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ISOLATION AND PROPERTIES OF *ESCHERICHIA COLI* ATPase MUTANTS WITH ALTERED DIVALENT METAL SPECIFICITY FOR ATP HYDROLYSIS

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SUMMARY

A method was devised for isolation of large numbers of energy-transducing ATPase (coupling factor) mutants based on a modification of the procedure of Hong and Ames (Hong, J. and Ames, B. N. (1971) Proc. Natl. Acad. Sci. U.S. 68, 3158–3162) for localized mutagenesis of any small region of the bacterial chromosome using transducing phages. The principle of this procedure is to mutate P1-transducing phage particles carrying the ATPase genes (Unc (uncoupled) DNA) using the strong chemical mutagen hydroxylamine. By transducing *ilv*$^-$ auxotrophs, a marker closely linked to Unc, to prototrophs, mutated Unc DNA can be introduced into the chromosome. We have used this method in conjunction with suitable selection procedures to isolate about 90 Unc$^-$ strains which have been classified by physiological, genetic, and biochemical criteria into three different phenotypes (Unc A, B, D). Mutants of the Unc D phenotype which were studied in detail were found to have the following properties: (1) aerobic growth yields on glucose are considerably lower than the wild type; growth occurs on glucose under anaerobic conditions; (2) Unc D lesions map near the *ilv* operon; (3) O_2 uptake is comparable to the rate of wild type; (4) vesicles catalyze respiratory-dependent transhydrogenation, but show very low levels of Ca^{2+} ATP-dependent transhydrogenation; Mg^{2+} is ineffective; (5) oxidative phosphorylation is almost completely blocked irrespective of which metal ion is used; (6) the specific activity of ATPase is only about 20 % of the wild type; (7) purified ATPase was found to have a marked specificity for Ca^{2+} as a divalent metal for ATP hydrolysis. A summary of properties of the new Unc mutants is discussed.

INTRODUCTION

Recent progress on the biochemical-genetic properties of energy-transducing ATPase (coupling factor, Unc) mutants of *Escherichia coli* has been reviewed by Cox and Gibson [1]; also a summary of experiments using oxidative phosphorylation

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mutants of yeast has appeared [2]. Gibson and co-workers [3, 4] have described types of Unc (uncoupled) mutants called Unc A and B. Unc A mutants produce little if any ATPase hydrolytic activity while Unc B strains synthesize ATPase hydrolytic activity but are unable to catalyze oxidative phosphorylation. It is clear from this work that mutants of the coupling factor are becoming a valuable tool for studies of the mechanism of action, mode of assembly and insertion into the membrane, and regulation of this crucial, membrane-bound enzyme. The availability of a wide spectrum of different mutant phenotypes is essential for these studies. The approach described in this paper involves the saturation of the ATPase genes localized near the *ilv* operon on the *E. coli* genetic map with mutations in order to generate a variety of mutant types suitable for study of enzyme function and to generate eventually a fine-structure map of the Unc region.

MATERIALS AND METHODS

Bacteriological. All mutants were derived from *E. coli* K12 (3110). The growth and handling of Unc strains were as described previously [6]. The generalized transducing phage P1 was used for mutagenesis and transduction [5]. Transduction was carried out according to the procedure of Miller [7].

Mutagenesis of the Transducing Phage. Phage P1 was grown on wild-type strain *E. coli* K12 at 37 °C with shaking in a medium composed of L-broth supplemented with 0.2 % glucose and 0.005 M $CaCl_2$. The phage particles, a mixture of phage and bacterial DNA in phage heads, usually had a titer of 2–3 $\times 10^{10}$/ml. The crude lysate (60 ml), made to 0.5 M with NaCl and 7 % with polyethyleneglycol, was incubated at 0°C for 1 h for phage separation followed by centrifugation at 15 000 $\times g$ for 20 min to pellet the phage. This pellet was resuspended in 3 ml of physiological saline. Using this procedure, about 50 % of the starting phage was recovered. For mutagenesis, 1 part of concentrated phage, about 10^{12}/ml in 0.9 % saline, was added to 5 parts of 0.1 M sodium phosphate buffer (pH 6.0) containing 1 mM EDTA, and 4 parts of 1 M hydroxylamine · HCl (adjusted to pH 6.0 with NaOH) containing 1 mM EDTA. At the desired time of mutagenesis, the phage were collected by centrifugation (40 min, 38 000 $\times g$) in the SS-34 head of the Sorvall RC-2 centrifuge and washed once with potassium phosphate buffer (50 mM at pH 7.0). The mutagenized phages were stored in phosphate buffer at 4 °C.

