An additional acidic residue in the membrane portion of the *b*-subunit of the energy-transducing adenosine triphosphatase of *Escherichia coli* affects both assembly and function

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Glycine at position 9 is replaced by aspartic acid in the mutant *b*-subunit of *Escherichia coli* F_1F_0 -ATPase coded for by the *uncF476* allele. The mutant *b*-subunit is not assembled into the membrane in haploid strains carrying the *uncF476* allele, but, if the mutant allele is incorporated into a multicopy plasmid, then some assembly of the mutant *b*-subunit occurs. Two revertant strains were characterized, one of which (AN2030) was a full revertant, the other (AN1953) a partial revertant. DNA sequencing indicated that in strain AN2030 the *uncF476* mutation had reverted to give the sequence found in the normal *uncF* gene. The partial-revertant strain AN1953, however, retained the DNA sequence of the *uncF476* allele, and complementation analysis indicated that the second mutation may be in the *uncA* gene. Membranes prepared from the partial-revertant strain carried out oxidative phosphorylation, although the membranes appeared to be impermeable to protons, and the ATPase activity was insensitive to the inhibitor dicyclohexylcarbodi-imide.

The genes forming the *unc* operon in *Escherichia coli* code for the components of the energy-transducing adenosine triphosphatase complex and map at about 84 min on the *E. coli* chromosome (Bachmann, 1983). Genetic studies (Gibson *et al.*, 1978; Downie *et al.*, 1981) and DNA sequencing (Gay & Walker, 1981) have established that the *unc* operon consists of nine genes in the order *uncIBEFHAGDC*, the *uncI* gene being closest to the promoter. The protein coded for by the *uncI* gene does not appear to be a structural component of the F_1F_0 -ATPase complex (Gay & Walker, 1981). The *uncBEF* genes code for the *a*-, *c*- and *b*-subunits respectively of the membrane sector of the ATPase (Downie *et al.*, 1981), whereas the *uncHAGDC* genes code for the δ -, α -, γ -, β - and ϵ -subunits respectively of the F_1 -ATPase (Downie *et al.*, 1980; Gay & Walker, 1981; Gunsalus *et al.*, 1982).

The amino acid sequence of the *b*-subunit of the ATPase is unusual in that a hydrophobic region extends from residues 1 to 33, whereas the remainder of the protein is highly hydrophilic (Gay & Walker, 1981). The molecule appears to be largely

α -helical, with a number of β -turns (Walker *et al.*, 1982; Senior, 1983). The unequal charge distribution would suggest that the *N*-terminal region of the protein traverses the membrane, whereas the remainder is outside the membrane and interacts with subunits of the F_1 -ATPase. The *b*-subunit would then be seen to play a key role in the functional and structural integration of the F_1 -ATPase with the membrane sector of the enzyme (Senior, 1983). The *b*-subunit also plays a key role in a proposed pathway of assembly of the F_1F_0 -ATPase (Cox *et al.*, 1981).

In the present paper we describe an amino acid substitution, caused by the *uncF476* allele, that prevents the incorporation of the mutant *b*-subunit into the membrane of haploid strains. The properties of a partial-revertant strain, in which assembly of the mutant *b*-subunit does occur, are also described.

Materials and methods

Enzymes

Restriction endonucleases and virus- T_4 DNA ligase were obtained from Amersham Australia. Pronase was obtained from Boehringer-Mannheim Australia.

Abbreviation used: DCCD, dicyclohexylcarbodi-imide.

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Bacterial strains and plasmids

All of the bacterial strains used were derived from *E. coli* K12 and are described, together with the plasmids used, in Table 1.

Genetic techniques

The techniques used for genetic experiments were as outlined previously (Gibson *et al.*, 1977b, 1978; Downie *et al.*, 1980).

Preparation of plasmid and chromosomal DNA

Plasmid DNA was prepared as described by Selker *et al.* (1977). For the preparation of chromosomal DNA, cells were centrifuged from 1 litre of culture and lysed as described by Selker *et al.* (1977) in a volume of about 20 ml. Pronase (100 mg) was added and the cell lysate incubated at 37°C for 1 h. CsCl (1 g/ml of cell lysate) was added and DNA purified by equilibrium density-gradient centrifugation (Selker *et al.*, 1977).

