JOURNAL OF BACTERIOLOGY, Jan. 1983, p. 416–422
0021-9193/83/010416-07$02.00/0

Vol. 153, No. 1

Escherichia coli Mutants Defective in the *uncH* Gene

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Received 13 July 1982/Accepted 9 October 1982

Plasmids carrying cloned segments of the *unc* operon of *Escherichia coli* have been used in genetic complementation analyses to identify three independent mutants defective in the *uncH* gene, which codes for the δ subunit of the ATP synthetase. Mutations in other *unc* genes have also been mapped by this technique. ATPase activity was present in extracts of the *uncH* mutants, but the enzyme was not as tightly bound to the membrane as it was in the parental strain. ATP-dependent membrane energization was absent in membranes isolated from the *uncH* mutants and could not be restored by adding normal F_1 ATPase from the wild-type strain. F_1 ATPase prepared from *uncH* mutants could not restore ATP-dependent membrane energization when added to wild-type membranes depleted of F_1. Membranes of the *uncH* mutants were not rendered proton permeable as a result of washing with low-ionic-strength buffer.

The ATP synthetase (EC 3.6.1.3) of bacterial, mitochondrial, and chloroplast membranes is a reversible proton translocator which synthesizes ATP from ADP and inorganic phosphate by using the electrochemical gradient of protons generated by the electron transport chain. A peripheral portion (F_1) of the ATP synthetase can be separated from the membrane-bound portion (F_0) by washing membranes with low-ionic-strength buffer. The F_1 portion of the enzyme from *Escherichia coli* contains five types of polypeptides, designated α, β, γ, δ, and ε, with molecular weights of 55,300, 50,200, 31,400, 19,300, and 14,200, respectively (14, 31). F_0 has three types of subunits, *a*, *b*, and *c*, with molecular weights of 30,200, 17,200, and 8,500, respectively (14, 28). All of the known genes coding for the polypeptides of the complex are arranged in an operon (designated *unc* for uncoupled) located at 83.5 min on the *E. coli* genetic map (2). The structural genes, in order of transcription, with the subunits they code for in parentheses, are *uncB* (*a*), *uncE* (γ), *uncF* (*b*), *uncH* (δ), *uncA* (α), *uncG* (α), *uncD* (β), and *uncC* (ε) (8–11, 13–17, 22, 23, 28, 35).

Extensive genetic studies have been conducted, and mutants have been identified, for all the structural genes of the *unc* operon except *uncH* (9). Genetic and biochemical complementation of other *unc* mutants has been demonstrated with (i) F′ plasmids carrying normal or mutant *unc* alleles (6, 8, 11, 15, 16); (ii) specialized λ transducing phage carrying the entire *unc* operon (24) or portions of it (19); and (iii) an amplifiable plasmid carrying the *uncD* and *uncC* genes (7). Only the method with F′ factors carrying mutant *unc* alleles has been able to localize *unc* mutations to a single *unc* structural gene. A recent report from this laboratory (17) has described the cloning of various segments of *unc* operon DNA into plasmid vectors. These plasmids produce one or more *unc* polypeptides in an in vitro transcription-translation system (3). In this report, we have used these plasmids in genetic complementation analysis to determine the genes in which a number of *unc* mutations are located. In addition to mutants with defects in genes which have already been described, three mutants with defects in the *uncH* gene have been identified. Biochemical and genetic characterization of an *uncH* mutant is reported.

MATERIALS AND METHODS

Bacterial strains used in these experiments (Table 1) were derivatives of *E. coli* K-12. The plasmids used in this work are depicted in Fig. 1. Minimal medium containing glucose (0.5%) or potassium succinate (0.5%) has been described (32). LBG plates contained 1% tryptone, 0.5% yeast extract, 0.5% NaCl, 1% glucose, and 1.5% agar, pH 7.3. Luria broth contained 1% tryptone, 0.5% yeast extract, 0.05% NaCl, and 1% glucose, pH 7.3.

