucrose-resistant colonies were screened for sensitivity to kanamycin to identify possible candidates arising from a double recombination event. The desired recombination event took place at a frequency of roughly 60%. The cells were then cured of the plasmid by growth overnight in LB medium in the absence of chloramphenicol. This was easily accomplished due to plasmid instability in the recD background (20). Chloramphenicol-sensitive colonies were screened by replica plating and found at a frequency of roughly 10%. The presence of the uncB deletion in the chromosome was confirmed by PCR amplification of chromosomal DNA using primers GCTGTCGTCGTTCTGTTCTGTTCTAG and CTCAGTGTGTGTTTTTGTGTTTGCAGA, initiating DNA synthesis at unc nucleotides 810 and 2319, respectively, and DNA sequencing of the PCR-amplified product.

**Construction of R210A Mutant**—The oligonucleotide CACTCTGGTT-TGGCCTGTCTTTGCTAC was synthesized with the Quik-252E mutation along with the Q252E mutation were confirmed by DNA sequencing. Plasmid pSBV16 (11) contains uncB gene except that the Arg-210 codon is substituted by Glu codon. The megaprimer method (22) was used to clone the megaprimer between these sites in plasmid pSBV16. The resulting strain was confirmed by UV sensitivity (6).

**Construction of Glu-219 Mutants**—Plasmid pSBV16 derivatives containing the Glu-219 mutations along with the Q252E mutation were provided by Dr. S. B. Vik (Southern Methodist University, Dallas, TX) (11). The Glu-219 mutations were separated from the Gln-252E mutation by subcloning the BamHI fragment from the corresponding plasmid pSBV16 derivative into the BamHI sites in plasmid pCP1200 (6). Plasmid pCP1200 carries the wild type uncB gene on a HindIII(870) to AvaI(1976) fragment of unc DNA cloned between these sites in plasmid pRS22. The presence of the Glu-219 mutations in the resulting plasmids was confirmed by DNA sequencing. Plasmid pSBV16 (11) contains an uncB gene engineered to contain a number of restriction sites. Plasmid pVF247 was used as a wild type control for the Glu-219 mutant plasmids by cloning the BamHI fragment from plasmid pSBV16 into plasmid pCP1200.

**Construction of F0 Subunit**—To reinvestigate the role of subunit a in the assembly of the F0 sector, a strain was constructed that had a complete deletion of the chromosomal uncB gene. This was achieved in two steps. In the first step, the deletion was generated on a plasmid using the PCR protocol described in Fig. 1. The dele-
tion was then transferred from the plasmid into the chromosome using a positive selection for eviction of the sacB/R-hptI cartridge inserted into the chromosomal uncB gene. The presence of the chromosomal uncB deletion in the resulting strain VF245 was confirmed by DNA sequencing. As shown in Fig. 3, immunoblots of VF245 membranes confirm the absence of subunit a (panel A, lane 3) and the presence of subunit b (panel B, lanes 5 and 6) and subunit c (panel C, lanes 5 and 6). The amounts of subunits b and c present may be slightly reduced relative to wild type.

**Growth Characteristics of the uncB Deletion Strain**—The ΔuncB strain did not grow on succinate minimal medium, where a functional F_{1}F_{0}-ATP synthase is required. UncB^{+} transformants of strain VF245, which carry the wild type subunit a gene on a plasmid, were able to grow on succinate. The colony sizes after growth on succinate and growth yields in limiting glucose medium were studied to determine the relative function of F_{1}F_{0}-ATP synthase in vivo. The ΔuncB strain showed low growth yields in glucose medium when compared with an isogenic wild type strain while the complemented strain gave growth yields similar to the wild type strain (Table I). The complemented strain also produced colonies of sizes similar to the wild type strain on succinate. We conclude that the uncB deletion within the unc operon (unc I^ΔBC F^F H^G A^G D^C) does not have a major polar effect on expression of the downstream genes.