Table 1. *Bacterial strains and plasmids used*

(a) Bacterial strains

Strain no.	Relevant genotype	Notes and references
AN1882	pAN63/ <i>uncF476 argH pyrE entA recA</i>	Partial diploid strain carrying <i>uncF476</i> allele on both F-plasmid and chromosome. Isolated as described previously (Gibson <i>et al.</i> , 1977b)
AN727	<i>uncB402 argH pyrE entA recA</i>	Strain carrying <i>uncB402</i> allele in AN346 <i>recA</i> background (Butlin <i>et al.</i> , 1973; Gibson <i>et al.</i> , 1977b)
AN1977	pAN181/ <i>uncB402 argH pyrE entA recA</i>	The present paper
AN1521	<i>uncF476 argH pyrE entA recA</i>	Strain carrying <i>uncF476</i> allele in AN346 <i>recA</i> background (Downie <i>et al.</i> , 1981)
AN1989	pAN181/ <i>uncF476 argH pyrE entA recA</i>	The present paper
AN1570	<i>uncG428 thr leu hrdR hsdM recA</i>	Prepared by transduction of <i>uncG428</i> allele into an <i>ilvC</i> derivative of C600 (Downie <i>et al.</i> , 1980)
AN888	<i>unc416::MuB⁺ EFHAGDC argH pyrF entA recA</i>	Jans <i>et al.</i> (1983)
AN2050	pAN199/ <i>unc416::MuB⁺ EFHAGDC argH pyrE entA recA</i>	The present paper
AN2030	<i>argH pyrE entA</i>	Revertant of AN1515 (the present paper)
AN1953	<i>uncF476 argH pyrE entA</i>	Partial revertant of AN1515 (the present paper)
AN1515	<i>uncF476 argH pyrE entA</i>	Strain carrying <i>uncF476</i> allele in AN346 background (Downie <i>et al.</i> , 1981)
AN1983	pAN182/ <i>ilv arg purE pyrE recA</i>	Partial-diploid strain carrying <i>unc</i> alleles from AN1953 on an F-plasmid, constructed as described previously (Gibson <i>et al.</i> , 1977b)
AN2023	<i>uncF476 argH pyrE entA recA</i>	<i>recA</i> derivative of AN1953
AN2021	pAN2/ <i>uncF476 argH pyrE entA recA</i>	Obtained by mating AN780 (Cox <i>et al.</i> , 1978) with AN2033 (the present paper)
AN2022	pAN2/ <i>uncF476 argH pyrE entA recA</i>	Obtained by mating AN780 (Cox <i>et al.</i> , 1978) with AN1521 (Downie <i>et al.</i> , 1981) (the present paper)
AN2059	pAN7/ <i>uncF476 argH pyrE entA recA</i>	Obtained by mating AN819 (Cox <i>et al.</i> , 1978) with AN2023 (the present paper)
AN2060	pAN7/ <i>uncF476 argH pyrE entA recA</i>	Obtained by mating AN819 (Cox <i>et al.</i> , 1978) with AN1521 (Downie <i>et al.</i> , 1981) (the present paper)

(b) Plasmids

pACYC184	<i>Cm^r Tc^r</i>	Chang & Cohen (1978)
pAN51	<i>Cm^r Tc^s uncB⁺E⁺F⁺H⁺A⁺</i>	Downie <i>et al.</i> (1980)
pAN181	<i>Cm^r Tc^s uncB⁺E⁺F476 H⁺A⁺</i>	The present paper
pAN36	<i>Cm^r Tc^s uncD⁺C⁺</i>	Downie <i>et al.</i> (1980)
pAN45	<i>Cm^r Tc^s uncB⁺E⁺F⁺H⁺A⁺G⁺D⁺C⁺</i>	Downie <i>et al.</i> (1980)
pAN199	<i>Cm^r Tc^s uncB⁺E⁺F476 H⁺A⁺G⁺D⁺C⁺</i>	The present paper
pAN63	<i>pyrE⁺ uncB⁺E⁺F476 H⁺A⁺G⁺D⁺C⁺ argH⁺ ilv⁺</i>	F-plasmid carrying <i>uncF476</i> allele isolated as described previously (Gibson <i>et al.</i> , 1977b)
pAN205	<i>Cm^r Tc^s uncB⁺E⁺F⁺H⁺A⁺</i>	The present paper
pAN182	<i>pyrE⁺ uncB⁺E⁺F476 H⁺A⁺G⁺D⁺C⁺ argH⁺ ilv⁺</i>	F-plasmid carrying the mutant <i>unc</i> alleles from AN1953 isolated as described previously (Gibson <i>et al.</i> , 1977b)
pAN2	<i>pyrE⁺ uncB⁺E⁺F⁺H⁺A401 G⁺D⁺C⁺ argH⁺</i>	F-plasmid carrying <i>uncA401</i> allele (Cox <i>et al.</i> , 1978)
pAN7	<i>pyrE⁺ uncB⁺E⁺F⁺H⁺A⁺G⁺D409 C⁺ argH⁺ ilv⁺</i>	F-plasmid carrying <i>uncD409</i> allele (Cox <i>et al.</i> , 1978)