Plasmids carrying one or more *unc* genes were constructed by digestion of either λ *asn-5* (24) or *unc* plasmid DNA (17) with restriction endonucleases followed by insertion of the resulting DNA segments into amplifiable plasmid vectors (17). The *unc* gene product synthesis from *unc* plasmid templates was carried out either in vitro by a transcription-translation system (3) or in vivo by minicells (4). Transformation of *unc*

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TABLE 1. List of *E. coli* K-12 strains

Strain	Genotype	Source or reference
1100	*bglR thi-1 rel-1* HfrPO1	Laboratory stock
ER	*asnA31 asnB32 thi* F^+	18
RH75	*bglR*	Laboratory stock
RH50	*ilvA215 bglR thi-1 rel-1* HfrPO1	18
RH93	*uncE293*::Mu *c*(Ts) *bglR*	Insertion of *Mu* into RH75[a]
RH104	*uncA204 bglR thi-1 rel-1* HfrPO1	Phage mutagenesis[b]
RH139	*uncH239 bglR thi-1 rel-1* HfrPO1	Neomycin selection[b]
RH141	*uncH241 bglR thi-1 rel-1* HfrPO1	Phage mutagenesis[b]
RH142	*uncH242 bglR thi-1 rel-1* HfrPO1	Phage mutagenesis[b]
RH301	*uncF201 recA56 srl*::Tn*10 bglR thi-1 rel-1* HfrPO1	Derived from RH101[b,c]
RH304	*uncA204 recA56 srl*::Tn*10 bglR thi-1 rel-1* HfrPO1	Derived from RH104[c]
RH306	*uncB206 recA56 srl*::Tn*10 bglR thi-1 rel-1* HfrPO1	Derived from RH106[b,c]
RH339	*uncH239 recA56 srl*::Tn*10 bglR thi-1 rel-1* HfrPO1	Derived from RH139[c]
RH341	*uncH241 recA56 srl*::Tn*10 bglR thi-1 rel-1* HfrPO1	Derived from RH141[c]
RH342	*uncH242 recA56 srl*::Tn*10 bglR thi-1 rel-1* HfrPO1	Derived from RH142[c]
RH343	*uncG243 recA56 srl*::Tn*10 bglR thi-1 rel-1* HfrPO1	Derived from RH143[b,c]
RH344	*uncD244 recA56 srl*::Tn*10 bglR thi-1 rel-1* HfrPO1	Derived from RH144[b,c]
RH393	*uncE293*::Mu *c*(Ts) *recA56 bglR*	Derived from RH93[c]

[a] *unc* mutants form small bright red colonies on ribose MacConkey indicator plates in contrast to unc^+ strains, which form large pink colonies. Strain RH75 was mutagenized with bacteriophage Mu *c*(Ts) and plated on ribose MacConkey plates without selection for *unc*. Strain RH93 appeared as a small bright red colony which was found to be *unc* and temperature sensitive for growth at 42°C owing to the insertion of the Mu *c*(Ts) prophage. All P1 transductants to unc^+ were found to be temperature resistant, indicating loss of the prophage.

[b] The *uncF201*, *uncA204*, and *uncD244* mutations were isolated in a derivative of strain 1100 by phage mutagenesis (33). The *uncH241*, *uncH242*, and *uncG243* mutations were isolated in strain ER by phage mutagenesis. The *uncH239* mutation was derived from strain 1100 by neomycin selection (25). All *unc* mutations except the *uncE293*::Mu *c*(Ts) mutation were transferred out of the original isolate into strain RH50 by P1 transduction. The *uncB206* mutation in strain RH306 is the same as that present in strain BG31 (33).