Isolation of Unc Mutants. The method for isolation of Unc mutants is to transduce *ilv*$^-$ auxotrophic bacteria to prototrophy with mutagenized transducing phage carrying Unc genes.

Recipient bacteria used for transduction were grown to the stationary phase of growth in 12 ml of nutrient broth at 37 °C. The cells were collected by centrifugation at 1500 $\times g$ for 10 min and resuspended to a final density of about 2×10^9 bacteria/ml in 6 ml of a solution containing 0.005 M $CaCl_2$, 0.1 M $MgSO_4$. Recipient cells were "activated" by shaking at 37 °C for 15 min. To 2 ml of this cell suspension was added 0.1 ml of mutagenized phage, which had an initial titer of 1–2 $\times 10^{12}$ plaque-forming particles per ml before mutagenesis. After shaking for 20 min for phage absorption at 37 °C, further penetration of the phage was stopped by addition of 2 ml of 1 M sodium citrate at 0 °C; an aliquot of 0.1 ml of infected bacteria was spread

uniformly on the surface of plates containing glucose-salts medium. The plates were incubated at 42 °C for about 3 days to allow the growth of transductant colonies. It was found that Unc$^-$, *ilv*$^+$ transductants form smaller colonies than Unc$^+$, *ilv*$^+$ transductants. Each small colony was transferred in a pattern, with a sterile wooden applicator stick, to a minimal glucose agar plate which was incubated at 42 °C for 1–2 days. The patches of cells were transferred by replica plating to two succinate-minimal plates and two glucose minimal plates. One set of plates was incubated at 42 °C for 1 day while the second set was incubated at 30 °C for 2 days. Mutants obtained from the latter plates were used in this study. The purpose of incubating one set of replica plates at 42 °C was for selection of thermosensitive (ts) or cold sensitive (cs) strains whose properties will be described in a separate publication. Clones unable to grow on succinate as a sole source of energy were purified by several transfers onto L-broth plates and stored as stocks.

ATPase Assay. The lysozyme (Millipore) assay for ATPase described previously [6] was modified for quantitative analysis of large numbers of mutants as follows: samples of putative Unc mutant cells (0–250 μg total protein) are pipetted to a bank of Millipore filters (0.45 μm cellulose nitrate pads) containing about 10 ml of lysozyme solution (20 μg/ml lysozome prepared daily, 1.0 mM EDTA, 0.1 M Tris-HCl buffer, pH 6.4, sucrose 20%). After incubation in the lysozyme solution at room temperature for 10 min the cells are collected on the filters, washed with 10 ml 0.01 M Tris buffer at pH 8.0, and the filters are transferred to assay tubes containing ATPase assay mixture (7.5 μmol ATP, 2.5 μmol $MgCl_2$, and 60 μmol Tris buffer at pH 9.0 in a total volume of 0.5 ml). The filter pads containing ATPase are immersed in the assay mix using a glass rod and incubated for 30 min at 37 °C. Release of inorganic phosphate is measured as described previously [6]. For monitoring ATPase activity during enzyme purification the enzyme fractions were added directly to the ATPase assay mix. Specific activity is expressed as μmol of inorganic phosphate released per mg protein per minute.

Anaerobic Growth. Anaerobic growth measurements were carried out in glucose Davis minimal medium, supplemented with L-broth 0.05%; for nitrate-grown cells KNO_3 (0.2%) was added [6]. Growth yields for Unc mutants were determined according to the procedure of Butlin et al. [3].

O_2 Uptake. Oxygen uptake in whole cells was monitored with an oxygen electrode (No. 5331, Yellow Springs Instrument Co.) at 30 °C in a 2-ml vessel.

Preparation of membrane vesicles and purification of ATPase. The French pressure cell method of Futai et al. [8] was used for preparation of membrane vesicles of Unc mutant and wild type, except that the pressure of the press was set at 10 000 lb/inch^2. In some experiments, $MgCl_2$ was replaced by $CaCl_2$ in the buffer solution used to resuspend the vesicle pellet fraction. Membrane preparations were stored under liquid nitrogen. ATPase was purified starting with membrane vesicles according to the procedure of Futai et al. [8]. Protein was determined using the procedure of Lowry et al. [9].