DNA sequencing

Nucleotide sequences were determined by the method of Maxam & Gilbert (1980). End-labelling of DNA was carried out by using DNA polymerase and deoxyadenosine [α - 32 P]triphosphate (Maxam & Gilbert, 1980).

Media and growth of organisms

The mineral-salts minimal medium used and additions were as described previously (Gibson *et al.*, 1977b). Cells for the preparation of membranes were grown in 14-litre fermenters essentially as described previously (Cox *et al.*, 1970). The medium in the fermenter vessels was supplemented with 5% (v/v) Luria broth (Luria & Burrous, 1957). Growth yields were measured as described previously (Cox *et al.*, 1970). Turbidities of cultures were measured with a Klett-Summer son colorimeter.

Preparation of cell membranes

The preparation and treatment of membranes were as described previously (Cox *et al.*, 1983).

ATPase activity and DCCD inhibition

ATPase activity was measured by using the method described by Gibson *et al.* (1977a). DCCD-sensitivity of the ATPase activity was determined as described by Cox *et al.* (1983).

Atebrin-fluorescence quenching

Atebrin-fluorescence quenching was measured as described by Gibson *et al.* (1977a).

Oxidative phosphorylation

Oxidative phosphorylation was measured as described by Cox *et al.* (1973).

Protein determination

Protein concentrations were determined by using Folin's phenol reagent (Lowry *et al.*, 1951), with bovine serum albumin (fraction V, Sigma) being employed as standard.

Results

Cloning and sequencing of the uncF476 allele

DNA was prepared from the partial-diploid strain AN1882 (*uncF476/uncF476*) and digested with the restriction endonuclease *Hind*III. The DNA preparation was mixed with *Hind*III-digested pACYC184-plasmid DNA and the mixture treated with virus- T_4 DNA ligase. Strain AN727 (*uncB402, recA*) was then transformed with the ligated-DNA mixture and selection made for growth on succinate/chloramphenicol minimal medium. Colonies were screened for plasmids, which, on the basis of size and restriction-enzyme analysis, were similar to the plasmid pAN51

Fig. 1. Restriction fragments from plasmids used for DNA sequencing

Arrows indicate the direction and approximate length of the DNA fragments sequenced from the *Sau*3A or *Hinf*I restriction sites. The restriction enzymes *Pvu*II, *Rsa*I and *Alu*I were used to cleave restriction fragments labelled at both ends with [32 P] P_i . The location of the DNA segment coding for the *b*-subunit is indicated.

(*uncB⁺E⁺F⁺H⁺A⁺*) (Downie *et al.*, 1980). One such colony was purified (strain AN1977) and the plasmid carried by this strain was designated pAN181.

The DNA sequence of the *uncF476* allele was determined by using the strategy outlined in Fig. 1 and compared with the DNA sequence of the *uncF* gene present on plasmid pAN51. The DNA sequence of the *uncF476* allele differed from normal in that a G→A base change occurred at nucleotide 26, resulting in glycine being replaced by aspartic acid at position 9 of the *b*-subunit (see Fig. 2).

Self-complementation of the *uncF476* allele

Strain AN1521 (*uncF476, recA*) was transformed with plasmid pAN181 (*uncB⁺E⁺F476H⁺A⁺*) and selection made for growth on rich medium containing chloramphenicol. Twelve colonies were tested for growth on succinate/minimal medium containing chloramphenicol, and all colonies were able to grow on this medium. One strain (AN1989) was selected for further study.