**Biochemical Characterization of the uncB Deletion Strain**—The level of membrane bound ATPase activity present in the ΔuncB strain was about 50% of that observed in the isogenic wild type strain (Table II). The ATPase activity was activated to a similar extent by LDAO, as compared with the wild type, which indicates that the reduction in ATPase activity was not due to an abnormal inhibition of the membrane-bound form. Rather the reduction is probably due to a slight polar effect of the uncB deletion on downstream gene expression. The presence of membrane-bound ATPase in the uncB deletion strain indicates proper insertion of the b and c subunits into the membrane and assembly into a complex that is competent in binding F_{1}. The UncB^{+} plasmid complemented strain showed ATPase activity similar to that in the wild type membranes. The ATPase activity of the ΔuncB strain was DCCD insensitive, whereas the complemented strain had a DCCD sensitivity similar to wild type. The complemented strain showed normal ATP-driven proton translocation activity as indicated by quenching of ACA fluorescence (Fig. 4).

**Characterization of the aGlu-219 Mutants**—The ΔuncB strain was transformed with plasmids containing single Glu-219 mutations. Growth of the transformants was tested on succinate minimal medium. The E219D and the E219K mutants showed growth similar to wild type and the E219G mutant showed reduced growth relative to wild type (Table III). The E219A mutant was unable to grow on succinate after 3 days but pinpoint colonies were observed after incubation for 6 days. The growth yields in glucose liquid medium were also determined and correspond roughly with the colony size on succinate, i.e. wild type > E219D > E219K > E219G > E219A (Table III). Each of the Glu-219 mutants showed membrane-bound ATPase activity with a DCCD sensitivity similar to the wild type, indicating normal assembly of the F_{0} sector in all the mutants (Table III). Immunoblots indicated the presence of roughly similar amounts of subunit a in the membranes of the VF245(ΔuncB) strains carrying the various Glu-219 mutant plasmids (Fig. 5). Each of the Glu-219 mutants tested also showed ATP-driven proton translocation as indicated by quenching of ACA fluorescence (Fig. 6). E219D mutant membranes gave a quenching response similar to wild type membranes as has been previously demonstrated (9). The E219G mutant membranes consistently showed greater quenching than the E219K mutant membranes. E219A mutant membranes also showed significant ATP-driven quenching despite being derived from cells that could not grow on succinate. The cell culture used in preparation of these membranes was checked for succinate positive revertants and none were found. The NADH-driven quinacrine quenching response of each of the mutant membrane preparations was equivalent to that of the wild type preparation. The reduced ATP-driven quenching response therefore cannot be attributed to increased proton leakiness of the membrane.

**Characterization of the aR210A Mutant**—The ΔuncB strain was transformed with plasmids carrying the R210A mutation or an UncB^{+} control plasmid. The R210A mutant was unable to grow on succinate and gave low growth yields on glucose (Table IV). The R210A mutant showed very low levels of membrane bound ATPase (Table IV). The ATPase could be activated to wild type levels by LDAO treatment which indicated that F_{1} was inhibited when bound to the R210A mutant F_{0}. The R210A

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**TABLE II**

| Strain/plasmid | Mutation | ATPase specific activity | DCCD sensitivity* |
|---------------|----------|-------------------------|-------------------|
|               |          | –LDAO | +0.5% LDAO | % |
| MM833         | None     | 0.53 | 2.10 | 63 |
| VF245         | ΔuncB    | 0.24 | 1.02 | 10 |
| VF245/pcP1200 | ΔuncB/uncB | 0.38 | 1.80 | 61 |

* Fraction of ATPase activity lost after treatment with 30 μM DCCD for 10 min at 30 °C in ATPase assay buffer.
The interdependence of insertion of the various F$_o$ subunits into the membrane was tested by Hermolin and Fillingame (8).

**DISCUSSION**

The interdependence of insertion of the various F$_o$ subunits into the membrane was tested by Hermolin and Fillingame (8).

Subunits b and c were found to insert independently, whereas stable insertion of subunit a required the presence of both subunits b and c. These studies were carried out in a strain carrying a truncated subunit a with a W231stop mutation. The truncated subunit was not detected in immunoblots of membranes from this strain. The lack of the truncated subunit may be strain dependent as truncated subunits could be detected in the experiments of Eya et al. (31), who used the same antiserum. Recently, subunit a was shown to insert into the membrane in the absence of the other F$_o$ subunits where it is subject to degradation by the FtsH protease (32). In the experiments of Hermolin and Fillingame (8), it is possible that a truncated subunit a was inserted into the membrane and then degraded in the absence of subunits b and/or c. Furthermore, if the W231stop truncated subunit a was inserted transiently, then conclusions regarding the independence of insertion of subunits b and c would need to be re-evaluated. The study here of the Unc$_b$ strain clearly indicates that subunit a is not required for the membrane insertion of subunits b and c or the assembly of the subunits into a F$_o$ like complex capable of binding F$_1$.