[c] Tetracycline-resistant transductants were screened for UV sensitivity, indicating presence of the *recA56* mutation.

mutants with indicated plasmid DNA was performed as described (18). Transformants were selected on LBG plates containing ampicillin (20 μg/ml), kanamycin (20 μg/ml), or chloramphenicol (25 μg/ml). Colonies were transferred to LBG antibiotic plates and were replicated after 1 day to minimal glucose and minimal succinate plates minus antibiotics. Transformed cells and control (untransformed) cells were scored for growth on succinate medium periodically from 2 to 6 days after transfer.

Cells used for the preparation of extracts and membranes were grown to 100 to 150 Klett units of turbidity (red filter) in Luria broth, harvested, and washed with 50 mM Tris-hydrochloride buffer, pH 7.5, containing 10 mM $MgSO_4$. The cells were then suspended in the same buffer to 1 g of cells per 4 ml of buffer and were broken by passage through a French pressure cell at 12,000 to 20,000 lb/in^2. Cellular debris was removed by centrifugation for 5 min at 4,000 rpm to produce a crude extract. Membranes were sedimented by centrifugation at 100,000 × *g* for 1 h and were washed once in the same buffer. The supernatant fraction remaining after the centrifugation of the crude extract is termed soluble extract. Membranes were suspended in a low-ionic-strength buffer containing 1 mM Tris-chloride buffer (pH 7.5), 0.5 mM EDTA, and 10% glycerol. They were next incubated for 1 h at room temperature and sedimented at 100,000 × *g* for 1 h to remove F_1 from the membrane. The pellet which contained membranes depleted of the F_1 portion of the ATP synthetase was suspended in 2 ml of buffer per initial g of cells to give low-ionic-strength washed membranes. For fluorescence quenching experiments, membranes were washed twice with low-ionic-strength buffer. For experiments examining the ability of F_1 to restore ATP-dependent membrane energization, a crude F_1 preparation was made by concentrating high- and low-ionic-strength wash from parental or mutant strains 20- to 50-fold by ultrafiltration.

Assays of ATPase (33) and of protein with bovine serum albumin used as a standard (26) have been described. Energy-dependent fluorescence quenching with 9-amino-6-chloro-2-methoxyacridine (ACMA) was as described (30). Purification of F_1 was as described (36), except that 20 mM benzamidine was used in initial washes to retain increased amounts of F_1 on the membrane.

RESULTS

Gibson, Cox, and co-workers (6–11, 15, 16) have characterized mutants of the ATP synthetase which are defective in each subunit of the complex except the δ subunit. The phenotype and physiological consequences of mutations in the δ subunit are of interest to further our understanding of the role of this subunit in the function of the ATP synthetase complex.

Isolation of *unc* mutants. Several *unc* mutants were independently isolated (Table 1), some by neomycin selection (25), some with mutagenized lysates of bacteriophage P1 (33). One additional mutant was obtained by inserting bacteriophage