The plasmid pAN181 was mixed with plasmid pAN36 (*uncD⁺C⁺*) (Downie *et al.*, 1980), and the mixture digested with the restriction endonuclease *Hind*III. The mixture was then treated with virus-*T₄* DNA ligase and used to transform strain AN1570 (*uncG428, recA*) selecting for growth on succinate/chloramphenicol-containing medium. A

number of colonies were screened for plasmids corresponding in size to the plasmid pAN45 (*uncB⁺E⁺F⁺H⁺A⁺G⁺D⁺C⁺*) (Downie *et al.*, 1980). Such a plasmid (pAN199) was used to transform strain AN888 (*unc-416::MuB⁺EFHAGDC*) and selection made for growth on rich medium containing chloramphenicol. Twelve colonies were tested for growth on succinate/minimal medium containing chloramphenicol and all colonies were able to grow on this medium. One colony was purified (strain AN2050) and retained for further study.

Membranes were prepared from strains AN1989 (pAN181/*uncF476*) and AN2050 (pAN199/*unc-416::MuB⁺EFHAGDC*), and ATPase activities, oxidative-phosphorylation activities and fluorescence-quenching activities examined (Table 2 and Fig. 3). Each of these activities was only about 20–30% of those obtained with normal membranes (Table 2), but it is clear that some assembly of the *F₁F₀*-ATPase has occurred in response to the increased dosage of the *uncF476* allele. The ATPase activities, however, were insensitive to the inhibitor DCCD (see Fig. 4).

Isolation of revertant strains from strain AN1515 (*uncF476*) and their membrane properties

Cells of strain AN1515 (*uncF476*) were spread on solid succinate/minimal medium supplemented with 0.05% casamino acids and incubated at 37°C.

Fig. 2. Nucleotide sequence of the DNA coding for the *b*-subunit of the *F₁F₀*-ATPase of *E. coli* together with the corresponding amino acid sequence

The base change and the corresponding amino acid change due to the *uncF476* allele are indicated.

Table 2. *ATPase and oxidative phosphorylation activities of membranes from various strains of E. coli*
 ATPase activities were determined on membrane preparations as described previously (Gibson *et al.*, 1977a). Oxygen uptake by the membrane preparations was measured by using an oxygen electrode. ATP formed from ADP in the presence of [32 P]P_i was trapped as glucose 6-phosphate. The reaction was stopped by the addition of trichloroacetic acid, and P_i was extracted after the addition of molybdate. For further details of strains, see Table 1.

Strain	Activity			P/O
	ATPase (μ mol/min per mg of protein)	NADH oxidase (ng-atoms of O/min per mg of protein)	ATP formation (nmol/min per mg of protein)	
AN1515 (<i>uncF476</i>)	0	178	<0.1	0
AN1989 (pAN181, <i>uncF476</i>)	0.29	72	3.9	0.05
AN2050 (pAN199, <i>uncF476</i>)	0.27	85	2.8	0.03
AN2030 (<i>unc</i> ⁺)	1.5	109	16.2	0.15
AN1953	0.7	89	10.3	0.12

Fig. 3. *Atebrin-fluorescence quenching in membranes prepared from various strains of E. coli*
 Atebrin-fluorescence quenching was measured as described previously (Gibson *et al.*, 1977a). Atebrin was added to give a final concentration of 4 μ M, NADH to 2 mM, NaCN to 2.5 mM, ATP to 1 mM and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) to 2 μ M. (a) Membranes from strain AN1515 (*uncF476*); (b) stripped membranes from strain AN1515; (c) membranes from strain AN1989 (pAN181, *uncF476/uncF476*); (d) stripped membranes from strain AN1989; (e) membranes from strain AN2030 (*unc*⁺); (f) stripped membranes from strain AN2030; (g) membranes from the partial-revertant strain AN1953 (*uncF476*); (h) stripped membranes from the partial-revertant strain AN1953 (*uncF476*).

Fig. 4. Inhibition of ATPase activity by DCCD. Membranes (0.3 mg of protein) were incubated at 30°C in 5 ml of the ATPase assay mixture together with the indicated amount of DCCD. The mixture was sampled at intervals, and the rate determined for each DCCD concentration and the percentage inhibition calculated. ●, Membranes from strain AN2030 (*unc*⁺); ■, membranes from the partial-revertant strain AN1953 (*uncF476*); ▼, membranes from strain AN1989 (pAN181, *uncF476/uncF476*).