Subunit a is required for the assembly of a functional F$_o$ and therefore the strain carrying the unc$_b$ deletion is unable to grow on succinate. The unc$_b$ deletion strain gives abnormally low growth yields on glucose for an unc mutant. A similar effect on glucose growth yield has been observed in an unc$_C$ mutant lacking the $\epsilon$ subunit (33). The low growth yield in the case of the unc$_C$ mutant was thought to result from an increase in active ATPase in the cytoplasm due to the lack of the $\epsilon$ subunit. The Unc$_b$ strain described here shows levels of cytoplasmic

**FIG. 5. Comparison of the levels of subunit a in membranes of the various Glu-219 mutants.** Subunit a was detected using anti-a antibody after SDS-acrylamide gel electrophoresis of 10 $\mu$g of membrane protein and blotting onto nitrocellulose paper. Lane 1, VF245/pVF237 (unc$_b$); lane 2, VF245/pVF232 (E219G); lane 3, VF245/pVF233 (E219A); lane 4, VF245/pVF235 (E219D); lane 5, VF245/pVF236 (E219K); lanes 6 and 7, F$_o$ standard 0.5 and 0.1 $\mu$g.

**FIG. 6. Comparison of ATP-driven quenching of ACMA fluorescence by Glu-219 mutants.** Membranes were diluted to 0.25 mg/ml in HMK buffer containing 0.3 $\mu$g/ml ACMA. ATP was added to 0.94 mm and the uncoupler SP6847 was added to 0.3 $\mu$mol at times indicated in the figure. Traces: 1, VF245/pVF247 (unc$_b$); 2, VF245/pVF235 (E219D); 3, VF245/pVF232 (E219G); 4, VF245/pVF236 (E219K); 5, VF245/pVF233 (E219A); 6, VF245 (unc$_b$).

**TABLE III**

| Strain/plasmid | Mutation | Growth on succinate | Growth yield in limiting glucose | ATPase specific activity | DCCD sensitivity |
|----------------|----------|---------------------|----------------------------------|------------------------|------------------|
| VF245/pVF247   | None     | 2                   | 84                               | 0.36                   | 59.37            |
| VF245/pVF232   | E219G    | 1                   | 54                               | 1.976                  | 65.37            |
| VF245/pVF233   | E219A    | 0$^d$               | 39                               | 1.670                  | 63.37            |
| VF245/pVF235   | E219D    | 2                   | 79                               | 2.106                  | 60.37            |
| VF245/pVF236   | E219K    | 1.5                 | 68                               | 1.601                  | 66.37            |

$^a$ Colony size after 72 h of incubation at 37 $°$C on minimal plates containing 22 mm succinate.

$^b$ Growth yield in 0.04% glucose minimal medium, calculated relative to wild type (MM833).

$^c$ Fraction of ATPase activity lost after treatment with 30 $\mu$mol DCCD for 10 min at 30 $°$C in ATPase assay buffer.

$^d$ On incubation of plates for 6 days at 37 $°$C, pinpoint sized colonies were observed.

Subunit a did not show any ATP-driven proton translocation (data not shown). The relative proton permeability of stripped membranes was determined by NADH-driven quenching of quinacrine fluorescence. Stripped membranes from the Unc$_b$ strain showed a maximal NADH-driven quenching response which indicated that they were impermeable to protons (Fig. 7). Stripped membranes from the Deltaunc$_b$ strain transformed with plasmid pDF163 showed reduced NADH-driven quenching, which indicated that the membranes were proton permeable and contained an F$_o$ that was functional in proton translocation. Stripped membranes from the Unc$_b$ strain transformed with plasmid pVP270, carrying the R210A mutation, showed the smallest NADH driven quenching response which suggested that the R210A mutant F$_o$ was fully functional in passive proton translocation. The proton permeability of the R210A stripped membranes could be inhibited by venturicidin or DCCD treatment and this confirmed that the proton leak took place through the mutant F$_o$. The R210A F$_o$ is therefore not blocked by the binding of F$_1$.