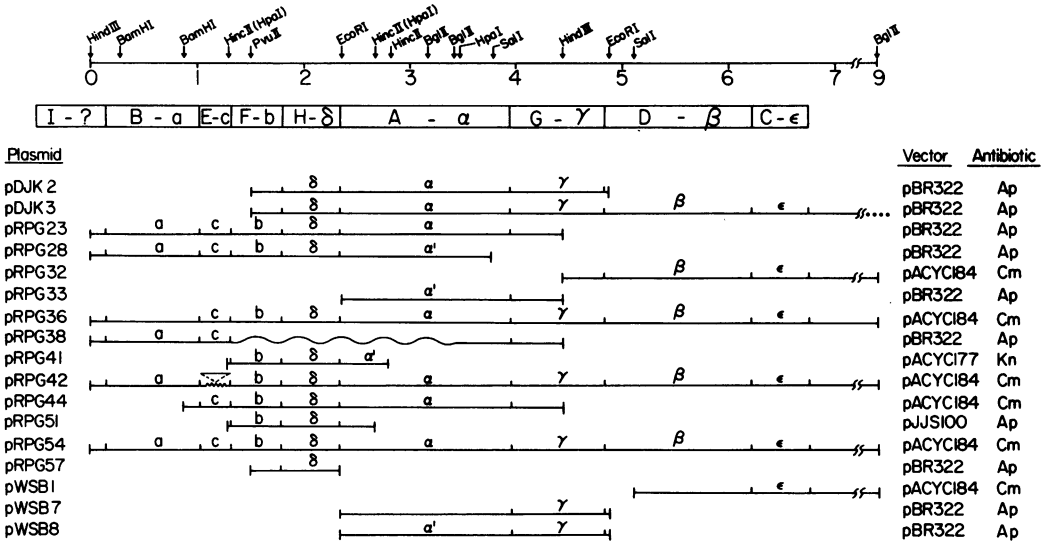

FIG. 1. Restriction map of the *unc* operon with plasmids used to determine the sites of *unc* mutations. The top line shows the locations of the restriction endonuclease recognition sites, with distances given in kilobases. The locations of the *unc* genes, the gene designations, and the polypeptides that each gene codes for are shown below the line. The start of each gene was determined from the DNA sequence (13, 14, 22, 23, 27, 31). The location of the open reading frame called gene I is included, although no polypeptide or function has yet been associated with this region (14). The promoter region for the operon is at the left of the figure. Regions of the *unc* operon present on the various plasmids are indicated. The plasmid designations are listed on the left; the vector used for each plasmid and the antibiotic resistance encoded are listed on the right. The ends of the lines indicate the restriction sites within the operon used to clone each region. The individual polypeptides coded for by each region and the limits of the genes for those polypeptides are indicated, as are the translation products of incomplete *uncA* genes (α'). Plasmid pRPG38 contains a deletion indicated by the wavy line. Plasmids were constructed by standard methods (16). Plasmids pRPG36 and pRPG42 were obtained from the same cloning which produced pRPG54. Plasmid pRPG36 contains the *uncB* gene but does not express it, and pRPG42 contains a 0.8-kilobase insertion in *uncE*, the exact location of which has not been determined.

Mu *c*(Ts) (5). None was able to grow on succinate, malate, or acetate as the sole carbon source. All had reduced growth yields with glucose as a carbon source and were 40 to 60% cotransducible with the *ilvC* locus in experiments performed with bacteriophage P1.

Plasmids containing portions of the *unc* operon. We have reported the construction of plasmids containing various segments of *unc* DNA derived from the specialized transducing phage λ *asn-5* (17). These plasmids contain well-defined portions of the *unc* operon and were identified by their ability to produce individual *unc* gene products in vitro rather than by their ability to complement *unc* mutants (17). The amount of *unc* operon DNA present in each plasmid and the *unc* gene products produced by each plasmid are indicated in Fig. 1. In some cases, plasmids contain portions of adjacent *unc* genes which may be expressed to give hybrid proteins encoded by fused vector and *unc* sequences. Any of several partial α proteins detected in S-30 incubations is designated α'.

Complementation of *unc* mutants with *unc* plasmids. Transformation of *unc recA*$^+$ and *unc recA* strains was performed with a number of the plasmids shown in Fig. 1. Good growth of most *unc* mutant strains complemented by *unc* plasmids was observed after 2 days of incubation on minimal succinate medium at 37°C. Strains not complemented by a plasmid did not grow on minimal succinate medium. Knowledge of the *unc* gene products produced by each plasmid allowed assignment of each *unc* mutation to a single *unc* gene. Mutations in genes other than *uncH* have been reported previously, so we have not reported the characterization of these mutants in detail here. We have reported briefly the method by which mutations in seven of the eight *unc* structural genes have been mapped. All mutants tested were complemented by plasmid pRPG54 (*a*, *c*, *b*, δ, α, γ, β, ε), a plasmid which contains all eight ATP synthetase structural genes.