After several days, revertant colonies were subcultured and growth yields on limiting concentrations of glucose determined. Two strains selected for further study were strains AN2030, which had a normal growth yield of 210 [measured as turbidity in Klett units after growth in 5 mM-glucose/mineral salts medium supplemented with 5% (v/v) Luria broth], and AN1953, which had a growth yield of 190. Strain AN1953 was therefore a partial-revertant strain with a growth yield less than the value obtained for a normal strain but considerably greater than the value of 140 obtained for the mutant strain AN1515 (*uncF476*).

ATPase, atebirin-fluorescence quenching and oxidative-phosphorylation activities of membranes prepared from strain AN1515 (*uncF476*) and revertant strains AN2030 and AN1953 were examined (Table 2 and Fig. 3). The properties of the membranes from strain AN2030 (Table 2 and Fig. 3) were normal (Fimmel *et al.*, 1983), confirming that this strain was a full revertant. Membranes from the partial-revertant strain AN1953 were capable of carrying out oxidative phosphorylation, giving a P/O ratio of about 80% of that obtained for the full-revertant strain AN2030 (*unc*⁺).

The AN1953 membranes also had low ATP-dependent atebirin-fluorescence-quenching activity but, unlike membranes from strain AN2030 (*unc*⁺), retained the NADH-dependent atebirin-fluorescence-quenching activity after removal of the F₁-ATPase (Fig. 3). A further difference in the properties of the membranes from strains AN1953 and AN2030 (*unc*⁺) may be found in the sensitivities of the respective ATPase activities to the inhibitor DCCD (Fig. 4). The ATPase activity of the membranes from strain AN1953 was not inhibited by DCCD.

DNA sequences of the *uncF* alleles present in strains AN2030 and AN1953

The *Hind*III-restriction-endonuclease fragments covering the *uncBEFHA* genes from strains AN2030 (*unc*⁺) and AN1953 were cloned into the vector pACYC184 by using the procedure described above for the cloning of the *uncF476* allele. The DNA sequences of the *uncF* alleles in plasmids pAN188 (derived from the partial-revertant strain AN1953) and pAN205 (derived from the full-revertant strain AN2030) were determined by using the sequencing strategy outlined in Fig. 1. The DNA sequence of the *uncF* allele present in strain AN2030 was identical with that obtained for the *uncF* gene on plasmid pAN51 (see Fig. 2). The DNA sequence of the *uncF* allele present in strain AN1953 was identical with the DNA sequence of the *uncF476* allele (see Fig. 2). The appropriate sections of the sequencing gels from plasmids pAN188 and pAN205 are compared in Fig. 5. The second mutation in strain AN1953 causing the partial reversion must therefore be in a gene other than the *uncF* gene.

Complementation between the *unc* alleles present in strain AN1953 and mutations in each gene of the *unc* operon

The *unc* alleles present in strain AN1953 were incorporated into an F-plasmid by the method described previously (Gibson *et al.*, 1977b). The partial-diploid strain (AN1983) carrying the AN1953 *unc* alleles on the F-plasmid (pAN182) was used as a donor strain in complementation tests with female strains carrying mutations in each of the genes of the *unc* operon, with the exception of the *uncI* gene. Complementation was concluded to have occurred when colonies were formed on the succinate medium in the region where the donor and recipient strains mixed in the cross-streaking test described previously (Gibson *et al.*, 1977a). The AN1953 *unc* alleles complemented mutations in the *uncBEFHGD* and *C* genes but not in the *uncA* gene, although in some tests very small colonies were observed after prolonged incubation. In order to confirm these observations, the F-

Fig. 5. Sections of DNA sequencing gels from plasmids pAN188 (derived from partial-revertant strain AN1953) and pAN205 (derived from full-revertant strain AN2030)

(a) Section of 20% (w/v)-polyacrylamide sequencing gel of *Hinf*I-restriction-endonuclease-generated fragment from plasmid pAN205 digested with the restriction endonuclease *Alu*I. (b) Section of 8% (w/v)-polyacrylamide sequencing gel of *Sau*3A-restriction-endonuclease-generated fragment from plasmid pAN188 digested with *Pvu*II.

Table 3. ATPase and atebrin-fluorescence-quenching activities of various partial-diploid strains of *E. coli*
ATPase activities were determined on membrane preparations as described previously (Gibson *et al.*, 1977a). Fluorescence-quenching activities were determined as described in the legend to Fig. 4. The mutant allele appearing first in the parentheses after the strain numbers is present on the F-plasmid and the second mutant alleles are present on the chromosome. The *uncF*476 PR refers to the *uncF*476 allele in the partial-revertant background of strain AN1953.