Assay of aerobic and ATP-dependent transhydrogenase and oxidative phosphorylation. The energy-dependent reduction of NADPH catalyzed by membrane vesicles was measured according to the procedure of Futai et al. [8]. Respiratory transhydrogenase refers to the reaction driven by oxidative energy using NADH as an electron donor in contrast to the ATP-driven transhydrogenase which requires ATP as a

substrate. The increase in absorbance of NADPH at 340 nm was followed in a Gilford spectrophotometer. Oxidative phosphorylation activity of membrane vesicles using D-lactate as an electron donor containing 0.2–0.4 mg protein was measured according to the procedure of Ninio and Yamamoto [10], except that the concentration of metal ions used was changed to 2 mM.

RESULTS

NH_2OH Mutagenesis of P1

Hydroxylamine was chosen as a mutagen because of its successful use for generalized mutagenesis of the *Salmonella* chromosome using transducing phage P22 [5]. An NH_2OH survival curve for P1 is plotted in Fig. 1. The mutagenesis was monitored by examination of the fraction of surviving phage (plaque-forming units), which is used as an index of the mutagenesis of transducing DNA. As seen from Fig. 1, the time required for killing 99% of the phage is about 6 h. The arrow on the curve illustrates the time point that P1 samples were removed for transduction. It is assumed that the level of killing of the phage particles is an indicator of the approximate level of mutagenesis of the transducing particles which carry the ATPase genes. Mutants with a lethal hit in any given gene (or operon) appear to be common by this procedure. For example, Hong and Ames [5] mentioned that mutants of glycogen biosynthetic genes appeared at a frequency between 2.1–4.5% of the total

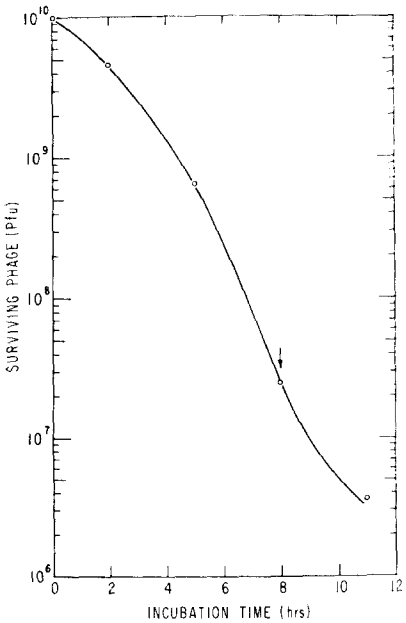

Fig. 1. Survival curve of phage P1 during hydroxylamine mutagenesis. The surviving fraction of viable phage particles (plaque-forming units, PFu) was determined by removing small aliquots at the times indicated, diluting in saline, plating with a suitable host strain and counting the remaining plaques. The arrow refers to the time point at which a large aliquot of mutagenized phage particles was removed and washed free of hydroxylamine for transduction experiments.

transductants. We have found that about 10% (90/880) of the small transductant clones derived from mutagenized P1 particles are succinate auxotrophs (Unc^-); however, this figure cannot be compared to the value for glycogen synthetase since in the initial screening, primarily small colonies were chosen for testing for the Unc^- phenotype.

Unc A and Unc B phenotypes

Approximately 90 putative Unc^- phenotypes were selected using the localized mutagenesis of the chromosome with hydroxylamine. Of these, 26 were tentatively classified as belonging to the Unc A phenotype [3] based on the following criteria: (1) all 26 strains were unable to utilize succinate as a sole source of energy; (2) the 26 strains did not grow anaerobically; (3) anaerobic growth was restored by addition of substrate levels of nitrate ion as a terminal electron acceptor; (4) ATPase hydrolytic activity measured in all strains was less than 10% of the wild type value; (5) four of the 26 strains chosen at random were found to map with a P1 cotransduction frequency of 46–57% with *ilv*. (6) O_2 uptake measured in 4 out of 4 strains chosen at random ranged from equivalent to slightly higher than the respiration rate observed with the parent strain. From these results it was concluded that about 1/3 of the mutants obtained by localized mutagenesis of the Unc region display the Unc A phenotype. About 10% of the Unc mutants examined showed properties similar to the Unc B phenotype described by Cox et al. [4]. For instance, the ATPase hydrolytic activity of seven out of seven of the Unc B strains examined was found to be from 80–100% that of the wild type strain. Unc B strains were able to grow anaerobically in the absence of terminal electron acceptors. Since the Unc A and Unc B strains mentioned above displayed properties similar to known Unc phenotypes they were not studied further. Instead, our attention was directed to the unusual phenotypes of the collection.