Mutations in the *uncH* gene. Strains RH139 (*uncH239*), RH141 (*uncH241*), and RH142 (*uncH242*) gave *unc*$^+$ transformants when transformed by plasmid pRPG23 (*a*, *c*, *b*, δ, α). No *unc*$^+$ transformants were obtained when these strains were transformed with plasmids pRPG32

(β, ε), pRPG33 (α'), or pRPG38 (*a*, *c*). Strains RH339 (*uncH239 recA*), RH341 (*uncH241 recA*), and RH342 (*uncH242 recA*) were complemented by plasmids pRPG57 (δ), pRPG41 (*b*, δ, α), pRPG51 (*b*, δ) and pDJK2 (δ, α, γ) but not by plasmids pRPG32 (β, ε), pRPG33 (α'), pRPG38 (*a*, *c*), or WSB7 (γ), indicating that the mutations in these strains are located in the *uncH* gene. Strain RH139 (*uncH239*) is characterized below.

Mutations in other *unc* genes. Strain RH301 (*uncF201 recA*) was found to possess a mutation in the *uncF* (subunit *b*) gene since it was complemented by plasmids pRPG41 (*b*, δ, α') and pPRG51 (*b*, δ) but not by pRPG57 (δ).

Strain RH104 (*uncA204*) was determined to be an *uncA* (subunit α) mutant. Recombinants which grew on minimal succinate medium were isolated from this strain after transformation with plasmids pWSB8 (α', γ), pRPG41 (*b*, δ, α'), pRPG33 (α'), pRPG28 (*a*, *c*, *b*, δ, α'), and pRPG23 (*a*, *c*, *b*, δ, α). The only region of *unc* operon DNA common to all of these plasmids is a promotor-proximal portion of the *uncA* gene; the mutation in strain RH104 (*uncA204*) must be located in this portion of the *uncA* gene. A *recA* derivative of this strain, RH304 (*uncA204 recA*), was complemented by plasmid pRPG36 (*c*, *b*, δ, α, γ, β, ε) or pDJK3 (δ, α, γ, β, ε). Surprisingly, strain RH304 (*uncA204 recA*) was not complemented by plasmid pRPG23 (*a*, *c*, *b*, δ, α) or pDJK2 (δ, α, γ), which produce subunit α in vitro as along with other ATP synthetase polypeptides. Strain RH304 (*uncA204 recA*) was not complemented by plasmids which produce only a partial α subunit in vitro or by other plasmids which do not produce α in vitro.

Strain RH306 (*uncB206 recA*) was found to be an *uncB* mutant since it was complemented by plasmids pRPG23 (*a*, *c*, *b*, δ, α), pRPG38 (*a*, *c*), and pPRG42 (*a*, *b*, δ, α, γ, β, ε) but not by pRPG36 (*c*, *b*, δ, α, γ, β, ε) or pRPG44 (*c*, *b*, δ, α).

We would predict that *uncC recA* (subunit ε) mutants would be the only mutant class to be complemented by plasmid pWSB1 (ε). No *uncC* mutants were detected among our *unc* strains. Strain RH344 (*uncD244 recA*) was complemented by plasmid pRPG32 (β, ε) but not by pWSB1 (ε) or other plasmids not producing subunit β in vitro. Thus, it is tentatively classified as an *uncD* mutant.

The Mu insertion in strain RH393 (*uncE293*:: Mu *c*(Ts) *recA*) was determined to be in the gene *uncE* (subunit *c*). Strain RH393 was able to grow on minimal succinate medium when transformed with plasmid pRPG36 (*c*, *b*, δ, α, γ, β, ε) but not when transformed with pRPG42 (*a*, *b*, δ, α, γ, β, ε), pRPG32 (β, ε), or pDJK3 (δ, α, γ, β, ε).

Strain RH343 (*uncG243 recA*) was complemented well by plasmid pDJK2 (δ, α, γ) and weakly by pWSB7 (γ) and thus appears to be an *uncG* mutant. Plasmid pWSB8 (α', γ) did not complement strain RH343.