Strain	ATPase activity (nmol/min per mg of protein)	ATP-dependent atebrin- fluorescence-quenching activity (%)
AN2021 (<i>uncA</i> 401/ <i>uncF</i> 476 PR)	0.6	39
AN2022 (<i>uncA</i> 401/ <i>uncF</i> 476)	1.2	75
AN2059 (<i>uncD</i> 409/ <i>uncF</i> 476 PR)	1.2	52
AN2060 (<i>uncD</i> 409/ <i>uncF</i> 476)	1.0	48

plasmid pAN2 (*uncA401*) was transferred into strains AN2023 (*recA* derivative of AN1953) and AN1521 (*uncF476*, *recA*), forming strains AN2021 and AN2022 respectively. The F-plasmid pAN7 (*uncD409*) was also transferred into strains AN2023 and AN1521, forming strains AN2059 and AN2060 respectively. Membranes were prepared from each of the four strains, and ATPase activities and fluorescence-quenching activities examined (Table 3). Complementation between the *uncA401* allele and those alleles present in the partial-revertant strain AN1953 was poor compared with that between the *uncA401* and *uncF476* alleles. Complementation between the *uncD409* allele and those alleles present in strain AN1953 was even slightly better than that obtained between the *uncD409* allele and the *uncF476* allele, confirming that the poor complementation between the *uncA* mutant allele and the alleles present in the partial-revertant strain AN1953 was a specific effect (Table 3).

Discussion

A pathway for the assembly of the F_1F_0 -ATPase into the cell membrane has been proposed (Cox *et al.*, 1981) in which α - and β -subunits are bound to the membrane before the assembly of the *b*-subunit. Decker *et al.* (1982) obtained evidence for the association of the *b*-subunit with membranes, after formation of the *b*-subunit in an 'in vitro' transcription-translation system, which appeared to be independent of other ATPase subunits. However, further experimentation indicated that both the α - and β -subunits of the F_1 -ATPase were required for the assembly *in vivo* of the *b*-subunit into the F_0 portion of the ATPase (Klionsky *et al.*, 1983). Friedl *et al.* (1983) also studied the integration of the *b*-subunit into the membrane by using various mutant strains and plasmid-bearing strains and, although they had some problems with proteolysis, reported data consistent with the assembly pathway previously reported by Cox *et al.* (1981). However, Friedl *et al.* (1983) also obtained some evidence for the membrane association of the *b*-subunit in the absence of other subunits. Whether or not the membrane-associated *b*-subunit, present in strains with high-copy-number plasmids carrying a normal *uncF* gene (Friedl *et al.*, 1983), is meaningful, in terms of the normal assembly of the F_1F_0 -ATPase, remains to be determined.

The present data provide support for the proposition that the assembly of the *b*-subunit into the membrane requires the presence of subunits of the F_1 -ATPase. The mutant *b*-subunit formed from the *uncF476* allele has an aspartic acid residue rather than a glycine residue at position 9. This change increases the number of charged residues

that are located in the hydrophobic *N*-terminal region of the protein from one to two. It is this region of the protein that is most likely to interact with the hydrophobic transmembrane helices of the *a*- and *c*-subunits (Walker *et al.*, 1982; Senior, 1983). According to the calculations of Engelman & Steitz (1981), two charged amino acids should not present a significant thermodynamic barrier to the spontaneous insertion of the α -helical *N*-terminal region of the *b*-subunit into the membrane. That a thermodynamic barrier to assembly does not exist is supported by the observation that increasing the mutant gene dosage, which presumably increases the amount of the mutant *b*-subunit in the cell, allows some assembly to occur. It seems likely, therefore, that it is the interaction of the mutant *b*-subunit with another subunit of the ATPase that is affected in strains carrying the *uncF476* allele, thus preventing assembly of the mutant *b*-subunit. It is suggested, on the basis of the complementation data, that the second mutation in strain AN1953 is in the *uncA* gene, and that it is the interaction between the mutant *b*-subunit and the α -subunit that is affected in strains carrying the *uncF476* allele.