Unc D

As reported in this section, three mutants (Unc 369, 372, 373) were found to display such unusual properties that a new phenotype designation, Unc D, seemed warranted. (Unc C as a phenotype has been omitted in naming the new mutant classes because of the possibility that Unc C (control) mutants may be forthcoming.)

The growth characteristics of Unc D mutant strains are similar to those reported for other Unc^- strains. For example, the mean generation time in glucose-salts medium was longer for the Unc D mutants, being 1.6 h compared with about 1 h for the wild-type strain. Determination of the growth yields, measured as turbidities, of the Unc D strains growing on media containing limiting concentrations of glucose showed (Fig. 2) that the aerobic growth yield of strain Unc D 373 was lower than that of the parent strain. However, the anaerobic growth yield of Unc D 373 compared to its parent was only slightly reduced. The anaerobic growth yield of two additional Unc D strains (Unc D 369 and Unc D 372) was found to be nearly identical with the wild type.

Previous workers have mapped several Unc mutations including Unc A and Unc B near *ilv* on the linkage map of *E. coli*. As seen from Table I, P1-mediated crosses between three different Unc D isolates (Unc D 369, 372, 373) as donors with an ilv^- strain as recipient yielded a majority of recombinant ilv^+ colonies car-

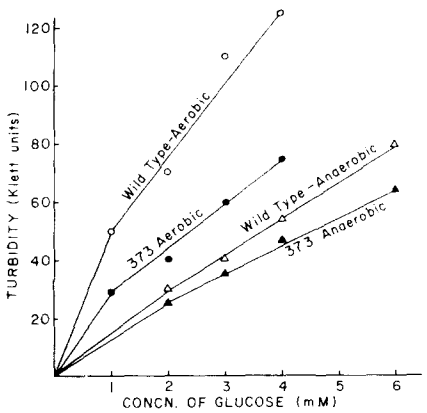

Fig. 2. Growth yields (turbidity) for Unc D 373 and wild type grown on limiting concentrations of glucose. Cultures were aerated by shaking at 37 °C and anaerobic cultures were incubated as described in the Methods section.

TABLE I

P1 COTRANSDUCTION FREQUENCIES OF DIFFERENT Unc PHENOTYPES WITH *ilv*⁻

See text for experimental details.

Donor	Recipient	Number of *ilv*⁺ transductants examined	Number of succinate minus	Cotransduction frequency (Unc-*ilv*)
Unc D⁻, ilv⁺				
Unc D 369	Unc⁺, *ilv*⁻	600	399	66.5
Unc D 372	Unc⁺, *ilv*⁻	300	186	62
Unc D 373	Unc⁺, *ilv*⁻	250	130	52

rying the Unc D phenotype. Cotransduction frequencies ranging from 52 to 62 % were observed for the different strains.

Experiments on O_2 uptake were conducted in order to rule out the possibility that Unc D mutations alter the respiratory properties of the mutants. O_2 uptake by the five strains using glucose as a carbon source was found to be in the range of 200–230 ng atom per min per mg protein, slightly higher than that of wild type, which was found to be about 160 ng atom per min per mg protein.

Using the lysozyme assay for ATPase, it was found that the activities of Mg^{2+} ATPase in whole cells of three Unc D mutants respectively was only about 25 % of wild type activity (Table II). However, as will be discussed in more detail below, addition of Ca^{2+} in place of Mg^{2+} stimulated the hydrolytic activity of ATPase to a level approx. 60–80 % of the wild type Mg^{2+} ATPase (Table II).

Unc D 373 has been selected for further biochemical studies. Several workers have reported that ATPase is required for ATP-dependent transhydrogenase activity, catalyzed by membrane vesicles [8, 11–15]. Summarized in Table III, vesicles prepared from wild type and mutant catalyze respiratory-dependent transhydrogenase activities with Mg^{2+} or Ca^{2+}. However, vesicles of Unc D 373 were found to catalyze very

TABLE II

ATPase ACTIVITY OF Unc D MUTANTS

The lysozyme assay procedure was used as described in the Methods.