ATPase activity of an *uncH* mutant. Results of ATPase assays from one of the three *uncH* mutants are described here; the other two *uncH* mutants had essentially identical physiological and biochemical phenotypes. Cell extracts and membranes were prepared, and the ATPase activity of the fractions was determined. The results of these experiments are shown in Table 2. Extracts of strain RH139 (*uncH239*) had approximately 70% as much ATPase activity as the parental strain, 1100. The amount of ATPase activity associated with the membrane fractions of strain RH139 (*uncH239*) was only 55% that of strain 1100. At every step in the fractionation procedure, the *uncH* mutant lost proportionately more membrane-bound ATPase activity than the parental strain, indicating that the F_1 ATPase is not as tightly attached to the membrane in *uncH* mutants as in the parental strain. Inclusion of 20 mM benzamidine in the buffer in which cells of strain RH139 (*uncH239*) were lysed and membranes were prepared did not increase the amount of ATPase activity attached to the membrane (data not shown). The ATPase activity of crude F_1 from strain RH139 (*uncH239*) did not differ in stability from that of the parental strain, 1100, when kept on ice for 10 h or when heated to 68°C for 10 or 30 min (data not shown).

The ATPase activity of an extract of strain RH93 was found to be only 2 to 3% that of strain 1100 (data not shown). This is presumably due to the strongly polar nature of the Mu insertion in this strain.

Reversion of the *uncH* mutant strain RH139.

TABLE 2. ATPase activity of cell fractions

Fraction	Strain 1100 (*unc*$^+$)		Strain RH139 (*uncH239*)	
	Activity[a]	Sp act[b]	Activity[a]	Sp act[b]
Crude extract	22.4	1.3	17.0	0.9
Soluble extract	7.6	0.7	12.6	1.2
Unwashed membranes	10.8	1.8	5.9	0.8
High-ionic-strength wash	1.4	1.5	3.1	1.9
Washed membranes	10.4	1.8	2.6	0.4
Low-ionic-strength wash 1	3.5	1.6	2.0	0.8
Low-ionic-strength wash 2	4.8	3.8	0.3	0.1
Low-ionic-strength washed membranes	1.3	0.6	0.2	0.1

[a] Micromoles of PO_4 released per minute per initial milliliter of extract.

[b] Micromoles of PO_4 released per minute per milligram of protein.

The *unc*$^+$ revertants of strain RH139 (*uncH239*) were selected by plating a cell suspension on minimal succinate plates and isolating colonies which appeared after a few days. When membranes were prepared from these revertants and ATPase activity was measured, the revertants were found to have normal amounts of membrane-bound ATPase activity (data not shown). The frequency and phenotype of these revertants suggests that the mutation in strain RH139 is a point mutation.

Energization of membranes from strain RH139. Assays of NADH- and ATP-dependent membrane energization were made with the fluorescent dye ACMA (Fig. 2). Membranes of wild-type strain 1100 showed typical quenching of ACMA fluorescence with NADH and ATP. The NADH-dependent fluorescence quenching was reduced, and the ATP-dependent fluorescence quenching was eliminated, in membranes of strain 1100 from which F_1 was removed by washing with low-ionic-strength buffer. Membranes of strains RH139 (*uncH239*) and RH93 (*uncE293*::Mu *c*(Ts)) demonstrated normal NADH-dependent fluorescence quenching but lacked ATP-dependent fluorescence quenching. Membranes from strain RH139 (*uncH239*) with F_1 ATPase activity removed by washing with low-ionic-strength buffer did not show the substantial decrease in NADH-dependent fluorescence quenching that is reproducibly observed with the parental strain, indicating that the membranes of this strain are not rendered proton permeable by low-ionic-strength washing. The addition of purified F_1 or crude F_1 from the parental strain to membranes from the parental strain depleted of F_1 restored ATP-dependent fluorescence quenching and increased NADH-