**Comparison of the levels of subunit a in membranes of the various Glu-219 mutants.** Subunit a was detected using anti-a antibody after SDS-acrylamide gel electrophoresis of 10 $\mu$g of membrane protein and blotting onto nitrocellulose paper. Lane 1, VF245/pVF237 (unc$_b$); lane 2, VF245/pVF232 (E219G); lane 3, VF245/pVF233 (E219A); lane 4, VF245/pVF235 (E219D); lane 5, VF245/pVF236 (E219K); lanes 6 and 7, F$_o$ standard 0.5 and 0.1 $\mu$g.

**Comparison of ATP-driven quenching of ACMA fluorescence by Glu-219 mutants.** Membranes were diluted to 0.25 mg/ml in HMK buffer containing 0.3 $\mu$g/ml ACMA. ATP was added to 0.94 mm and the uncoupler SP6847 was added to 0.3 $\mu$mol at times indicated in the figure. Traces: 1, VF245/pVF247 (unc$_b$); 2, VF245/pVF235 (E219D); 3, VF245/pVF232 (E219G); 4, VF245/pVF236 (E219K); 5, VF245/pVF233 (E219A); 6, VF245 (unc$_b$).
TABLE IV

| Strain/plasmid  | Mutation | Growth on succinate | Growth yield in limiting glucose | ATPase specific activity |
|----------------|----------|---------------------|-------------------------------|-------------------------|
| VF245/pVF163    | UncB     | 2                   | 50                            | 0.36, 1.73              |
| VF245/pVF270    | R210A    | 0                   | 55                            | 0.33, 1.48              |

- Colony size after 72 h of incubation at 37°C on minimal plates containing 22 µM succinate.
- Growth yield in 0.04% glucose minimal medium, calculated relative to wild type (MM833).

Fig. 7. Proton permeability of Arg-210 stripped membranes as estimated by NADH-driven quenching of quinacrine fluorescence. Stripped membranes were diluted to 0.125 mg/ml in HMK buffer containing 0.375 µg/ml quinacrine. NADH was added to a final concentration of 50 µM at the time indicated. Panel A, VF245(DuncB); Panel B, VF245(pDF163(UncB)); Panel C, VF245(pVF170(aR210A)). Trace 1 is the untreated sample, trace 2 is the sample after treatment with 12.5 µg of venturicidin, trace 3 is the sample after treatment with 30 µM DCCD, and trace 4 is the sample after treatment with 45 µM DCCD.

Fig. 8. Proton permeability of Arg-210 whole membranes as estimated by NADH-driven quenching of ACMA fluorescence. Membranes were diluted to 0.25 mg/ml in HMK buffer containing 0.375 µg/ml quinacrine. NADH was added to a final concentration of 50 µM at the time indicated. Traces: 1, VF245(DuncB); 2, VF245(pDF163(UncB)); 3, VF245(pVF270(aR210A)).

ATPase similar to a wild type strain (data not shown) so excess, unregulated cytoplasmic ATPase is apparently not the explanation for the low growth yield. The effect on growth yield is clearly related to the loss of subunit a since close to wild type growth yields are observed when the uncB deletion is complemented by a wild type subunit a gene carried on a plasmid.

Previous mutagenesis studies on Glu-219 of subunit a indicated that the residue was not tolerant to mutation. For example, the E219Q mutation led to almost complete loss of function (10), and the Glu-219 residue was concluded to be a likely participant in the proton translocation pathway. It was therefore surprising to find that the E219K mutation demonstrated such robust growth and function. The E219G mutation is also functional. A third mutant, the E219A mutant, did not form colonies on succinate during a prolonged incubation of 3 days, but did show significant ATP-driven quenching. We have no explanation for the imperfect correlation between growth on succinate and ATP-dependent quenching in these mutants. However, in summary, these results clearly indicate that Glu-219 cannot play a critical part in the proton translocation pathway. If the residue were a critical component of the proton translocation pathway, then mutations which alter or eliminate the charge on the side chain, i.e., E219K or E219G mutations, would be expected to be non-functional.