Unc strain	ATPase activity (μmol Pi/mg protein/30 min)	
	Mg^{2+} ATPase	Ca^{2+} ATPase
369	3.0	6.0
372	2.8	6.7
373	2.3	8.1
Wild type (3110)	10.5	3.0

TABLE III

ENERGY-DEPENDENT PYRIDINE NUCLEOTIDE TRANSHYDROGENASE ACTIVITIES AND OXIDATIVE PHOSPHORYLATION OF AN Unc D MUTANT

The transhydrogenase assay mixture containing 0.15–0.25 mg of protein was as described by Futai [8]. The reaction mixture was incubated for 2 min at 23 °C and the reaction was initiated by the addition of NADP. For the aerobic reaction, the increase in absorbance at 340 nm was followed for about 4 min; at this point ATP was added to measure the ATP-driven transhydrogenase and the increase in absorbance was followed for 3 min. The aerobic rate was subtracted from this increase, and the result was expressed as ATP-driven rate. For the Ca^{2+}-activity measurements, $CaCl_2$ replaced $MgCl_2$ in the assay mixture. The assay mixture for oxidative phosphorylation containing 0.2–0.4 mg vesicle protein was as described by Ninio [10]. The reaction was followed for 15 min and ATP measured by using the luciferase assay [16]. O_2 uptake was measured using an O_2 electrode as described in Methods. Transhydrogenase activity was measured as nmol NADPH/mg protein/min.

E. coli strain	Mg^{2+} activity			Ca^{2+} activity		
	Respiratory transhydrogenase activity	Oxidative phosphorylation		Respiratory transhydrogenase activity	Oxidative phosphorylation	
		ATP	P/O		ATP	P/O
Wild type 3110	52	41	0.088	52	19	0.046
Unc D 373	75	8	0.002	75	24	0.002

low levels of ATP-dependent transhydrogenase activities (with Mg^{2+}) compared to the wild type. Ca^{2+} appears to function to some extent for ATP-dependent transhydrogenase catalyzed by mutant and wild type vesicles, and it is the preferred ion for the mutant system.

The rates of oxidative phosphorylation of vesicles prepared from Unc D 373 and wild type were compared in order to determine whether the energy-transducing system of the mutant would respond to Ca^{2+} in preference to Mg^{2+}. As seen in Table III, only trace levels of oxidative phosphorylation were found with the mutant regardless of the divalent metal ion used. For the wild type, the P/O ratio of 0.046 for Ca^{2+} is about half that with Mg^{2+}. We cannot rule out the possibility that part of this Ca^{2+} activity is due to Mg^{2+} in the preparation. Thus, Unc D 373, like other classes of Unc mutants, shows a very poor capacity for oxidative phosphorylation.

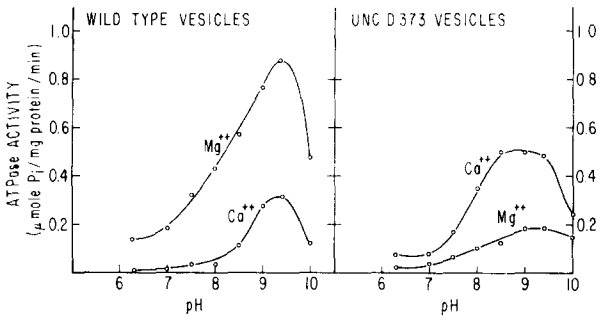

Fig. 3. Altered divalent metal ion specificity for ATP hydrolysis catalyzed by ATPase of membrane vesicles of Unc 373 compared to wild type. Membrane vesicles were prepared as described in Methods. About 250–300 mg protein was added to the assay mixture and the released inorganic phosphate was determined as described previously (6). WT, wild type; 373, Unc D 373.

The next experiments are concerned with the hydrolytic properties of ATPase of the mutant Unc D 373. The marked difference in divalent metal specificity of ATPase activity of vesicles of Unc D 373 versus wild type is illustrated in Fig. 3, in which ATP hydrolytic activity is plotted as a function of pH. The curves labeled Ca^{2+} or Mg^{2+} refer to the hydrolytic activity obtained using these metals. Note from Fig. 3 that the Mg^{2+}/Ca^{2+} activity ratio to the ATP hydrolytic activity with Mg^{2+} divided by the specific activity with Ca^{2+} at pH 9 is about 2.4 for wild-type vesicles. In contrast, the Mg^{2+}/Ca^{2+} ratio for Unc D 373 was found to be 0.4.