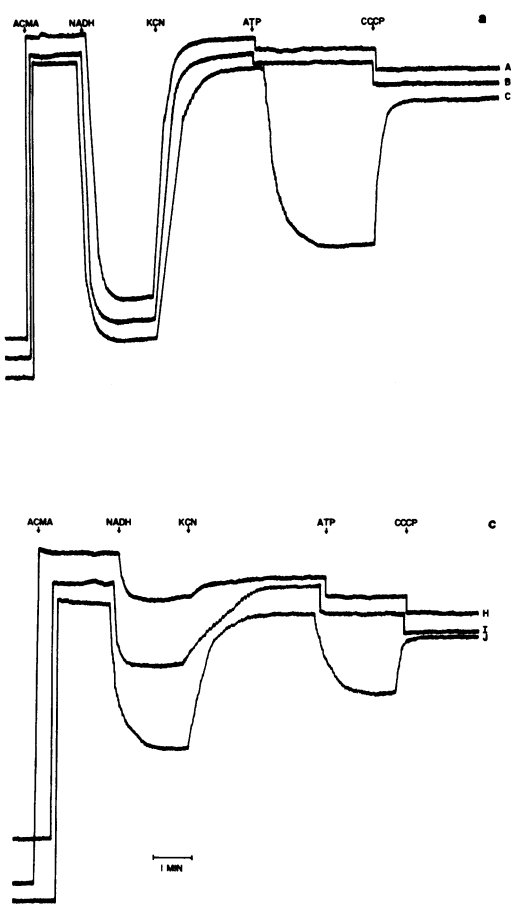

FIG. 2. Quenching of ACMA fluorescence by membranes from wild-type and mutant cells. Arrows mark the addition of (from left to right) 2 μM ACMA, 0.5 mM NADH, 0.5 mM KCN, 0.25 to 0.4 mM ATP, and 10 μM carbonyl cyanide-*m*-chlorophenyl hydrazone (CCCP). Traces represent membranes of: (Panel a) A, strain RH93 (*uncE293*::Mu *c*(Ts); B, RH139 (*uncH239*); C, strain 1100 (wild type); (Panel b) D, strain 1100 (membranes washed with low-ionic-strength buffer); E, RH139 (*uncH239*) (membranes washed with low-ionic-strength buffer); F, RH139 (*uncH239*) (membranes washed with low-ionic-strength-buffer and purified F_1 [80 μg of protein] added); G, strain 1100 (membranes washed with low-ionic-strength buffer and purified F_1 [80 μg of protein] added); (Panel c) H, strain 1100 (membranes washed with low-ionic-strength buffer); I, strain 1100 (membranes washed with low-ionic-strength buffer and crude F_1 [1.1 U of ATPase activity] from strain RH139 (*uncH239*) added); J, strain 1100 (membranes washed with low-ionic-strength buffer and crude F_1 [1.1 U of ATPase activity] from strain 1100 added). One unit of ATPase releases 1 μmol of PO_4 per min. In these experiments 0.7 mg of membrane protein was used for traces A and B, 0.6 mg for trace C, 1.1 mg for traces D and G–J, and 0.8 mg for traces E and F.

dependent fluorescence quenching. In contrast, no restoration of ATP-dependent fluorescence quenching occurred when purified F_1 from the parental strain was added to membranes of strain RH139 (*uncH239*). This result indicates that the membranes of strain RH139 (*uncH239*) do not bind F_1 productively. Crude F_1 from strain RH139 (*uncH239*) did not restore ATP-dependent fluorescence quenching when added to membranes of strain 1100 depleted of wild-type F_1, indicating that the F_1 of this strain is also defective.