Hartzog and Cain (34) have shown that the functionless H245G mutation is suppressed by substitution of Asp or Lys for Gly-218. Furthermore, the experiments of Hatch et al. (12) indicate that the functionless R210Q mutation can be suppressed by a Q252R substitution. These two sets of results suggest a close interaction between the final two transmembrane helices in subunit a, i.e., transmembrane helix-4 and helix-5 in the model of Hatch et al. (12), with Gly-218 opposite His-245 and Arg-210 opposite Gln-252. If Arg-210 and Glu-219 exist on a continuous stretch of α-helix, then these residues would be positioned on opposite faces of helix-4. The helices would also be oriented so that Arg-210 on helix-4 and Gln-252 on helix-5 are in structurally similar positions. This orientation would preclude any interaction between Glu-219 and Gln-252 as was suggested by Vik and Antonio (11). Residues adjacent to Gly-218 and Glu-219 have been identified by suppressor analysis as being important for the functional interaction between subunit a and subunit c (35). The substitutions at Glu-219 and His-245 which disrupt function may do so by perturbing interactions between the a and c subunits.

The aR210A mutation was initially described by Hatch et al. (12). They reported an abnormally low NADH-driven quenching response for mutant membranes and suggested that the mutant membranes were partially permeable to protons, although other possible explanations were not ruled out. The data reported by Hatch et al. (12) led us to re-investigate the properties of the aR210A substitution. Using the DuncB complementation system developed here, we observe significantly greater proton leakiness in the aR210A mutant membranes than was suggested by the work of Hatch et al. (12). Indeed, our experiments consistently indicate that aR210A mutant-stripped membranes have a greater proton permeability than wild type-strippred membranes. The proton conductance is mediated by F0 as it is blocked by the specific inhibitors, venturicidin and DCCD. Higher concentrations of DCCD are required for inhibition of proton conductance by the aR210A membranes than with wild type membranes. This may indicate that the aR210A mutation directly effects the chemical reactivity of cAsp61, or that it alters the DCCD-binding site, although less direct effects are also possible. The proton leakiness of the aR210A mutant membranes indicates that Arg-210 is not essential for passive proton conductance through F0. Previously, several reports have suggested that Arg-210 may directly contribute to the proton conductance pathway, based upon the properties of the mutants studied (12, 31, 36, 37). All other mutations in this residue (i.e., R → K, Q, E, V, I) were concluded to block passive proton translocation mediated by F0.

We have previously discussed a model for ATPase-coupled proton transport by F1F0-ATPase in which the H+ carrier, i.e.,...
the carboxyl side chain of Asp-61 of subunit c, undergoes a $pK_a$ change during the active transport cycle (2). The key aspect of this model, namely the change in $pK_a$ during ATP synthesis is now supported by kinetic studies (38). In the proposed model, the high $pK_a$ form of Asp-61 is proposed to facilitate the passive proton conductance of uncoupled $F_0$, an idea also supported by kinetic evidence (39, 40). The passive proton conductance through $F_0$ would normally be prevented by the binding of $F_1$ to $F_0$. The passive proton translocation cycle is proposed to be mediated by a single subunit c, whereas the translocation of 3-4 $H^+$ during coupled synthesis/hydration of one ATP is proposed to be mediated by multiple subunit c. The model explicitly makes the passive $H^+$ translocation cycle a side path that need not share kinetic characteristics with the $H^+$ transport coupled ATP synthesis/hydration cycle. In the model, we have proposed that the role of the aArg-210 may be to transiently lower the $pK_a$ of the subunit c. The translocation conformation where the Asp-61 carboxyl is locked in a high $pK_a$ conformation where the Asp-61 carboxyl group has access to conducting channels on both sides of the membrane. As a result, the mutant cannot carry out coupled $H^+$ translocation but would be unaffected in passive $H^+$ transport. In this high $pK_a$ conformation, there are structural changes in the polar loop of subunit c which result in an uncoupling of $F_1$ from $F_0$, i.e. generate a proton leaky membrane due to improper binding of $F_1$ to $F_0$. The uncoupled phenotype observed here is also seen in mutations in subunit c. In a R210A mutation described.

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