In order to establish that the ATPase itself is altered in the Unc D mutants, ATPase was purified from one of the Unc D mutants (Unc D 373) according to the procedure of Futai et al. [8] and tested for divalent metal specificity at different steps of the purification (Table IV).

The column of Table IV labeled Fraction refers to the different stages of enzyme fractionation [8]. The major point to note is that the Mg^{2+}/Ca^{2+} ratio for the ATP

TABLE IV

PURIFICATION OF Mg^{2+}, Ca^{2+} ATPase FROM Unc D 373 AND WILD TYPE

Starting material	Fraction	Total protein (mg)	Total ATPase activity		Ratio of Mg^{2+} activity/ Ca^{2+} activity	% Recovery
			Mg^{2+} ATPase (units)*	Ca^{2+} ATPase (units)*		
Wild type (3110)	EDTA extract	306	398	147	2.7	100**
(55 g)	DEAE pooled	140	154	63	2.4	39
	Biogel A 0.5 M	3.8	53	22	2.4	13
Unc D 373	EDTA extract	320	64	130	0.49	100***
(55 g)	DEAE pooled	140	28	77	0.37	59
	Biogel A 0.5 M	7	12	20	0.6	15

* Unit = μmol P1 per min.
** Mg^{2+} activity
*** Ca^{2+} activity.

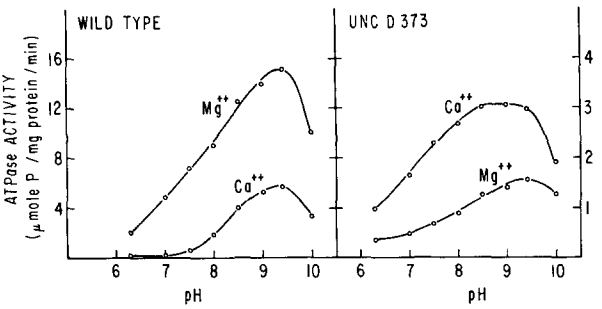

Fig. 4. Mg^{2+} and Ca^{2+} specificity of ATP hydrolysis by purified ATPase of wild type and Unc D 373. ATPase assay as described in the legend for Fig. 2. ATPase of membrane vesicles was solubilized and purified as described in Methods. WT, wild type; 373, Unc D 373.

hydrolytic activity of ATPase of Unc D 373 and wild type remain relatively constant during enzyme fractionation. For wild type, the values varied from a ratio of 2.4 to 2.7, whereas the ratio for the mutant ranged from 0.4 to 0.6. The remaining data of Table IV are presented in the form of an enzyme purification chart in order to permit a comparison of specific activities, enzyme recovery, etc., between wild type and the mutant enzyme at each stage of purification. As noted earlier, the specific activity of Mg^{2+} ATPase of crude extract of the mutant is considerably lower than the wild type. However, the enzymes behaved similarly during purification.

Enzyme fractions representing the peak of activity from the Biogel column were pooled and assayed for hydrolytic activity as a function of pH as summarized in Fig. 4. Results were similar to Fig. 3. The major conclusion from these experiments is that the purified ATPase of the Unc D 373 has a strong specificity for Ca^{2+} over Mg^{2+} as a divalent metal for ATP hydrolysis.

DISCUSSION

Energy-transducing ATPase as isolated from the membrane of *E. coli* is a complicated enzyme of about $3\text{–}4 \times 10^5$ mol. wt. containing either four or five subunits (α, β, γ, δ, ε) depending on the method of isolation [4, 8, 12, 14]. Bragg et al. [15] were the first to observe that removal of the δ-polypeptide resulted in complete loss of coupling activity as measured by the restoration of ATP-dependent transhydrogenase activity of ATPase-stripped respiratory particles. ATPase hydrolytic activity was only slightly affected in the complex missing the δ subunit. The key role of the δ subunit has further been elucidated by Futai et al. [8] and Smith and Sternweis [17], who observed that ATPase missing the δ subunit was ineffective for reconstituting energy coupling and also did not bind to an ATPase-deficient membrane. The molecular weight of the four-subunit ATPase was found to be significantly lower but the activity was not appreciably affected. These findings point to a role of the δ subunit in the interaction of ATPase with the membrane. The role of the remaining subunits in *E. coli* coupling factor activity is just beginning to come to light. For example, Nelson et al. [18] (see also Salton and Schor [19]) have recently observed that trypsin treatment of the four-subunit ATPase of *E. coli* results in a further reduction of a two-subunit protein apparently containing only the two larger subunits

(α and β). Interestingly, the two-subunit complex still catalyzed ATP hydrolysis, suggesting that the active site for ATP breakdown was present on either the α or β subunits or both.