DISCUSSION

Knowledge of the DNA sequence of the *unc* operon (13, 14, 23, 27, 28, 31) and of the restriction sites used to construct each plasmid allows a precise determination of the portion of any *unc* gene carried by any plasmid. According to the nucleotide sequences reported, which indicate an open reading frame preceding the *uncB* gene (14, 22, 28, 31), the *unc* promoter may not be present on any of the plasmids used in this study. The production of *unc* polypeptides from our plasmids which do not contain this region may be due to promoters on the plasmid vectors (17).

It has been shown that individual *unc* gene products produced by chromosomal and plasmid-located *unc* genes can assemble to give a functional ATP synthetase (6, 9, 15, 16; this report). We have extended these complementation studies at a more detailed level by using well-defined plasmids containing one or more of the individual *unc* genes. Transformation of any of several *recA unc* mutants with the appropriate antibiotic selection of plasmids yields results in a complementation test which can only be explained by locating the *unc* defects in a single *unc* gene.

The *unc* mutants we have studied were complemented readily by plasmids bearing a normal copy of the defective chromosomal *unc* gene in all but two instances. The *uncG* (subunit γ) mutants were only complemented weakly by plasmid pWSB7 (γ) but were complemented well by pDJK2 (δ, α, γ). The *uncG* mutants were not complemented by plasmid pWSB8 (α', γ). The *uncA* (subunit α) mutants were not complemented by plasmid pRPG23 (*a*, *c*, *b*, δ, α) or pDJK2 (δ, α, γ) but were complemented by pRPG36 (*c*, *b*, δ, α, γ, β, ε) and pDJK3 (δ, α, γ, β, ε). The reasons for poor complementation or lack of complementation of *uncG* and *uncA* mutants in these instances is unknown, but it could include regulatory effects, degradation of unassembled subunits, formation of inactive hybrid ATP synthetase containing both mutant and wild-type subunits, an abortive assembly event, or an insufficient level of *unc* polypeptide synthesis to allow normal function.

Screening of our collection of *unc* mutants by the complementation procedure we have described has revealed mutants defective in the *uncH* (subunit δ) gene, a class of mutants which has not previously been described. The F_1 portion of the ATP synthetase of *uncH* mutants is active in the ATPase reaction but does not mediate membrane energization. F_1 is less strongly attached to the membrane in *uncH* mutants than in the parental strain. Membranes of *uncH* mutants depleted of ATPase activity do not bind wild-type F_1 productively. Purification of F_1 lacking the δ subunit has been reported (20, 29). These preparations do not restore energy-linked functions to wild-type membranes (12); preparations containing the δ subunit are able to restore energy-linked functions to membranes. Binding of δ-deficient F_1 to membranes was observed in some cases, depending on which wild-type strain was used for the preparation of F_1 and membranes (12). Studies of the reassembly of *E. coli* F_1 polypeptides in vitro indicate that δ functions to attach F_1 to F_0 (34). F_1 from chloroplast thylakoids can bind to F_0 when the δ subunit has been removed, but energy-linked functions are not restored (1). Addition of purified δ to F_1 which has been depleted of δ restores proton accumulation to chloroplast membranes (1). We have not determined whether the δ subunit produced by *uncH* mutants, if any, is associated with F_1 released from the membrane or whether it remains attached to F_0. The lack of proton permeability of membranes of *uncH* mutants washed with low-ionic-strength buffer and the apparent defects in both F_1 and F_0 suggest that the mutant δ subunit remains attached to F_0. This mutant δ subunit may be partially active in the attachment of F_1 to F_0 but inactive in energy transduction. It has recently been suggested that assembly of the *uncF* gene product is defective in a mutant with a polar *uncD* mutation (7). It is also possible that the assembly of F_0 requires the presence of a normal δ subunit. Experiments are under way to examine these possibilities.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant GM18539 from the National Institutes of Health. W.S.A.B. was supported by postdoctoral fellowship GM07598, and D.J.K. is a predoctoral trainee supported by grant GM07276, both from the National Institutes of Health.

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