The properties of the membranes from the haploid strain AN1953 are also of considerable interest. The oxidative phosphorylation obtained with membrane preparations from strain AN1953, relative to the ATPase activity, was greater than that obtained with membranes from the full-revertant strain AN2030. However, the membranes remained proton-impermeable on removal of the F_1 -ATPase. In addition, the membrane-bound ATPase activity of membranes from strain AN1953 was insensitive to the inhibitor DCCD, although oxidative phosphorylation and ATP-dependent atebirin-fluorescence quenching remained DCCD-sensitive (G. Cox, unpublished work). These properties are similar to those of membranes prepared from strains carrying multiple copies of the *uncE408* or *uncE463* alleles (Cox *et al.*, 1983) in that oxidative phosphorylation occurred in apparently proton-impermeable membranes and the ATPase activity was insensitive to DCCD.

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References

- Bachmann, B. J. (1983) *Microbiol. Rev.* **47**, 180-230
- Butlin, J. D., Cox, G. B. & Gibson, F. (1973) *Biochim. Biophys. Acta* **292**, 366-375
- Chang, A. C. Y. & Cohen, S. N. (1978) *J. Bacteriol.* **135**, 1141-1156
- Cox, G. B., Newton, N. A., Gibson, F., Snoswell, A. M. & Hamilton, J. A. (1970) *Biochem. J.* **117**, 551-562

- Cox, G. B., Gibson, F. & McCann, L. (1973) *Biochem. J.* **134**, 1015-1021
- Cox, G. B., Downie, J. A., Gibson, F. & Radik, J. (1978) *Biochem. J.* **170**, 593-598
- Cox, G. B., Downie, J. A., Langman, L., Senior, A. E., Ash, G., Fayle, D. R. H. & Gibson, F. (1981) *J. Bacteriol.* **148**, 30-42
- Cox, G. B., Jans, D. A., Gibson, F., Langman, L., Senior, A. E. & Fimmel, A. L. (1983) *Biochem. J.* **216**, 143-150
- Decker, K. P., Brusilow, W. S. A., Gunsalus, R. P. & Simoni, R. D. (1982) *J. Bacteriol.* **152**, 815-821
- Downie, J. A., Langman, L., Cox, G. B., Yanofsky, C. & Gibson, F. (1980) *J. Bacteriol.* **143**, 8-17
- Downie, J. A., Cox, G. B., Langman, L., Ash, G. R., Becker, M. & Gibson, F. (1981) *J. Bacteriol.* **145**, 200-210
- Engelman, D. M. & Steitz, T. A. (1981) *Cell (Cambridge, Mass.)* **23**, 411-422
- Fimmel, A. L., Jans, D. A., Langman, L., James, L. B., Ash, G. R., Downie, J. A., Senior, A. E., Gibson, F. & Cox, G. B. (1983) *Biochem. J.* **213**, 451-458
- Friedl, P., Hoppe, J., Gunsalus, R. P., Michelson, O., von Meyenburg, K. & Schairer, H. V. (1983) *EMBO J.* **2**, 99-103
- Gay, N. J. & Walker, J. E. (1981) *Nucleic Acids Res.* **9**, 3919-3926
- Gibson, F., Cox, G. B., Downie, J. A. & Radik, J. (1977a) *Biochem. J.* **162**, 665-670
- Gibson, F., Cox, G. B., Downie, J. A. & Radik, J. (1977b) *Biochem. J.* **164**, 193-198
- Gibson, F., Downie, J. A., Cox, G. B. & Radik, J. (1978) *J. Bacteriol.* **134**, 728-736
- Gunsalus, R. P., Brusilow, W. S. A. & Simoni, R. D. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 320-324
- Jans, D. A., Fimmel, A. L., Langman, L., James, L. B., Downie, J. A., Senior, A. E., Ash, G. R., Gibson, F. & Cox, G. B. (1983) *Biochem. J.* **211**, 717-726
- Klionsky, D. J., Brusilow, W. S. A. & Simoni, R. D. (1983) *J. Biol. Chem.* **258**, 10136-10143
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Luria, S. E. & Burrous, J. W. (1957) *J. Bacteriol.* **74**, 461-476
- Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499-560
- Selker, E., Brown, K. & Yanofsky, C. (1977) *J. Bacteriol.* **129**, 388-394
- Senior, A. E. (1983) *Biochim. Biophys. Acta* **726**, 81-96
- Walker, J. E., Saraste, M. & Gay, N. J. (1982) *Nature (London)* **298**, 867-869