Obviously there is much to be discovered regarding the mechanism of action and role of the subunits of ATPase in energy transduction. Studies of *E. coli* mutants altered in the various subunits of the enzyme may prove to be an important tool for this work. For example, Gibson and co-workers [12, 13] have recently described strains (Unc A) deficient in ATPase hydrolytic activity mutations which may alter the subunits concerned with ATP hydrolysis. In addition, a second protein called the "Unc B protein" has been identified by these workers as a crucial membrane protein which is deficient in strains carrying the Unc B mutation. The finding that the Unc A and Unc B lesions occur very close to one another on the linkage map of *E. coli* is suggestive that several key energy transduction proteins may be clustered in the same region of the chromosome, perhaps on an operon. One mutation studied by Gibson and co-workers [3] apparently blocks the synthesis or assembly of all of the ATPase subunits, a result which led to the suggestion by the authors that this phenotype might be caused by a strongly polar mutation, such as is observed with nonsense-type mutants, which could simultaneously block the translation of several different Unc proteins of the operon.

From a biochemist's viewpoint, Unc D mutants appear to be an interesting phenotype since the mutation apparently alters the structure of the ATPase-enzyme complex observed as a change in divalent metal specificity for ATP hydrolysis. The simplest explanation is that one of the structural proteins concerned with ATP hydrolysis is altered in the mutant. Since the mutation responsible for this catalytic defect maps within the Unc cluster near Unc A, the argument can be made that a structural subunit of the ATPase enzyme complex responsible for metal ion specificity must be located within or near the Unc gene cluster. As mentioned above, there is the interesting possibility that some or all of the structural genes coding for the five subunits of ATPase as well as energy-transducing proteins working closely with ATPase, such as Unc B protein, may be located as an operon(s). If this turns out to be the case, then in the future it may be possible to correlate the various unique ATPase phenotypes (classes) with a particular subunit(s).

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REFERENCES

1 Cox, G. B. and Gibson, F. (1974) Biochim. Biophys. Acta 346, 1–25
2 Kovac, L. (1974) Biochim. Biophys. Acta 346, 101–135
3 Butlin, J. D., Cox, G. B. and Gibson, F. (1971) Biochem. J. 124, 75–81
4 Butlin, J. D., Cox, G. B. and Gibson, F. (1973) Biochim. Biophys. Acta 292, 366–375
5 Hong, J. and Ames, B. N. (1971) Proc. Natl. Acad. Sci. U.S. 68, 3158–3162

6 Yamamoto, T. H., Mevel-Ninio, M. and Valentine, R. C. (1973) Biochim. Biophys. Acta 314, 267–275
7 Miller, J. H. (1972) in Experiments in Molecular Genetics, Cold Spring Harbor Laboratory
8 Futai, M., Sternweis, P. C. and Heppel, L. A. (1974) Proc. Natl. Acad. Sci. U.S. 71, 2725–2729
9 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
10 Ninio, M. M. and Yamamoto, T. (1974) Biochim. Biophys. Acta 357, 63–66
11 Cox, G. B., Newton, N. A., Butlin, J. D. and Gibson, F. (1971) Biochem. J. 125, 489–493
12 Cox, G. B., Gibson, F. and McCann, L. (1973) Biochem. J. 134, 1015–1021
13 Cox, G. B., Gibson, F. and McCann, L. (1974) Biochem. J. 138, 211–215
14 Bragg, P. D., Davies, P. L. and Hou, C. (1972) Biochem. Biophys. Res. Commun. 47, 1248–2155
15 Bragg, P. D. and Hou, C. (1973) Biochem. Biophys. Res. Commun. 50, 729–736
16 Strehler, B. L. (1963) in, Methods in Enzymatic Analysis (Burgmeyer, H. V., ed.), p. 559, Academic Press, New York
17 Smith, J. B. and Sternweiss, P. C. (1975) Biochem. Biophys. Res. Commun. 62, 764–777
18 Nelson, N., Kanner, B. I. and Gutnick, D. L. (1974) Proc. Natl. Acad. Sci. U.S. 71, 2720–2724
19 Salton, M. R. J. and Schor, M. T. (1972) Biochem. Biophys. Res. Commun. 49, 350–357